Information on this journal can be accessed at http://wileyonlinelibrary.com/journal/mec

The journal is covered by AGRICOLA, Chemical Abstracts, Current Awareness Biological Sciences and Current Contents.

This journal is available at Wiley Online Library. Visit http://wileyonlinelibrary.com to search the articles and register for table of contents e-mail alerts.

MOLECULAR ECOLOGY
VOLUME 23, NUMBER 17, SEPTEMBER 2014

NEWS AND VIEWS
Perspectives
4185 Expanding the population genetic perspective of cnidarian–Symbiodinium symbioses
S. R. Santos

4188 Species integrity in trees
D. Ortiz-Barrionuevo & E. J. Bauck

4192 Take up the challenge! Opportunities for research evolution from resolving conflict in integrative taxonomy
R. C. Schild-Harder, W. Anthoff & F. M. Steinze

Opinion
4195 Reproductive identity in protozoan pathogens—truth or artefact?
J. D. Ramirez & M. S. Llewellyn

FROM THE COVER
4203 New insights into the dynamics between reef corals and their associated dinoflagellate endosymbionts from population genetic studies
I. B. Baums, M. K. Devlin-Durante & T. C. Lajeunesse

ORIGINAL ARTICLES
Population and Conservation Genetics
4216 What is genetic differentiation, and how should we measure it—Gst, D, neither or both?
R. Verity & R. A. Nichols

4226 Geographical differences in vertical connectivity in the Caribbean coral Montastrea annularis despite high levels of horizontal connectivity at shallow depth

4241 Inbreeding and inbreeding depression in endangered red wolves (Canis rufus)

Ecological Genomics
4256 Fine-scale population epigenetic structure in relation to gastrointestinal parasite load in red grouse (Lagopus scotica)
M. A. Wenzel & S. B. Piertney

4274 The role of plant and host species in structuring rhizosphere epifungal communities

4391 Flowering time QTL in natural populations of Arabidopsis thaliana and implications for their adaptive value
E. S. Dittmar, C. G. Oakley, J. Ågren & D. W. Schemske

4316 Unexpected ancestry of Populus seedlings from a hybrid zone implies a large role for postzygotic selection in the maintenance of species
D. Lindtke, Z. Gompert, C. Lexer & C. A. Buerkle

4322 Distinct male reproductive strategies in two closely related oak species
L. Lagache, E. K. Klein, A. Ducousso & R. J. Petit

4331 Genomics of the divergence continuum in an African plant biodiversity hotspot, I: drivers of population divergence in Restio capensis (Restionaceae)

4344 Integration of conflict into integrative taxonomy: fitting hybridization in species delimitation of Mesocarabus (Coleoptera: Carabidae)
C. Andújar, P. Arribas, C. Ruiz, J. Serrano & J. Gómez-Zurita

4362 Chromosomal variation segregates within incipient species and correlates with reproductive isolation
G. Charron, J.-B. Leducq & C. R. Landry

4373 Phylogeography of Chinese house mice (Mus musculus/ Castaneus): distribution, routes of colonization and geographic regions of hybridization
M. Jing, H.-T. Yu, X. Bi, Y.-C. Lai, W. Jiang & L. Huang

Ecological Interactions
4387 Contrasting soil fungal community responses to experimental nitrogen addition using the large subunit rRNA taxonomic marker and cellobiohydrolase I functional marker
R. C. Mueller, M. M. Balasch & C. R. Kuske

4398 Assessing Symbiodinium diversity in scleractinian corals via high throughput sequencing-based genotyping of the ITS2 rDNA region
C. A. Stol, C. Daniels, T. Bayer, E. Banguera-Hinestroza, A. Barbrook, C. J. Howe, T. C. Lajeunesse & C. R. Voolstra


Published by Wiley Blackwell
Abstract

The mutualistic symbioses between reef-building corals and micro-algae form the basis of coral reef ecosystems, yet recent environmental changes threaten their survival. Diversity in host-symbiont pairings on the sub-species level could be an unrecognized source of functional variation in response to stress. The Caribbean elkhorn coral, Acropora palmata, associates predominantly with one symbiont species (Symbiodinium ‘fitti’), facilitating investigations of individual-level (genotype) interactions. Individual genotypes of both host and symbiont were resolved across the entire species’ range. Most colonies of a particular animal genotype were dominated by one symbiont genotype (or strain) that may persist in the host for decades or more. While Symbiodinium are primarily clonal, the occurrence of recombinant genotypes indicates sexual recombination is the source of this genetic variation, and some evidence suggests this happens within the host. When these data are examined at spatial scales spanning the entire distribution of A. palmata, gene flow among animal populations was an order of magnitude greater than among populations of the symbiont. This suggests that independent micro-evolutionary processes created dissimilar population genetic structures between host and symbiont. The lower effective dispersal exhibited by the dinoflagellate raises questions regarding the extent to which populations of host and symbiont can co-evolve during times of rapid and substantial climate change. However, these findings also support a growing body of evidence, suggesting that genotype-by-genotype interactions may provide significant physiological variation, influencing the adaptive potential of symbiotic reef corals to severe selection.

Keywords: climate change, Cnidarians, coevolution, contemporary evolution, coral reefs, ecological genetics, population genetics—empirical, species interactions, Symbiodinium

Introduction

Mutualistic associations between host and symbiont species are ubiquitous in nature (Douglas 2010). Tropical reef ecosystems are rich in animals that require dinoflagellate endosymbionts (Symbiodinium) for their survival. Phylogenetic approaches facilitated a turning point and transformed the study of these globally important symbioses (Rowan & Powers 1991). Presently, the genus Symbiodinium comprises numerous species grouped into several divergent sub-generic clades (referred as Clades A, B, C, etc.; Rowan & Powers 1991; Pochon & Gates 2010). We now know that the ecology (e.g. host specificity, water depth), biogeography and physiology are decidedly different among Symbiodinium spp. within and between clades. However, a full understanding of the ecology and evolution of host and symbionts requires sub-species-level resolution of the partners. This is because individual genotypes are the fundamental units of selection. The resolution of individual host and symbiont genotypes over large geographical ranges provides information about population structure, gene flow and patterns of
association between partners: evidence that is critical to furthering our understanding of the ecological and evolutionary processes important to these mutualisms.

Resolving *Symbiodinium* and hosts to the level of individual genotypes, or clones, can generate insights into how the symbiosis is established and maintained with potential functional consequences. Sub-species-level functional variation has been attributed to the interaction between host and mutualists in a variety of systems such as insects and bacteria (Feldhaar 2011), grasses and endophytes (Gundel et al. 2012), and legumes and *Rhizobia* (Parker 1995). The variation in stress responses observed among adjacent colonies of the same host species harbouring the same *Symbiodinium* type (Császár et al. 2010; Lajeunesse et al. 2010) suggests that sub-species-level variation in stress responses exists. Individual (genotype or strain)-level differences among host genets on the one hand and symbiont strains on the other, as well as the interactions between them, likely account for physiological variation.

The functional diversity contributed by inter-individual combinations of host and symbiont genotypes can also be the target of selection (Rodriguez et al. 2009) and may contribute to the adaptation of mutualisms to environmental change. The spread of better-adapted host and symbiont genotypes depends on the scale over which they successfully disperse. Marked differences in life history between coral and microbe lead to expectations that gene flow and dispersal patterns differ. Branching and massive coral hosts can reproduce asexually through fragmentation over small spatial scales (tens of metres; Highsmith 1982; Willis & Ayre 1985; Foster et al. 2007) as well as sexually through planktonic larvae with much larger potential dispersal ranges (tens of metres to thousands of kilometres; Baums et al. 2005; Underwood et al. 2007). The single-celled symbionts divide and multiply asexually and occasionally reproduce sexually (Lajeunesse 2001; Santos et al. 2004). *Symbiodinium* cells are constantly emitted/expelled, but the effective dispersal of these cells remains largely unknown. A full understanding of the adaptive potential of host and symbionts requires resolution of the fundamental units of selection in each partner, and knowing the spatial scales over which differently adapted genotypes can disperse.

The development and analysis of higher-resolution population genetic microsatellite markers has provided early insights into sub-species-level diversity of symbionts (Andras et al. 2011; Pettay et al. 2011; Pinzón et al. 2011; Thornhill et al. 2014). The initial results from multilocus genotyping recognized the existence of numerous *Symbiodinium* clones distributed among the colonies of a host population. Different patterns of distribution and abundance of *Symbiodinium* clones have been described including that a coral colony often associates with multiple genotypes (Howells et al. 2013) to evidence that indicates most colonies harbour a dominant clonal population represented by a single multilocus genotype (MLG; Thornhill et al. 2009, 2014; Andras et al. 2011; Pettay et al. 2011).

Here, we provide an analysis of gene and genotypic diversity across the entire geographic range of a coral–dinoflagellate symbiosis to identify patterns of genotype-by-genotype combinations, dispersal and gene flow that provide insight into the dynamics of animal–microbe mutualisms (Thompson & Cunningham 2002; Mihaljevic 2012). We investigated the individual-level diversity in the *Symbiodinium* sp. associated with the once dominant but now endangered Caribbean reef-building coral, *Acropora palmata*. *A. palmata’s* population genetic structure across its entire distribution range consists of just two large populations, one in the eastern and one in the western Greater Caribbean (Baums et al. 2005). Repeated genetic analyses of their dinoflagellate symbionts suggest that the coral is highly specific for the ITS-2 type A3 (Lajeunesse 2002; Thornhill et al. 2006; tentatively designated *Symbiodinium* ‘fitti’, nomen nudum) despite the fact that the larvae must acquire symbionts from the environment (horizontal transmission). We applied microsatellite markers developed for *Symbiodinium* ‘fitti’ that allow us to investigate how a combination of geographic, environmental and biotic factors affects the dispersal, formation and stability of specific combinations of host and symbiont genotypes across the Greater Caribbean.

**Methods**

*Microsatellite development*

Microsatellite multi-locus genotypes (MLGs) for *A. palmata* were of Baums et al. (2005; n = 5 loci). *Symbiodinium* ‘fitti’ DNA was amplified with primers of Pinzón et al. (2011; n = 10 loci, Table S1, Supporting information). Three additional loci for *S. fitti* were developed here (Table S1, Supporting information). Alleles were fluorescently visualized and sized with internal standards on a PRISM 3100 Genetic Analyzer (Applied Biosystems). Briefly, 25–50 ng of template DNA was added to PCRs containing 1× Standard Taq Buffer (New England Biolabs), 2.5 mM MgCl₂ (New England Biolabs), 0.5 mg/mL Bovine serum albumin (New England Biolabs) and 0.75 U of Taq (0.325 U for primers 31, 32 and 41; New England Biolabs). Primer concentrations of 200 nM of each primer were added to reactions involving loci A3Sym_01, 18, 27 and 28, whereas a primer concentration of 93 nM was added to reactions for primers 31, 32, 41. Primer concentrations of 50 nM of the
tained forward primer (see Table S1, Supporting information), 150 nt of the reverse primer and 75 nt of the dye labelled T-oligonucleotide were used for amplifying loci A3Sym_01, 02, 03, 07, 08, 09 and 48. All loci were amplified using the following thermal cycle profile: 94 °C for 2 min (1 cycle); 94 °C for 15 s, primer-specific annealing temperature (Table S1, Supporting information) for 15 s, 72 °C for 30 s (31 cycles); 72 °C for 30 min on a Mastercycler gradient thermal cycler (Eppendorf, Westbury, NY, USA). PCR primer sets designed for each S. ‘fitti’ locus were screened against DNA from Symbiodinium ITS-2 type A3 cultures. Additionally, S. ‘fitti’ primers were tested on symbiont-free Acropora egg DNA. No amplification of host DNA was observed.

**Sampling schemes**

We characterized the population genetic and clonal structure of S. ‘fitti’ at various spatial scales. First, we took samples from multiple locations within a colony and then we considered reef- and region-scale clonal reproduction using randomly sampled polar plots (15 m diameter). We utilized A. palmata samples archived from around the Caribbean basin (Table 1 and Table S2, Supporting information, Baums et al. 2005). A subset of these samples (~700) was collected to examine the extent to which A. palmata propagate through fragmentation (Baums et al. 2006). In a stand of A. palmata, 24 colonies were mapped and sampled randomly using a circular transect (polar plot). Each plot consisted of nested 5-, 10- and 15-m-radius circles resulting in spatially explicit random sampling biased towards detection of genet diversity within the 5-m-radius circle. If the size of the A. palmata stand allowed it, two plots were collected per reef. Samples were collected from several reefs per region (Table 1 and Table S2, Supporting information). This design kept sampling effort constant across sites and thus allowed us to compare the contribution of clonal reproduction to the population of host versus symbionts. Additional samples were collected haphazardly to investigate gene flow patterns. Details of the sampling procedure are given in Baums et al. (2006).

**Genotype diversity**

We refer to an assemblage of genetically identical colonies (clones) that are descendants of a single zygote as a ‘genet’. Physiologically distinct colonies that can function and survive on their own but belong to the same genet are termed ‘ramets’. Symbiodinium ‘fitti’ is a haploid, single-celled eukaryote that replicates via cell division, thus generating clonal cell lines. Different MLGs of S. ‘fitti’ are referred to as strains. The microsatellite markers for the host are highly heterozygous (mean observed heterozygosity = 0.77); thus, there is a low probability of identifying two colonies as clonemates when in fact they are distinct genets (this is called the probability of identity (PI) and equals $10^{-7}$ for A. palmata). The PI for the diploid A. palmata was calculated in GENALEX version 6.4 (Peakall & Smouse 2006). The PI for S. ‘fitti’ was calculated as the sum of each allele’s frequency squared in each population and multiplied across loci. For the haploid S. ‘fitti’, the probability of identity equalled $10^{-5}$. After inspecting a plot of pairwise differences in genetic distance among all samples (Fig. S1, Supporting information), we determined that only those MLGs were clonemates that shared identical alleles at all loci (see Appendix S1 for justification of approach, Supporting information). Genet identification and calculation of diversity indices were performed in GENALEX version 6.4 and GENODIVE (Table S3, Supporting information, Meirmans & Van Tienderen 2004).

**Table 1 Acropora palmata host ($N_{Ap}$) and symbiont ($N_{Sf}$) genotypes were obtained from colonies located throughout the Greater Caribbean**

<table>
<thead>
<tr>
<th>Region</th>
<th>Population</th>
<th>$N_{Plots}$</th>
<th>$N_{Ap}$</th>
<th>$N_{Sf}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>East</td>
<td>Bonaire</td>
<td>2</td>
<td>38</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>Curacao</td>
<td>6</td>
<td>132</td>
<td>117</td>
</tr>
<tr>
<td></td>
<td>Puerto Rico</td>
<td>13</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SVG</td>
<td>8</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>USVI</td>
<td>3</td>
<td>67</td>
<td>61</td>
</tr>
<tr>
<td>West</td>
<td>Bahamas</td>
<td>5</td>
<td>105</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>Dominican Republic</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Florida</td>
<td>4</td>
<td>173</td>
<td>155</td>
</tr>
<tr>
<td></td>
<td>Mona</td>
<td>2</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Mexico</td>
<td>1</td>
<td>37</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Navassa</td>
<td>1</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Panama</td>
<td>3</td>
<td>66</td>
<td>55</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>12</td>
<td>25</td>
<td>664</td>
</tr>
</tbody>
</table>

Some reefs were sampled randomly in circular plots ($N_{Plots}$) with constant sampling effort ($n = 24$ colonies per plot). Colonies sampled multiple times are listed only once, and colonies with multiple infections are excluded (Fig. 1).

**Linkage disequilibrium**

Tests of linkage disequilibrium (LD) were conducted using Genepop on the web 4.0 (Rousset 2008) to ascertain whether recombination occurs in Symbiodinium ‘fitti’ genotypes (Santos & Coffroth 2003). We tested the null hypothesis of random association between two loci via a log-likelihood ratio test. High values of LD are expected when clonal reproduction is high and all MLGs are considered. Once repeated MLGs are removed, LD is expected to decline in recombining...
organisms. Indeed, *S. ‘fitti’* appears to undergo sexual recombination: LD was nearly absent when considering only the 176 unique MLGs (10 of 78 tests were significant at \( P < 0.05 \) after Bonferroni correction). As expected for a species with mixed asexual and sexual reproduction, almost all pairwise locus comparisons became significant for LD when including all 664 samples with complete MLGs (71 of 78 tests were significant at \( P < 0.05 \) after Bonferroni correction).

**Clonal structure**

Considering all genotypes sampled in random plots, we performed a spatial autocorrelation analysis assuming 15 even distance classes ranging from 1 to 15 m (the radius of the plots) in GENALEX version 6.5. Spatial autocorrelation analysis describes the spatial extent or neighbourhood size of genets by calculating the pairwise genetic distances among samples for each of a number of distance classes and correlates those with the geographic distance between the samples. The resulting correlogram depicts the change in correlation (\( r \)) between genetic and geographic distance over the range of distance classes. A spatial heterogeneity test (Banks & Peakall 2012) was used to test the null hypothesis of no difference between the correlograms derived from *S. ‘fitti’* and from *A. palmata* (999 permutations).

**Genetic population structure**

Clustering methods that use unique MLGs were employed to estimate the number of different populations in the data set albeit the symbiont is haploid, violating model assumptions (Pritchard et al. 2000). We then used analysis of molecular variance (AMOVA) to compare the amount of variation distributed within and between populations when grouping *S. ‘fitti’* populations by the Structure results versus grouping *S. ‘fitti’* populations according to the previously identified eastern and western host population (Table 2). A similar AMOVA was performed on all genotypes, and pairwise \( \Phi_{PT} \) values were calculated among populations (Peakall & Smouse 2006). Results were qualitatively similar to the unique MLG data set (Table S4, Supporting information). We also constructed a median-joining Table 2 Population differentiation of *Symbiodinium ‘fitti’* across 10 populations in the Greater Caribbean

<table>
<thead>
<tr>
<th>(A) Source</th>
<th>d.f.</th>
<th>SS</th>
<th>MS</th>
<th>Var.</th>
<th>%</th>
<th>( \Phi )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among population</td>
<td>9</td>
<td>185.20</td>
<td>20.58</td>
<td>1.07</td>
<td>30</td>
<td>0.30**</td>
</tr>
<tr>
<td>Within population</td>
<td>166</td>
<td>408.72</td>
<td>2.46</td>
<td>2.46</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>175</td>
<td>593.92</td>
<td>3.53</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(B) Source</th>
<th>d.f.</th>
<th>SS</th>
<th>MS</th>
<th>Var.</th>
<th>%</th>
<th>( \Phi )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between regions</td>
<td>1</td>
<td>38.77</td>
<td>38.77</td>
<td>0.14</td>
<td>4</td>
<td>0.04**</td>
</tr>
<tr>
<td>Among population</td>
<td>8</td>
<td>146.43</td>
<td>18.307</td>
<td>0.99</td>
<td>27</td>
<td>0.29**</td>
</tr>
<tr>
<td>Within population</td>
<td>166</td>
<td>408.72</td>
<td>2.46</td>
<td>2.46</td>
<td>69</td>
<td>0.32**</td>
</tr>
<tr>
<td>Total</td>
<td>175</td>
<td>593.92</td>
<td>3.59</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(C) ( \Phi_{PT} )</th>
<th>Bonaire</th>
<th>Curacao</th>
<th>SVG</th>
<th>USVI</th>
<th>Bahamas</th>
<th>Florida</th>
<th>Mexico</th>
<th>Navassa</th>
<th>Puerto Rico</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curacao</td>
<td>0.127</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SVG</td>
<td>0.407</td>
<td>0.258</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>USVI</td>
<td>0.168</td>
<td>0.165</td>
<td>0.362</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bahamas</td>
<td>0.382</td>
<td>0.235</td>
<td>0.414</td>
<td>0.334</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Florida</td>
<td>0.410</td>
<td>0.320</td>
<td>0.491</td>
<td>0.393</td>
<td>0.182</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mexico</td>
<td>0.397</td>
<td>0.305</td>
<td>0.462</td>
<td>0.409</td>
<td>0.421</td>
<td>0.383</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Navassa</td>
<td>0.351</td>
<td>0.214</td>
<td>0.425</td>
<td>0.203</td>
<td>0.245</td>
<td>0.339</td>
<td>0.375</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Panama</td>
<td>0.226</td>
<td>0.142</td>
<td>0.330</td>
<td>0.225</td>
<td>0.353</td>
<td>0.441</td>
<td>0.349</td>
<td>0.272</td>
<td></td>
</tr>
<tr>
<td>Puerto Rico</td>
<td>0.126</td>
<td>0.135</td>
<td>0.385</td>
<td>0.159</td>
<td>0.325</td>
<td>0.340</td>
<td>0.411</td>
<td>0.247</td>
<td>0.211</td>
</tr>
</tbody>
</table>

Dominican Republic (\( n = 1 \)) and Mona (\( n = 2 \)) were excluded because of small sample sizes. Given are the results of an AMOVA when considering all populations separately (A) and according to the east/west regions of the host (B). Pairwise comparisons (C) of population differentiation (\( \Phi_{PT} \)) were significant between all populations (\( P < 0.05 \)).

d.f., degrees of freedom; SS, sum of squares; MS, mean sum of squares; Var., estimated variance; \( \Phi \), fixation index. **\( P < 0.001 \).
network on the MLG data from \textit{S. \textit{fitti}} using program
defaults in Network 4.6 (Bandelt \textit{et al.} 1999) albeit \textit{S. \textit{fitti}} microsatellite loci show evidence of recombina-
tion, violating model assumptions. Structure, AMOVA and
Network analyses produced congruent results; hence,
analyses were deemed robust against violations of
model assumptions.

Multiple alleles

To assess the prevalence of multiple infections per \textit{A. palmata} colony, we considered a data set containing all sam-
pies for which we attempted genotyping of primers 1, 3,
7, 9, 18, 27 and 28 (\textit{n} = 759). Any samples that showed
multiple alleles at those loci were not run for the remain-
ing loci and thus the analysis would be skewed if we
were to include samples that had complete or nearly
complete MLGs for all 13 loci. In this data set, we found
\textit{n} = 124 samples with multiple alleles at one or more loci
(16\%). We tested the hypothesis of no difference in the propor-
tion of multiple infections per colony per region
(Bonferroni adjusted) and between the eastern and wes-
tern Greater Caribbean using a Pearson chi-square test.
See Appendix S2 (Supporting information) for further
details and justification of approach.

Repeated sampling over space and time

Repeated sampling of \textit{S. \textit{fitti}} strains over time (interval
1 year, \textit{n} = 17 or 2 years, \textit{n} = 2) and space (2–7 times
within a colony, \textit{n} = 9) was conducted in sand island
and horseshoe reefs, Florida (\textit{n} = 18 colonies) and Sea
Aquarium reef, Curacao (\textit{n} = 4 colonies) to address the sta-
bility of the host-symbiont association (Table S5,
Supporting information). Repeated sampling within a
colony usually included three samples from growing
tips, one sample from the underside of a branch and one
from the base of the colony.

Co-infection detection sensitivity

For two \textit{S. \textit{fitti}} strains with different allele sizes at a
locus, DNA was combined to constitute from 5\% to
95\% of the total. Singleplex PCR was performed as
described above. Allele sizes were scored for all dilu-
tions and detection thresholds determined (Fig. S2,
Supporting information).

Results

Are host-symbiont associations stable within a colony?

Most \textit{A. palmata} samples were dominated by a single
genotype, or strain, of a \textit{Symbiodinium} type A3 (as
defined by 13 microsatellite loci; Fig 1a) across the dis-
tributional range of this coral (Fig 1a) across the dis-
tributional range of this coral (Table 1 and Table S2,
Supporting information). This symbiont type was
recently designated a provisional binomial \textit{Symbiodini-
un} \textit{fitti} and is awaiting formal description by the
authors. PCR amplification of these loci was often sen-
sitive enough to distinguish between two co-occurring
\textit{S. \textit{fitti}} strains when the DNA concentration of the
minor strain was >5\% depending on locus and allele
size differences (Fig. S2, Supporting information). We
acknowledge that additional strains may have occurred

![Fig. 1](attachment:image_url)

Fig. 1 (a) The proportion of \textit{Acropora palmata} samples showing
single vs. mixed infections of \textit{Symbiodinium \textit{fitti}} strains from
different locations across the Greater Caribbean (124 of 759
samples, or 16\%, were mixtures). These proportions varied lit-
tle across sampling locations (Pearson chi-square = 18.368,
d.f. = 11, \textit{P} = 0.073) but differed significantly between the east-
ern and western regions, corresponding to the main genetic
division of \textit{A. palmata} (Pearson chi-square = 12.080, d.f. = 1,
\textit{P} = 0.001). Pie graphs were scaled to relative sample size,
and samples with missing data at some loci were included. (b)
Repeated sampling of colonies over time (1 year interval,
\textit{n} = 17 or 2 years, \textit{n} = 2) and space (2–7 times within a colony)
revealed that most colonies stably associated with one \textit{S. \textit{fitti}}
strain. A few colonies contained two genotypes that persisted
for at least 1 year (\textit{n} = 2). In two other colonies, a second geno-
type was detected 1 year after initial sampling. Co-infecting
strains appeared to be present at different ratios throughout
the colony.
in these samples at lower concentrations. When two strains of *S. fitti* were detected in one colony, as indicated by two alleles at one to several loci, that colony was excluded from gene flow and clonal structure analysis. The proportion of multiple infections differed to some extent between eastern (23.1%) and western (12%) Caribbean host populations (Baums et al. 2005; Fig. 1a; \( \chi^2 = 12.24, \text{d.f.} = 1, P < 0.001 \)).

Within-colony diversity was investigated further by sampling colonies multiple times (Table S5, Supporting information). Typically, this included samples from the tips of three separate branches, one sample from the underside of a branch, and one sample from the base of the colony. In these samples, we observed a single strain 82% of the time (Fig. 1b). Additionally, colonies sampled over time (1 or 2 year intervals, \( n = 19 \)) harboured the same strain(s), yet occasionally the presence of a second strain was detected at the later time point (Fig 1b). We never observed the loss of a strain (Table S5, Supporting information). Single-step changes in allele sizes involving a single locus were observed between time points and within colonies in rare cases. These minor differences may be due to somatic mutations. In two cases, allelic changes were consistent with sexual recombination between co-occurring strains (Table S5, Supporting information).

**Are host-symbiont associations stable across host genets?**

Genotypic diversity (i.e. the number of strains or genets, \( N_g \)) was comparatively low in *S. fitti*. Of 664 samples from the Greater Caribbean, only 176 strains remained after collapsing identical MLGs. Indeed, among locations where colonies were sampled randomly with comparable effort over a circular plots (Table 1 and Table S2, Supporting information), genotypic diversity (Table S3, Supporting information) was significantly higher in the coral host (Caribbean-wide \( N_g/N = 0.44 \)) than it was for the dinoflagellate symbiont (Caribbean-wide \( N_g/N = 0.26 \)). Host and symbiont genotypic diversity was positively correlated at locations sampled throughout the range (east: \( r^2 = 0.57, P < 0.01 \); west: \( r^2 = 0.45, P < 0.01 \); Fig. 2).

*Acropora palmata* is capable of reproducing asexually through physical breakage. This creates patches of spatially separate colonies (ramets) that are descendants of a single zygote (the genet). In Florida, three of four *A. palmata* stands distributed over tens of metres were monoclonal (colonies in each stand belonged to just one genet). In each case, the colonies from a particular genet shared a single symbiont strain (Fig. 3a). Moreover, in the US Virgin Islands and the Bahamas, whole *A. palmata* stands comprising numerous genets were often populated by a single *S. fitti* strain (Fig 3b). In several cases, we found genetically identical colonies (ramets) with distinct *S. fitti* strains, indicating that changes in the dominant symbiont genotype may occur over the life of a colony (Fig 3c). Clonemates (ramets) of a particular *A. palmata* genotype (genet) did not always harbour the same *S. fitti* genotype: two cases of 24 genets with multiple ramets in the eastern region and nine of 33 genets with multiple ramets in the western region were found to associate with different symbiont genotypes.

**Are patterns of clonal propagation and gene flow similar in host and symbiont?**

Patterns of clonal propagation were assessed in plots sampled with comparable sampling effort (Table 1, \( n = 25 \) plots). On average, colonies belonging to the same *A. palmata* genet were dispersed over small spatial scales as measured by the geographic distance among ramets (mean = 7.07 m ± 1.3 SE, median = 4.90 m, max = 60.4 m) and spatial extent of genets (neighbourhood size; Fig 3d). While some colonies belonging to the same genet of *A. palmata* were found in neighbouring sampling plots (radius = 15 m) within a reef, none were shared across reefs. The range of clonally propagated *S. fitti* strains was at least an order of magnitude greater (257 m ± 168 SE, median = 12 m, max = 7844 m; Fig. 3d). Across a particular sample region, many

---

**Fig. 2** Genotypic diversity (expressed as Corrected Nei’s Diversity) of *Acropora palmata* predicts the genotypic diversity of its symbiont, *Symbiodinium fitti*. The correlation is stronger in eastern \( r = 0.76, \text{d.f.} = 11, F = 12.10, P < 0.001 \) than western \( r = 0.67, \text{d.f.} = 13, F = 9.82, P < 0.001 \) populations of *A. palmata*. These analyses were conducted on samples obtained randomly from circular plots (\( n = 17-23 \) colonies per plot) with the same sampling effort (\( N_{\text{east}} = 11 \text{ plots}, N_{\text{west}} = 14 \text{ plots} \), Table 1 and Table S2, Supporting information). Data were checked for collinearity.
S. ‘fitti’ strains occurred in more than one plot and some were found on more than one reef, indicating the capacity of some strains to successfully disperse distances of 2000 m, or more. No single strain of S. ‘fitti’ occurred in reef systems separated by >100 km. Thus, clonal propagation occurred over smaller spatial scales in host populations than in populations of the symbiont.

Gene flow among locations across the Greater Caribbean was assessed for S. ‘fitti’ using an expanded, hap-hazardly sampled data set but including only unique MLGs (n = 176). Symbiont gene flow was moderately influenced by geographic distance (Mantel test between geographic distance and genetic distance, $r^2 = 0.44$, $P < 0.01$). Much stronger genetic differentiation was observed in S. ‘fitti’ compared to its host (Baums et al. 2005) based on the analysis of 176 strains recovered from ten regions (Table 2, $\Phi_{PT} = 0.30$, $P < 0.001$). Structure identified $K = 7$ as the most likely number of S. ‘fitti’ clusters (Fig. 4a) compared to two host clusters (Baums et al. 2005). S. ‘fitti’ strains from Florida, the Bahamas, Panama and Mexico comprised separate cohesive genetic units. The Virgin Islands and St. Vincent and the Grenadines belonged mostly to the same inferred population. Navassa had admixed genotypes. One set of genotypes (i.e. the ‘blue’ cluster in Fig. 4a, $K = 7$) contributed to populations in Puerto Rico, Bonaire and Curacao. An additional cluster (‘yellow’) was found at low frequency but with high individual membership probabilities in Curacao (Fig 4a). A network analysis of S. ‘fitti’ (Fig 4b) differentiated northern (Bahamas, Florida) and southwestern (Mexico, Panama) populations. Pairwise comparisons of $\Phi_{PT}$ values were significant in all cases (ranging from 0.126 between Bonaire and Puerto Rico to 0.491 between Florida and St. Vincent and the Grenadines, Table 2c).
The genetic structure of *Symbiodinium fitti* (based on 13 microsatellite loci, *n* = 176 distinct multilocus genotypes, MLGs) obtained from locations throughout the Greater Caribbean. The results from Structure analyses (a) of unique MLGs at *K*-values of 2, 4, and 7 show increasing population differentiation that mostly relates to regions. Initially, the Florida Keys and Bahamas cluster as distinct from the rest of the Caribbean (*K* = 2). At the optimal *K* = 7 (Evanno *et al.* 2005), *S. fitti* MLGs from the Bahamas were distinguished from the Florida Keys, Yucatan Mexico arose as unique from the rest of the Greater Caribbean, and there is indication that some proportion of the populations in Panama (light brown membership) and Curacao (yellow membership) are distinctive. Host MLGs (*Acropora palmata*) are divided into just two populations (West and East, Baums *et al.* 2005). Given is the probability of membership (y-axis, from 0–1) in each cluster, *K* for each multilocus genotype (x-axis). (b) A median-joining network analysis shows the genetic variation and relative similarity among *S. fitti* MLGs from all sample locations. Circle size is proportional to the number of times a MLG was observed and circle colour corresponds to geographic origin. MLGs were never found in more than one location. Locations with <8 MLGs excluded.

**Discussion**

The interactions between organisms are progressively illuminated by increasing the resolution used to examine genetic diversity (Wimp *et al.* 2004; Bickford *et al.* 2007; Barbour *et al.* 2009). Data presented here on the distribution of sub-species-level diversity provide critical new insight into the establishment and maintenance of cnidarian-*Symbiodinium* mutualisms. Associations between *A. palmata* and *Symbiodinium fitti* were relatively stable over time and space even at the genotype/strain level, and only occasional displacement of one strain by another was observed. In cases where strains did co-infest colonies, we found evidence of sexual recombination between them. Such temporal and spatial uniformity of many *A. palmata* host-symbiont genotype combinations, especially at the edges of *A. palmata*’s northern range, may be explained by either a lack of symbiont diversity in these regions or environmental selection for certain pairings. By recognizing that genotypic diversity (numbers of strains) and effective gene flow among symbiont populations are considerably less than the host, we may begin to infer the ecological and evolutionary response of these symbioses to climate change with greater precision.

**Genotype-by-genotype correspondence between colonies of *A. palmata* and their symbionts**

Genetic uniformity is deemed necessary for the stability of mutualistic symbioses. If unchecked, the presence of multiple symbionts may favour selection of parasitic genotypes (Wilkinson & Sherratt 2001; Douglas 2008). Genetically homogenous symbionts within individual colonies may provide greater stability than heterogeneous symbiont populations. Under a scenario where multiple strains occur commonly in a host, selection should favour the symbiont strain that maximizes use of the host before its competitors. Symbiont strains may differ in physiological attributes that over time will cause one to displace others unless conditions change frequently enough to limit competition (Hutchinson 1961; Maina *et al.* 2011). These interactions played out over time may select for less mutualistic strains (Maynard Smith & Szathmary 1995). The competitive arms race among strains would therefore negatively affect the host population (Douglas 1994). Consistent with theory, the *S. fitti*’ population found in any one colony of *A. palmata* usually comprised a single haploid genotype (strain), despite the availability of other symbiont genotypes occurring at many of the sampling sites. Whether inferred from one sample, multiple samples taken from different locations on the colony, or multiple samples from separate yet genetically identical colonies, a single *S. fitti* strain was recovered in 80–90% of cases (Fig. 1). These findings are consistent with several earlier studies utilizing numerous microsatellites (*n* ≥ 8) to investigate the genetic identity and homogeneity of *Symbiodinium* contained within cnidarian colonies in the Caribbean, Eastern Pacific, and Indo-West Pacific (Andras *et al.* 2011; Pettay *et al.* 2011; Pettay & LaJeunesse 2013; LaJeunesse *et al.* in press) albeit more complex patterns were reported along the Great Barrier Reef (Howells *et al.* 2013).
Colony homogeneity in the genetic identity of the dominant symbiont genotypes has been reported in other scleractinian corals (Pettay et al. 2011; LaJeunesse et al. in press) and octocorals (Goulet & Coffroth 2003; Andras et al. 2013). This apparent fidelity in genotype-genotype combinations, in at least some if not most coral–Symbiodinium associations, has important consequences in the study of reef coral physiology. We propose that genotype-genotype interactions may significantly influence the functional performance of the coral–algal symbiosis as observed in terrestrial mutualisms (Schweitzer et al. 2008; Marshall & Morgan 2011). Several lines of evidence suggest that performance differences exist among host genets alone (Meyer et al. 2009; Polato et al. 2010, 2013; Baums et al. 2013) and in association with Symbiodinium (Howells et al. 2012; J. E. Parkinson & I. B. Baums, submitted). Comparative studies conducted on multiple genotypic combinations are needed to assess the effects of genetic variation on the physiology of individual associations (Császár et al. 2010; Kenkel et al. 2013). A separate line of inquiry should assess conditions resulting in more complex coral–Symbiodinium genotype combinations (Howells et al. 2013).

While colonies of A. palmata exhibit remarkable specificity for S. ‘fitti’, some may, at times, contain background levels of a different Symbiodinium species (<5% of the total symbiont population as assessed via qPCR) (Silverstein et al. 2012) with unknown, if any, functional significance to A. palmata colonies. Many Caribbean Scleractinia are symbiotic with up to several physiologically distinct Symbiodinium spp. that correspond to the host’s habitat distribution (water depth, reef zone) or geographic location (Rowan et al. 1997; Warner et al. 2006; Finney et al. 2010). Acropora spp. from the central GBR exhibit similar variations in their dominant symbiont populations that often relate to external environmental conditions (Ulstrup & Van Oppen 2003). The principal association with a single Symbiodinium sp. may explain, in part, the shallow depth distribution of A. palmata.

Stability and change in host-symbiont genotype combinations over space and time

The large monoclonal stands of A. palmata present in the Florida Keys provided a natural experiment to examine the stability between individual genotypes of A. palmata with clonal populations of S. ‘fitti’ (Fig. 3). These stands can comprise 50–200 colonies spread over 70–200 m², with individual colonies exceeding 3 m in diameter. Linear growth rates of A. palmata are <1 cm/month, so these extensive clonal stands with large colonies (and therefore the host genet) must be exceptionally old. Clearly under certain conditions, genotype-by-genotype associations between host and symbiont remain stable for decades or more. An alternate explanation that all A. palmata colonies at these monoclinal reefs switched in unison to the same S. ‘fitti’ strain is less parsimonious and inconsistent with our results from temporal sampling. This stability suggests that the initial formation of certain A. palmata–S. ‘fitti’ combinations once established remains intact via internal biotic or external environmental drivers, that is particular combinations might be constrained by strong selection. Stability may also be influenced by the apparent lack of competition from few nearby S. ‘fitti’ genotypes (at least at present). The small number of symbiont genotypes occurring in the environment would limit opportunities for competitive displacement.

The distribution of S. ‘fitti’ genotypes observed in A. palmata stands from other regions indicates that local adaptation and competition may influence stability and genetic turnover in the symbiont cells of an individual colony. In the US Virgin Islands, large stands of genotypically diverse colonies of A. palmata were often dominated by few or a single S. ‘fitti’ strain (Fig 3b). A strain that dominates adult colonies on a particular reef might have the highest probability of first infecting recruited larvae, thereby reinforcing its ecological dominance (Orsini et al. 2013). This early establishment in host colonies may be all that is required for a strain of S. ‘fitti’ to maintain dominance on a reef. Alternatively, a strain may have risen to ecological dominance on a reef because it is particularly suited for the prevailing conditions. Without additional evidence, we cannot ascertain whether the dominance of so many different host genotypes by one symbiont clone is the result of deterministic or stochastic (indefinitrminant) processes.

By contrast, in Curacao, where A. palmata communities contain high genotypic diversity for both host and symbiont, we observed the highest number of host clonemates hosting different S. ‘fitti’ strains, indicating that the dominant symbiont strain within a colony can and does change occasionally (Pettay et al. 2011). During co-infection, sex appears to be possible inside the host – and not only outside the host as previously suggested (Appendix S2, Supporting information, Trench 1997). The presence of a large number of S. ‘fitti’ strains distributed throughout a host population may simply increase the frequency of one strain being displaced by another symbiont strain or may indicate that factors maintaining specific genotype-genotype combinations are relaxed in Curacao. Despite the high number of symbiont strains potentially compatible with A. palmata, most colonies were still dominated by a single strain, suggesting that biological mechanisms are in place to suppress the co-occurrence of multiple symbiont strains (Wilkinson & Sherratt 2001; Douglas 2008).

© 2014 John Wiley & Sons Ltd
Symbiont populations have a different genetic structure than A. palmata

Populations of S. ‘fitti’ exhibited more regional genetic structure throughout the Greater Caribbean compared to its host (Fig. 4a, b). S. ‘fitti’ from Florida, the Bahamas, Mexico, Panama and the Virgin Islands plus St. Vincent and the Grenadines each formed genetically differentiated populations resembling more the population genetic structure of its alternate host, A. cervicornis (Baums et al. 2010). The long-recognized biogeographic break between the eastern and western Caribbean had little effect on the variation in populations of S. ‘fitti’ (Fig. 4a; Table 2b). In contrast, A. palmata consists of just two genetically distinct populations occupying the eastern and western Caribbean, respectively (Baums et al. 2005). The populations of several other reef-dwelling species also show strong genetic differentiation between the eastern and western Caribbean, including other corals (Baums et al. 2010; Foster et al. 2012) and goby fish (Taylor & Hellberg 2003).

Acropora palmata possesses a horizontal mode of acquisition where larvae ultimately acquire compatible symbiont species from the environment. The successful dispersal of Symbiodinium is therefore not necessarily constrained by the dispersal of the host, per se, during times of spawning. Indeed, Symbiodinium spp. can exist free-living (Freudenthal 1962) and are sometimes found in the water column and on the benthos (Littman et al. 2008). The pattern of population differentiation among S. ‘fitti’ populations shares few similarities with those of the Symbiodinium BI populations analysed from the gorgonian sea fan, Gorgonia ventalina, also collected from sites around the Greater Caribbean (Andras et al. 2011). Clearly, species of Symbiodinium differ in their dispersal abilities that may correspond to their persistence in a range of external environments, availability of compatible hosts, and timing and frequency of host reproduction and dispersal.

Some of the genetic structuring observed for S. fitti may be influenced by the complex patchwork of environmental conditions found across the Greater Caribbean (Chollett et al. 2012). For example, samples of S. ‘fitti’ from Florida and the Bahamas differentiated early (K = 2) as analysis proceeded from low to high K-values in Structure and relate to a distinct thermal cline (Fig. 4a, Chollett et al. 2012). This northeastern region of the Greater Caribbean is about 1 °C colder in mean sea surface temperature than measured for other locations (Chollett et al. 2012) and suggests that temperature gradients can exert selective pressure on the genetic composition of Symbiodinium populations (Pettay & LaJeunesse 2013; LaJeunesse et al. in press), as they do on marine phytoplankton (Thomas et al. 2012).

Persistence of a species relies on the production of better-adapted offspring when selective pressures exceed the capacity for acclimatization of existing genotypes. The spread of better-adapted genotypes depends on the scale of effective dispersal. The smaller spatial scale of effective dispersal in Symbiodinium compared to the host implies that beneficial mutations can rise to high frequency only in some parts of the hosts’ range, at least in the short term. The effect this might have on the ability of the ecologically threatened A. palmata to survive climate change is as yet unknown.

Conclusion

The finding that colonies of some coral species contain clonally homogeneous populations of Symbiodinium, and that certain host and symbiont genotype combinations remain stable over long periods of time, has implications for how these organisms are physiologically examined and compared. The apparent fidelity in genotype-genotype combinations and geographic variation in genotypic diversity may have important consequences in the persistence of coral reef systems across regions. The local dominance of certain host-symbiont genet combinations suggests that host-symbiont genotypic combinations contribute to the performance of the holobiont and that this complex diversity, we speculate, can ultimately affect continued ecosystem function in times of major climate change. We propose that interactions at the individual level may significantly influence the performance of the coral-algal symbiosis as observed in terrestrial mutualisms (Schweitzer et al. 2008; Marshall & Morgan 2011). However, scales of effective dispersal between coral and dinoflagellate partners can differ substantially and may, to some extent, decouple the microevolution between host and symbiont populations. Given this possibility, corals and their dinoflagellate symbionts might respond independently to significant shifts in climate.

Acknowledgements

We thank the numerous researchers and government agencies that have collected Acropora palmata samples and contributed permits and funding over the years, including S. Gittings, A. Bruckner, A. Ortiz, E. Weil, A. Bourque, D. Williams, M. Miller, M. Vermeij, D. Swanson, K. Niedemyer, C.S. Rogers, E. Muller, R. Torres, E. Pugibet, R. Albright, E. Diaz. Funding for this study was provided by NSF Grant OCE 0928764 to T.C.L. and I.B.B.

References

Andras JP, Kirk NL, Drew Harvell C (2011) Range-wide population genetic structure of Symbiodinium associated with the


LaJeunesse TC, Wham DC, Pettay DT, Parkinson JE, Keshavmurthy S, Chen CA (in press) Ecologically differentiated, stress tolerant endosymbionts in the dinoflagellate genus *Symbiodinium* Clade D are different species. *Phycologia*.


Data accessibility

Microsatellite loci for *Symbiodinium* ‘fitti’ are available in GenBank (see Supporting information for accession numbers). All *S. fitti* multilocus genotypes, sampling locations for each colony, and host and symbiont clone identifications are available in Dryad (doi:10.5061/dryad.h2p05). Structure and Network analysis input files and settings are also available in Dryad (doi:10.5061/dryad.h2p05).

Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1 Identifying Genets.

Appendix S2 Detecting Multiple Infections.

Table S1 Microsatellite loci for Caribbean *Symbiodinium* ITS2 type A3 (*S. fitti*).

Table S2 *Acropora palmata* host and symbiont samples genotyped.

Table S3 Genotypic diversity of *Acropora palmata* and its symbiont *Symbiodinium fitti* in randomly sampled plots.

Table S4 Population differentiation of *Symbiodinium fitti* across ten sites in the Caribbean using a data set that included all genotypes (*n* = 664) instead of only unique genets as in Table 2.

Table S5 Within-colony diversity of *Symbiodinium fitti*.

Fig. S1 Pairwise genetic distances among (a) *Symbiodinium fitti* and (b) *Acropora palmata* multilocus genotypes (MLGs).

Fig. S2 Summary of the relative sensitivity of microsatellite loci to detect the presence of additional *Symbiodinium fitti* genotypes within a sample.
### Supplemental Figures and Tables

**Table S1** Microsatellite loci for Caribbean *Symbiodinium* ITS2 type A3 (*S. fitti*). Given are the locus name, fluorescent dye label, the primer sequence, repeat motif, the annealing temperature for the polymerase chain reaction, and the Genbank Accession number. Tails were added to some primers in which case the tail was fluorescently labeled instead of the forward primer. N alleles = number of different alleles; h = diversity calculated as 1 – the sum of the squared population allele frequencies based on 670 samples. Tails (5’ – 3’): T1 = GGCTAGGAAAGGTTAGTGCC, T2 = TCATACATGTCTCTCAGCGTAAAC, and T3 = ACCAACCTAGGAAACACAG, T4 = GACTATGGGCGTGAGTGCAT.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Dye</th>
<th>Primer (5’ – 3’)</th>
<th>Repeat</th>
<th>N alleles</th>
<th>h</th>
<th>Temp (°C)</th>
<th>Tail</th>
<th>Ascension Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>A3Sym_01*</td>
<td>VIC</td>
<td>F: AACATGACCTGAGAGAGTGGT</td>
<td>(AG) 9..20 bp. (AG) 11(AC) 8</td>
<td>8</td>
<td>0.763</td>
<td>55</td>
<td>T4</td>
<td>HM802880</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: TTGCACAAAGGCTTGCAAGAATCAC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A3Sym_03*</td>
<td>6FAM</td>
<td>F: GTACCTGCTCCCATGGCCGAT</td>
<td>(CGT) 8</td>
<td>4</td>
<td>0.556</td>
<td>56</td>
<td>T2</td>
<td>HM802881</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: ACATGTGCAAGATGCCTGACCA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A3Sym_07*</td>
<td>NED</td>
<td>F: CCTGCTAAGTTTTGTCGGCTAGG</td>
<td>(CTG) 8</td>
<td>4</td>
<td>0.547</td>
<td>56</td>
<td>T3</td>
<td>HM802882</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: GCAGACGTCAGAGCCACACAGCT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A3Sym_09*</td>
<td>PET</td>
<td>F: TCAGATGCCAAGAGGCGCAGG</td>
<td>(GAT) 9</td>
<td>5</td>
<td>0.465</td>
<td>56</td>
<td>T1</td>
<td>HM802883</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: CACTGTTTTGTTAGATGCGCTGAA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A3Sym_18*</td>
<td>HEX</td>
<td>F: CGATCAGGACACACACAGAAG</td>
<td>(AAC) 4</td>
<td>4</td>
<td>0.070</td>
<td>55</td>
<td>NA</td>
<td>HM802884</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: TGAACGTAGCGATACCCTTGGC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A3Sym_27*</td>
<td>NED</td>
<td>F: AACACAGCTAAGATCGCTGCT</td>
<td>(ACA) 5..11 bp. (AGC) 3</td>
<td>2</td>
<td>0.499</td>
<td>60</td>
<td>NA</td>
<td>HM802885</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: GTGTGTGTGTTGTTGTTGTTGTG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Locus</td>
<td>Dye</td>
<td>Primer (5’ – 3’)</td>
<td>Repeat</td>
<td>N alleles</td>
<td>h</td>
<td>Temp (°C)</td>
<td>Tail</td>
<td>Ascension Number</td>
</tr>
<tr>
<td>-----------</td>
<td>-----</td>
<td>---------------------------------------------------------------------</td>
<td>----------------</td>
<td>----------</td>
<td>--------</td>
<td>-----------</td>
<td>------</td>
<td>------------------</td>
</tr>
<tr>
<td>A3Sym_28*</td>
<td>PET</td>
<td>F: CGAGCCCGCCAAGCTTCGAGGTT</td>
<td>(GAT) 6</td>
<td>5</td>
<td>0.391</td>
<td>52</td>
<td>NA</td>
<td>HM802887</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: TGACCCAGAGTTATCAGTGATCG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A3Sym_31*</td>
<td>HEX</td>
<td>F: GTGGTATAGTGTAGTGGCTGG</td>
<td>(GACGC) 3</td>
<td>3</td>
<td>0.098</td>
<td>52</td>
<td>NA</td>
<td>HM802888</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: GACCGCATTTCGACAGTCTAGGCT</td>
<td>(AGC) 4..10 bp..(CAG) 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A3Sym_32*</td>
<td>6FAM</td>
<td>F: GGTCGCAGTGGCAGTGGCAGTA</td>
<td>(AGTAGC)5</td>
<td>14</td>
<td>0.815</td>
<td>55</td>
<td>NA</td>
<td>HM802889</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: ACTGACTGCTGCGCAACAAGG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A3Sym_41*</td>
<td>6FAM</td>
<td>F: CACCACACTACACTGAATGGCGAC</td>
<td>(CAC) 5</td>
<td>5</td>
<td>0.502</td>
<td>52</td>
<td>NA</td>
<td>HM802890</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: CGATGGCGATGGCGATGGCGATGGCGATGGGCATG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A3Sym_02</td>
<td>NED</td>
<td>F: GCATAAACACGTTTTCTTCCAA</td>
<td>(TATG) 7</td>
<td>6</td>
<td>0.657</td>
<td>55</td>
<td>T3</td>
<td>KF787091</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: TTGCACTCTTGCACTGTG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A3Sym_08</td>
<td>VIC</td>
<td>F: TGTTTGGTTAGGTTAGGGTCA</td>
<td>TAG(9)</td>
<td>6</td>
<td>0.681</td>
<td>55</td>
<td>T4</td>
<td>KF787092</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: GGGGGGCTCTACAAAAAGAAA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A3Sym_48</td>
<td>6FAM</td>
<td>F: CCACATGACACATCTGGCTA</td>
<td>TATC(6)</td>
<td>4</td>
<td>0.543</td>
<td>55</td>
<td>T2</td>
<td>KF787093</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: CCGCAAAAAGTGTACTCACC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* From Reference (Pinzón et al. 2011)
NEWS AND VIEWS

PERSPECTIVE

Expanding the population genetic perspective of cnidarian-Symbiodinium symbioses

SCOTT R. SANTOS*†
*Department of Biological Sciences and Molette Biology Laboratory for Environmental and Climate Change Studies, Auburn University, 101 Rouse Life Sciences Building, Auburn, AL 36849, USA; †Cellular and Molecular Biosciences Program, Auburn University, Auburn, AL 36849, USA

The modern synthesis was a seminal period in the biological sciences, establishing many of the core principles of evolutionary biology that we know today. Significant catalysts were the contributions of R.A. Fisher, J.B.S. Haldane and Sewall Wright (and others) developing the theoretical underpinning of population genetics, thus demonstrating adaptive evolution resulted from the interplay of forces such as natural selection and mutation within groups of individuals occupying the same space and time (i.e. a population). Given its importance, it is surprising that detailed population genetic data remain lacking for numerous organisms vital to many ecosystems. For example, the coral reef ecosystem is well recognized for its high biodiversity and productivity, numerous ecological services and significant economic and societal values (Moberg & Folke 1999; Cinner 2014).

Many coral reef invertebrates form symbiotic relationships with single-celled dinoflagellates within the genus Symbiodinium Freudenthal (Taylor 1974), with hosts providing these (typically) intracellular symbionts with by-products of metabolism and in turn receiving photosynthetically fixed carbon capable of meeting hosts’ respiratory demands (Falkowski et al. 1984; Muscatine et al. 1984). Unfortunately, the health and integrity of the coral reef ecosystem has been significantly and negatively impacted by onslaughts like anthropogenic eutrophication and disease in addition to global climate change, with increased incidences of ‘bleaching’ events characterized as the loss of photosynthetic pigments from the algal cell or massive reduction of Symbiodinium density from hosts’ tissue) and host mortality leading to staggering declines in geographic coverage (Bruno & Selig 2007) that have raised questions on the viability of this ecosystem as we know it (Bellwood et al. 2004; Parmesan 2006). One avenue towards anticipating the future of the coral reef ecosystem is by developing a broader and deeper understanding of the current genotypic diversity encompassed within and between populations of their keystone species, the scleractinian corals and dinoflagellate symbionts, as they potentially possess functional variation (either singularly or in combination) that may come under selection due to the ongoing and rapid environmental changes they are experiencing. However, such studies, especially for members of the genus Symbiodinium, are sparse. In this issue, Baums et al. (2014) provide a significant contribution by documenting the range-wide population genetics of Symbiodinium ‘fitti’ (Fig. 1) in the context of complementary data from its host, the endangered Caribbean elkhorn coral Acropora palmata (Fig. 2).

Notable results of this study include a single S. ‘fitti’ genotype typically dominates an individual A. palmata colony both spatially and temporally, gene flow among coral host populations is a magnitude higher to that of its symbiont populations, and the partners possess disparate patterns of genetic differentiation across the Greater Caribbean. The implications of such findings are discussed herein.

Keywords: Acropora, cnidarian, coral reef, population genetics, Symbiodinium

Received 20 June 2014; revised 04 July 2014; accepted 16 July 2014

Genotypic diversity of Symbiodinium ‘fitti’ within and among individual Acropora palmata colonies

The work of Baums et al. (2014) further defines and strengthens some recurring patterns from population genetic studies of Symbiodinium (Fig. 1) associated with scleractinian coral (their study and references within), sea anemone (e.g. Thornhill et al. 2013) and octocoral (e.g. Santos et al. 2003) species. For example, the fact that a majority (i.e. ~75–94%) of host individuals temporally harbour only a single symbiont genotype at detectable levels, and when multiple genotypes are present, there are no more than two simultaneously, implies (with apparent exceptions, e.g. Howells et al. 2013) the occurrence of competitive exclusion and/or competition among them (Fitt 1985). If this is the case, what scenario(s) may select for the infrequent residence of two symbiont genotypes within the same host? Here, Baums et al. (2014) present evidence that such co-occurrence can result at some frequency in sexual recombination between genotypes. This is an exciting development for (at least) two reasons. Firstly, although the genetic diversity and linkage equilibrium previously observed in various Symbiodinium lineages has been attributed to sexual recombination, potential answers to how...
often it occurs, the factors inducing it and where it takes place have been elusive (reviewed by Santos & Coffroth 2003). Secondly, any recombination within *Symbiodinium* ‘fitti’ or other *Symbiodinium* populations increases the possibility that novel genotypes may arise, be favoured by natural selection, and potentially play a role in the environmental resilience of future generations. Thus, data from Baums et al. (2014) provide a contextual framework towards experimentally and unequivocally demonstrating whether *Symbiodinium* genotypes compete and/or undergo sexual recombination within hosts’ tissues.

Another recurring pattern reflective in Baums et al. (2014) is the near dominance of a single symbiont genotype among multiple host individuals in many localized geographic areas. Such a situation could arise from mechanisms including clonal propagation of the host (predominately the case for *Acropora palmata* (Fig. 2)), selective sweeps, local adaptation and/or an inability of migrant symbionts to infiltrate established populations (Thornhill et al. 2009). While ‘successful’ under current ecological conditions, how such relatively monotypic *S. ‘fitti’* populations and their more genetically variable host *A. palmata* might respond to future environmental perturbations remains open to debate. In this case, Baums et al. (2014) will serve as an excellent baseline for subsequent studies on potential emergent functional differences due to genotype–genotype interactions between these hosts and their symbionts.

**The dichotomy in gene flow and population differentiation of *Symbiodinium* ‘fitti’ and *Acropora palmata***

Life history traits of organisms can greatly influence their geographic distributions, and comparative studies offer a means of highlighting just how dramatic differences might be. This is exemplified in Baums et al. (2014), where levels of gene flow among *Symbiodinium* ‘fitti’ and *Acropora palmata* populations differed by an order of magnitude. Thus, while *S. ‘fitti’* can successfully disperse via environmental routes, this capability is apparently highly limited relative to their animal host and additional studies are required to elucidate the particular attributes responsible for this. However, the restricted dispersal potential of *Symbiodinium* genotypes in general raises the spectre that even if novel and resilient symbiont genotypes arise via mutation and/or recombination and increase in frequency due to natural selection, they may not have the ability to emigrate into new populations. This possibility, which extends to other organisms with similar biological characteristics as well, deserves further consideration when discussing the future of populations and ecosystems in the face of global climate change.

Given the striking differences in gene flow, there is little surprise that Baums et al. (2014) identified disparate patterns of genetic differentiation between *S. ‘fitti’* and *A. palmata* populations across the Greater Caribbean, with >3X structure in the former compared to the latter.

Although known environmental conditions evidently drive some of the genetic breaks among symbiont populations, much of this differentiation appears intrinsic to the biology of *S. ‘fitti’* itself. Notably, Baums et al. (2014) comment that the genetic differentiation among *S. ‘fitti’* populations resembles that of an alternative host species, the staghorn coral *Acropora cervicornis*. While this might be purely coincidental, it could also signify a previously unrecognized facet to the ecology and evolution of *S. ‘fitti’* and these Caribbean *Acropora* species. Overall, there is little doubt that this system will continue to yield unique insight into cnidarian-dinoflagellate symbioses in the coming years.
Population genetics of cnidarian-algal symbioses – a call to arms

Along with defining the population genetics of *Symbiodinium* ‘fitti’ compared with its host *Acropora palmata*, Baums et al. (2014) should draw attention to the need for additional research into this area. As an illustration, a June 2014 search of Thomson Reuters’ Web of Science with the combined keywords ‘*Symbiodinium*’, ‘population’ and ‘microsatellite’ yielded just 39 indexed publications since 2001 (\(\bar{x} = 3.0/\text{year}\), including some false positives). By bringing this to the community’s attention, it is hoped that this will motivate others to produce similar works in an important and fascinating area of coral reef symbioses.

References


The article was written by S.R.S. Research in The Santos Lab utilizes molecular tools and computational approaches to address questions of population genetics, resource conservation, genomic evolution and symbiosis biology in aquatic (both freshwater and marine) and terrestrial microbes and multicellular organisms.

doi: 10.1111/mec.12865