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How old are you? Genet age estimates in a clonal animal

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Abstract

Foundation species such as redwoods, seagrasses and corals are often long-lived and clonal. Genets may consist of hundreds of members (ramets) and originated hundreds to thousands of years ago. As climate change and other stressors exert selection pressure on species, the demography of populations changes. Yet, because size does not indicate age in clonal organisms, demographic models are missing data necessary to predict the resilience of many foundation species. Here, we correlate somatic mutations with genet age of corals and provide the first, preliminary estimates of genet age in a colonial animal. We observed somatic mutations at five microsatellite loci in rangewide samples of the endangered coral, *Acropora palmata* (n = 3352). Colonies harboured 342 unique mutations in 147 genets. Genet age ranged from 30 to 838 years assuming a mutation rate of 1.195^{-04} per locus per year based on colony growth rates and 236 to 6500 years assuming a mutation rate of 1.542^{-05} per locus per year based on sea level changes to habitat availability. Long-lived *A. palmata* genets imply a large capacity to tolerate past environmental change, and yet recent mass mortality events in *A. palmata* suggest that capacity is now being frequently exceeded.

Keywords: clonal, longevity, microsatellite, population dynamics, somatic mutations

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Introduction

The population dynamics of a species depend in part on the longevity of each individual. However, in colonial organisms such as corals neither 'individual' nor 'age' are easy to define, making longevity the least accessible demographic trait to study for these organisms. Coral colonies consist of genetically identical polyps that each fulfil the function of an individual (reproduction, growth, defence), yet it is the collection of polyps in a colony that represent the ecologically significant unit (Santelices 1999). Hence, studies of coral population dynamics often track the fate of colonies rather than that of individual polyps. The very nature of the clonality of corals allows colonies to survive partial mortality (Hughes & Jackson 1980), propagate asexually through fragmentation (Highsmith 1982), and partake in clonal fission and fusion (Hughes & Jackson 1980). The result is independent colonies (ramets) not connected by live tissue that share the same genotype (clonemates of the same genet). Coral species where clonemates constitute a significant proportion of local populations are found in at least nine coral genera (Table S1, Supporting information). Ramets are produced throughout the lifetime of the genet, and hence, they can be of different chronological age and size although their genetic age (i.e. the time since meiosis and zygote formation) remains the same. Taken together these processes have the net effect of decoupling size of a ramet from its age (Hughes & Jackson 1980).

In noncolonial multicellular organisms, size is often a good proxy of genet age until adult size is attained.

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After adult size is reached, age determination becomes more challenging, but the incorporation of environmental signals into tissues (Prouty *et al.* 2011), the shortening of telomeres with increasing numbers of cell divisions (Barrett *et al.* 2013), decreasing reproductive output, and phenotypic changes (Caspari & Lee 2004) can be quantified as indicators of age in a wide range of multicellular organisms. Many of these approaches are not useful in plants and colonial invertebrates. Radiocarbon or U-series dating (Radtke *et al.* 2003) is an alternative to using size or phenotypic changes as a proxy for genetic age; however, this requires the identification and continued existence of the oldest portion of a genet because, as such, environmental signals reflect ramet age, not genet age (Eggins *et al.* 2005). This may be possible in some clonal plant species in which ramet attachment persists and the centre, typically the oldest portion of a genet, can be identified (Vasek 1980), and perhaps for coral species not prone to fragmentation (Table 1; Table S1, Supporting information). Furthermore, reproduction is tied to colony size so recently fragmented ramets belonging to previously fecund colonies might not produce gametes themselves (Okubo *et al.* 2007) and phenotypic changes are not obvious because a genetically old but small coral colony is not visually distinguishable from a genetically young and small colony.

A possible method for determining genet age is to use mutation accumulation in somatic tissues to estimate longevity. Despite their asexual origin, clonemates are not always exactly genetically identical. The concept is based on 'the somatic mutation theory of clonality' (Klekowski 1997) which reasons that continuous division of mitotic cells in a clonal organism will lead to the accumulation of somatic mutations over time. Somatic mutations convert a genetically homogenous individual into a mosaic with divergent cell lineages (mosaicism). Due to the stochastic nature of somatic mutations, the incidence of genetic mosaicism would be expected to increase with increasing longevity of the organism and also with a higher prevalence of asexual reproduction; gain in ramet number or size increases the total number of dividing cells available for mutation (Orive 2001). Thus, it should be possible to relate the accumulation of somatic mutations to genet age.

Utilizing genetic divergence generated by somatic mutations is a novel approach for calculating lifespans in clonal organisms (Heinze & Fussi 2008). The use of neutral microsatellites is ideal for divergence estimates due to their high mutation rates that range from 10^{-2} to 10^{-6} per sexual generation (Shimoda *et al.* 1999; Ellegren 2000; Peery *et al.* 2012). Genetic divergence in microsatellite loci has been used to model clonal age in the aspen tree *Populus tremuloides* (Ally *et al.* 2008) and the water flea *Daphnia magna* (Robinson *et al.* 2012).

Limitations of lifespan estimates based on genetic divergence include the necessity of clonality, the low frequency or absence of mutations in some species (Lanner & Connor 2001; Cloutier *et al.* 2003) and difficulties in measuring mutational rates that are often variable among loci (Chakraborty *et al.* 1997; Schug *et al.* 1998). It can also be challenging to distinguish somatic mutations from allelic variation (Heinze & Fussi 2008) if the species under consideration is inbred.

Furthermore, the rate of somatic mutational divergence not only differs between species (Klekowski & Godfrey 1989), but also among individuals (Haag-Liautard *et al.* 2007; Conrad *et al.* 2011) with intraspecies variation partly due to varying exposure to environmental stress (de Witte & Stöcklin 2010). Genetic homogeneity can be restored from a mosaic state through sexual reproduction, but also through parallel back-mutations or lineage selection (Klekowski & Kazarinova-Fukshansky 1984) which would lead to

Table 1 Published age estimates of coral colonies

Species	Age estimate (years)	Method	Region	Depth (m)	Year collected	Reference
Leiopathes	70–2040	^{14}C and growth ring measurements	Gulf of Mexico	304–317	Not stated	Prouty *et al.* (2011)
Gerardia sp. *Leiopathes*	300–2700 350–4200	$\delta^{13}C$	Hawaii	400–500	2004	Roark *et al.* (2009)
Keratoisis, *Isidella* or *Acanella* spp.	75–126	^{14}C	Gulf of Alaska	634–720	2002	Roark *et al.* (2005)
Lophelia pertusa	451 ± 36	^{14}C	West Ireland	840–1300	1995–1997	Hall-Spencer *et al.* (2002)
Pocillopora verrucosa	3.69 ± 0.48 3.82 ± 0.55 3.89 ± 0.42	U/Th	Kosrae and Lelu Island	Unknown	2012	Richards *et al.* (2015)

underestimates of mutational load and thus clonal age. Despite the limitations, genetic divergence estimates are the most promising technique to estimate genet age in colonial marine invertebrates.

To demonstrate the potential of using somatic divergence estimates to estimate genet longevity, we used genetic divergence in five microsatellite loci to calculate the age of 90 genets of the elkhorn coral, *Acropora palmata*. *A. palmata* is an ideal species for determining genet age based on somatic mutations because this species relies heavily on fragmentation for local population maintenance (Highsmith 1982; Baums *et al.* 2006a; Williams & Miller 2012) and some genets have >30 members (Baums *et al.* 2014). The process of fragmentation and regrowth of colonies from fragments has been documented photographically via quarterly surveys over the past decade or so (Fig. S1, Supporting information; Williams & Miller 2012) and fragments match donor colony genotypes. Furthermore, in a previous range-wide study of population genetic structure in *A. palmata* we noticed the occasional occurrence of three alleles per locus in this otherwise diploid species (Baums *et al.* 2005a). *A. palmata* is a self-incompatible hermaphrodite (Szmant 1986; Baums *et al.* 2005a), and population genetic data show that the species is genetically diverse and outbred (Baums *et al.* 2005b). Here, we investigate whether third alleles in *A. palmata* arose from somatic mutations and then use somatic mutations to estimate genet age in this species.

Methods

Study system

Acropora palmata is a fast-growing, branching coral that once dominated coral reefs in the Caribbean and northwest Atlantic. Adult colonies release egg-sperm bundles once a year after the August full moon during a synchronized mass-spawning event. Egg-sperm bundles float to the surface where they break apart. Successful fertilization requires the union of egg and sperm from different genets; that is, *A. palmata* is a self-incompatible hermaphrodite (Baums *et al.* 2005a). Gametes develop into nonfeeding planula larvae during a 3-day to several-week planktonic period. Mature larvae search for suitable habitat and metamorphose into primary polyps during a process generally referred to as settlement (Fig. 1). Once the primary polyp is established, it will bud repeatedly, a type of asexual reproduction, and eventually form a colony of genetically identical polyps. In some cases, two genetically distinct primary polyps (recently settled larvae) can fuse, resulting in colonies with mixtures of polyps of different genotypes (chimerism; Barki *et al.* 2002; Puill-Stephan *et al.* 2009; Work *et al.* 2011). Signals and resources are shared across the colony. There is also division of labour to a degree with some polyps primarily engaged with defence, reproduction or growth (Soong & Lang 1992). Because of this integration, the colony is usually considered as the ecologically significant unit. We refer to an assemblage of genetically identical colonies that are descendants of a single zygote as a 'genet' (Harper 1977; Hughes 1989; Carvalho 1994). Physiologically distinct colonies, formed from fragmentation, that can function and survive on their own but belong to the same genet are termed 'ramets' (Kays & Harper 1974).

Samples of *A. palmata* were collected from Florida and the Caribbean (2001–2012, $n = 3352$; Fig. 2 and Table 2). The time range of sample collection lends an error rate of ± 12 years to the age calculations. Previous population genetic evidence (Baums *et al.* 2005b) divided *A. palmata* samples into two largely isolated populations, the eastern Caribbean (including Bonaire, Curacao, St Vincent and the Grenadines, the US Virgin Islands) and the western Caribbean (including the Bahamas, Belize, Cuba, Dominican Republic, Florida, Mexico, Mona, Navassa and Panama). Samples from Puerto Rico were assigned to the eastern Caribbean but show some degree of admixture between the east and the west. A subset of the total data set ($n = 430$ from 14 reefs in the Bahamas, Bonaire, Curacao, Florida, Panama, the US Virgin Islands and Navassa) were sampled using a stratified, random sampling approach, as described in Baums *et al.* (2006a). Most colonies within our collection were only sampled once; however, 11 colonies from Florida were resampled in 2011 and 2014 at 2–8 locations within the colony (Table S1, Supporting information).

Microsatellite scoring. All samples were genotyped at five (166, 181, 182, 192 and 207) previously published, polymorphic microsatellite loci with Mendelian inheritance as shown by experimental crosses (Baums *et al.* 2005b). All five microsatellite loci are AAT trinucleotide repeats. Two 10-μL multiplex PCRs (M-I and M-II) were performed per sample. M-I consisted of 0.2 μL each of primer pairs 166-PET (5 μM), 192-6-FAM (5 μM) and 181-NED (5 μM), 1 μL 10× PCR buffer (Promega), 0.8 μL of $MgCl_2$ (25 mM), 0.2 μL of dNTPs (10 mM), 0.3 μL of *Taq* polymerase (5 U/μL, storage buffer B; Promega) and 6.1 μL H_2O. M-II consisted of 0.2 μL each of primer pairs 207-PET (5 μM) and 182-6-FAM (5 μM), 1 μL Promega 10× PCR buffer, 1.2 μL of $MgCl_2$ (25 mM), 0.2 μL of dNTPs (10 mM), 0.2 μL of *Taq* polymerase (5 U/μL) and 6 μL H_2O. DNA (100–200 ng, 1 μL) was added to each reaction. Thermal cycling was carried out with Eppendorf Mastercyclers with an initial denaturation step at 95 °C for 5 min followed by 35

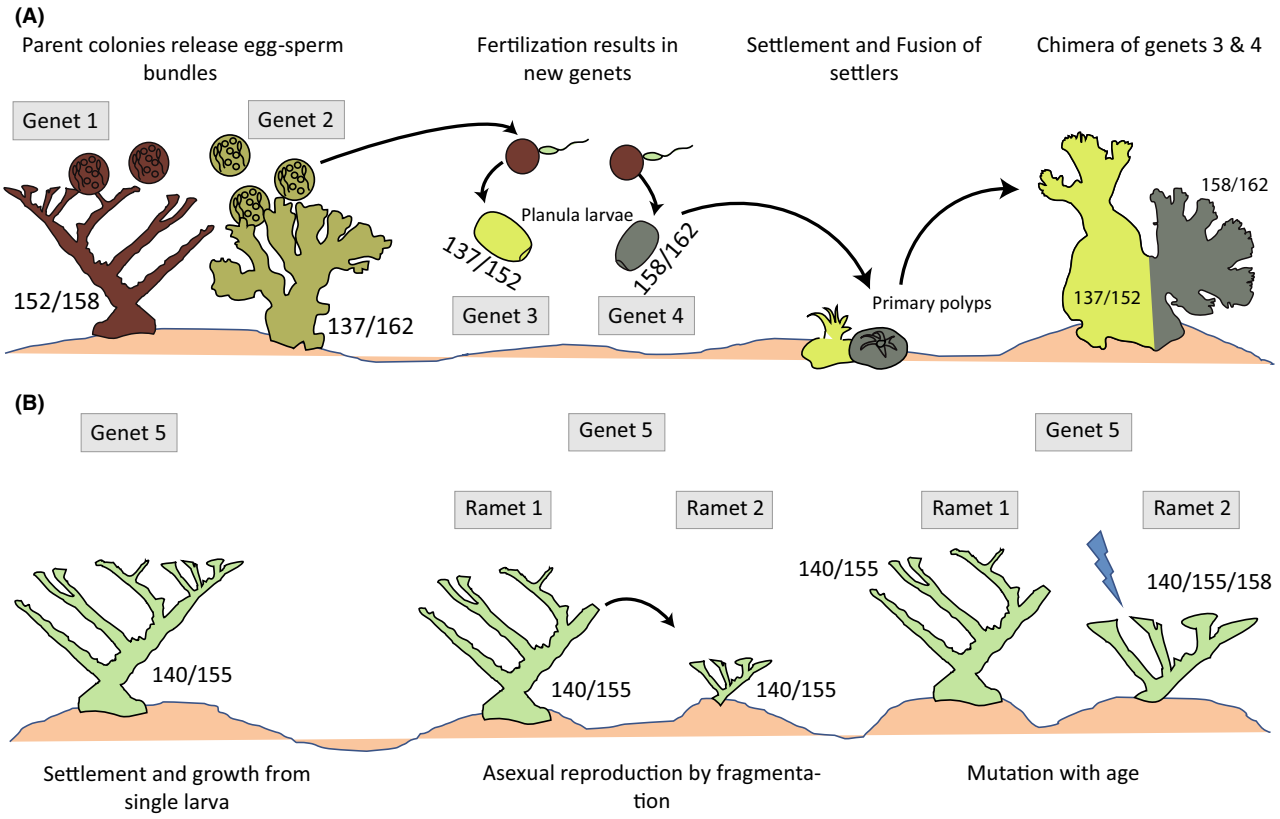

Fig. 1 Diagram depicting (A) the formation of a chimera from the settlement and fusion of gametes of different genets. (B) An illustration of asexual reproduction by fragmentation and the accumulation of mutations with age. See Fig. S1 (Supporting information) for a photograph time series of fragmentation. Example alleles at one locus are given in base pairs (three digit numbers separated by forward slashes). Diagram not to scale.

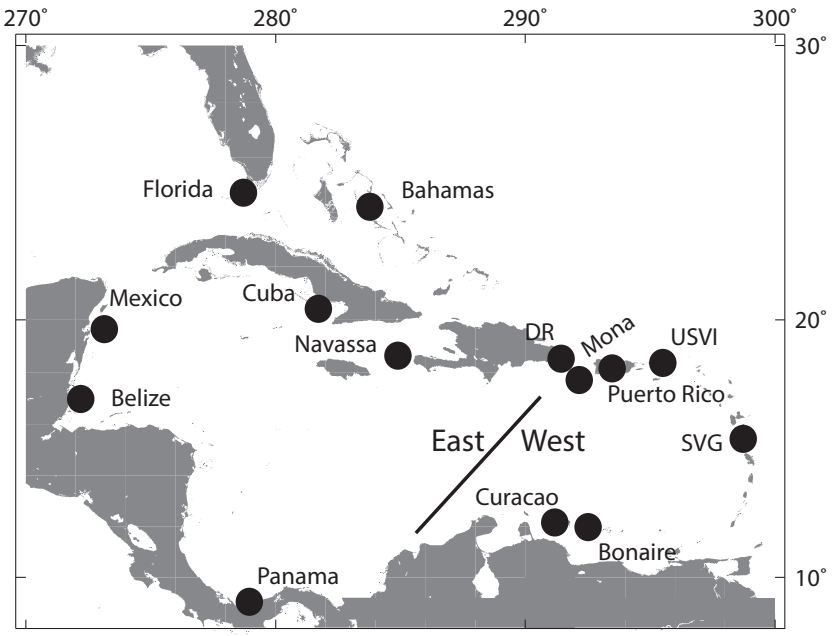

Fig. 2 Samples of *Acropora palmata* were collected throughout Florida and the Caribbean. DR = Dominican Republic, USVI = US Virgin Islands, SVG = St. Vincent and the Grenadines. See Baums *et al.* (2005b, 2006a) for sampling location details.

cycles of 95 °C for 20 s, 50 °C for 20 s, 72 °C for 30 s. A final extension of 30 min at 72 °C ensured that the majority of amplicons were +A (Brownstein *et al.* 1996). PCR products were visualized using an ABI 3730. An internal size standard (Gene Scan 500-Liz; Applied Biosystems) was used for accurate sizing.

Table 2 Summary table of *Acropora palmata* samples used in the various analyses

Region	Clonal richness vs. Nonmosaic samples: MLGs with $n \geq 1$ ramets	Mutational analysis: MLGs with $n \geq 2$ ramets				Genet age analysis: MLGs with $n \geq 5$ ramets	
	Samples	Samples	MLGs	UM	UM/MLG	Samples	MLGs
East							
Bonaire	43	8	3	4	1.3	0	0
Curacao	286	73	17	20	1.2	55	7
Puerto Rico*	308	41	12	16	1.3	46	7
SVG	210	33	12	18	1.5	10	2
USVI	464	65	9	14	1.6	64	7
West							
Bahamas	259	134	23	46	2.0	131	17
Belize	152	16	4	8	2.0	5	1
Cuba	2	0	0	0	NA	0	0
Dom. Rep.	49	4	1	2	2.0	0	0
Florida	1036	892	47	175	3.7	931	44
Mexico	180	33	3	7	2.3	0	0
Mona	70	18	3	11	3.7	0	0
Navassa	176	21	8	12	1.5	0	0
Panama	117	49	5	9	1.8	52	5
Total	3352	1387	147	342		1294	90

MLG, multilocus genotype; UM, unique mutations.
*Puerto Rico contains admixed *A. palmata* genets between the eastern and western Caribbean.

Electropherograms were analysed with GENEMAPPER Software 5.0 (Applied Biosystems).

A single genet designation (clonal ID) was assigned to corals that have exact matching multilocus genotypes (MLGs) or have exact matching MLGs (share all the same diploid state ancestral alleles) and have an additional allele(s). The exceptions to this rule were 4% of mutations that were either a full mutation (e.g. ancestral state 166/175 to 166/178) or a loss of heterozygosity (e.g. to 166/166; Table 3), but at the other four loci, all alleles were shared with other members of the genet (see Table S2, Supporting information, for an example genet).

Loci had an average of 19.6 alleles (SD ± 2.3). This level of polymorphism translated into a high power of distinguishing closely related (i.e. inbred) MLGs from those that were the product of asexual reproduction (i.e. clonemates) where the probability of identity = 10^{-5} (Baums *et al.* 2005b) (see Fig. S2, Supporting information). When considering only genotypes with two alleles per locus ($n = 2643$, i.e. those without somatic mutations) the average probability of encountering a genotype more than once by chance (psex) was 2.23^{-07} (MLGSIM 2.0, http://www.rug.nl/research/gelifes/tres/software), indicating that identical genotypes were the result of asexual reproduction. Once asexually produced, identical MLGs are removed from the data set, no heterozygote deficits are detected [i.e. all loci adhere to Hardy–Weinberg expectations (Baums *et al.* 2005a)], and thus, *A. palmata* shows no sign of inbreeding (Halkett *et al.* 2005).

Table 3 Ancestral alleles could be determined for some *A. palmata* genets with only two ramets

Clonal ID	Database ID	Locus	A1 (bp)	A2 (bp)	Mutated allele (bp)	2nd mutated allele (bp)
P2635	4597	192	166	175	169	
P2635	4602	192	166	175	172	178
P2634	1643	192	166	181	163	
P2634	1644	192	166	181	178	
P1084	1601	192	160	181	178	
P1084	1602	192	160	181	157	

A, allele size; bp, base pairs.

Mutation-step analysis

For all genets with at least two ramets each novel mutation was reported [referred to as a unique mutation (UM)]. A total of 342 UMs were found in 147 genets with 1387 ramets (Table 2; Fig. 3). To discriminate between a mutated allele and a PCR error, a singleplex PCR was performed for all UMs. Following a stepwise-mutation model (Kimura & Ohta 1978), the smallest

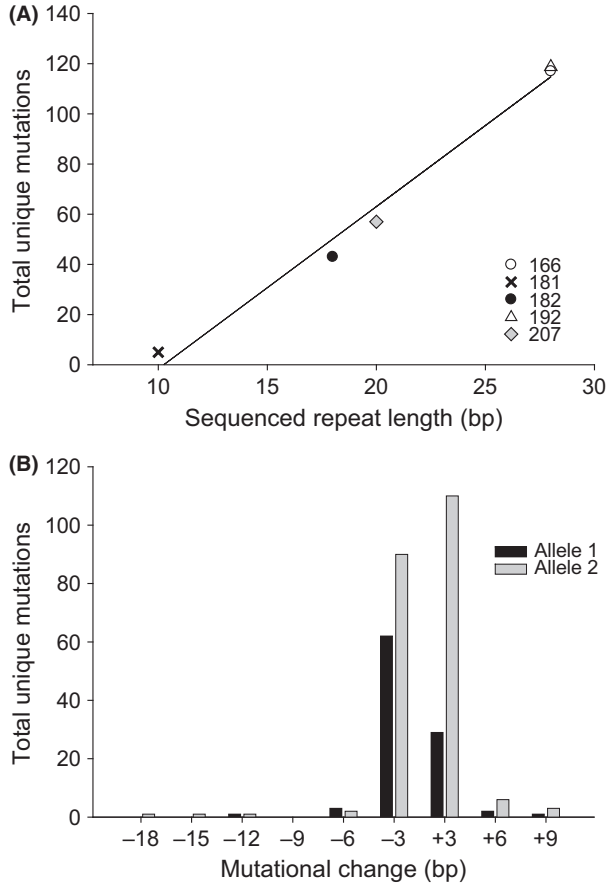

Fig. 3 Mutation-step analysis. In panel (A), as the repeat length of a microsatellite locus increases, the total number of unique mutations found within each locus increases linearly (slope = 6.47 ± 0.47 SD, $F_{2,3} = 186.63$, $P < 0.001$, adj. $R^2 = 0.98$). (B) Most mutations were one step away from the ancestral allele size (i.e. ± 3 bp) with allele 1 (the smaller of the two alleles) showing more repeat unit losses than gains and the larger allele (allele 2) showing more gains than losses of repeat units. Twenty-nine mutations were excluded from (B). Twenty-eight mutations were excluded because the mutation step was equidistant for alleles 1 and 2 so that the mutated allele could not be determined; one mutation was a dropped allele.

possible mutation step that could have resulted in the new allele was used to determine which of the two ancestral alleles mutated and the size of the mutation step (in repeat units). Mutations were excluded if there were no other samples within the genet that were biallelic at that locus making it impossible to determine the mutation step. However, sometimes a genet had only two ramets and both ramets had different mutations at the same locus. In that case the ancestral allele state was determined to consist of the two alleles found in both ramets (Table 3). The mutation-step analysis contained a reduced sample size of $n = 1387$ (Table 2).

Clustering analysis

To determine whether the samples with three alleles could be attributed to somatic mutations or chimerism, we applied a Bayesian clustering analysis using the program STRUCTURE 2.3.4 (Pritchard *et al.* 2000) to all genets with at least five ramets ($n_{genets} = 90$; Table 2). We forced a diploid state by replacing the ancestral allele with the third allele mutation. There were no missing genotype data. We assumed that ramets should only diverge from the ancestral genotype in one or two loci or alleles if somatic mutations were the cause, following previous studies (Puill-Stephan *et al.* 2009; Maier *et al.* 2011). Alternatively, colonies were defined as chimeras if genotypes differed by more than 60% in their major cluster assignment probability from other members of their genet as defined by Schweinsberg *et al.* (2015). STRUCTURE 2.3.4 (Pritchard *et al.* 2000) was run with a burn-in period of 100 000 and 1 000 000 MCMC repeats with three iterations per *K*, without a prior (Fig. 4). Because of their large number, Florida genets were run in two separate groups each containing 22 genets, with $K = 22$. The eastern Caribbean samples (23 genets, $K = 23$) and all other western Caribbean samples (23 genets, $K = 23$) were run in two additional groups. Results of the three runs per group were merged with CLUMPAK (Kopelman *et al.* 2015).

Clonal richness vs. mosaicism

We evaluated whether somatic mutations were found more often on reefs where little sexual recruitment was evident (and thus were presumably inhabited by older individuals) by tallying all mutations in all samples and comparing the number of mutations detected with the number of genets present. This was expressed as clonal richness. We did this analysis on two data sets. We compared the proportion of nonmosaic samples to clonal richness on reefs with ≥10 samples, with no limitations placed on the genet size (Table 2). Therefore, clonal and nonclonal samples were included in this analysis (i.e. all genotype samples $n = 3352$; Table 2). Then, we only compared reefs that were sampled with similar sampling effort [see Table 1 in Baums *et al.* (2006a)]. The clonal richness *R* is calculated as the number of genets *G* relative to the number of analysed ramets *N* with the modification by Dorken & Eckert (2001):

$$R = \frac{G - 1}{N - 1}.$$

A monoclonal stand has a clonal richness of $R = 0$, whereas the maximum clonal richness of $R = 1$ is reached when all samples from a reef are of a different

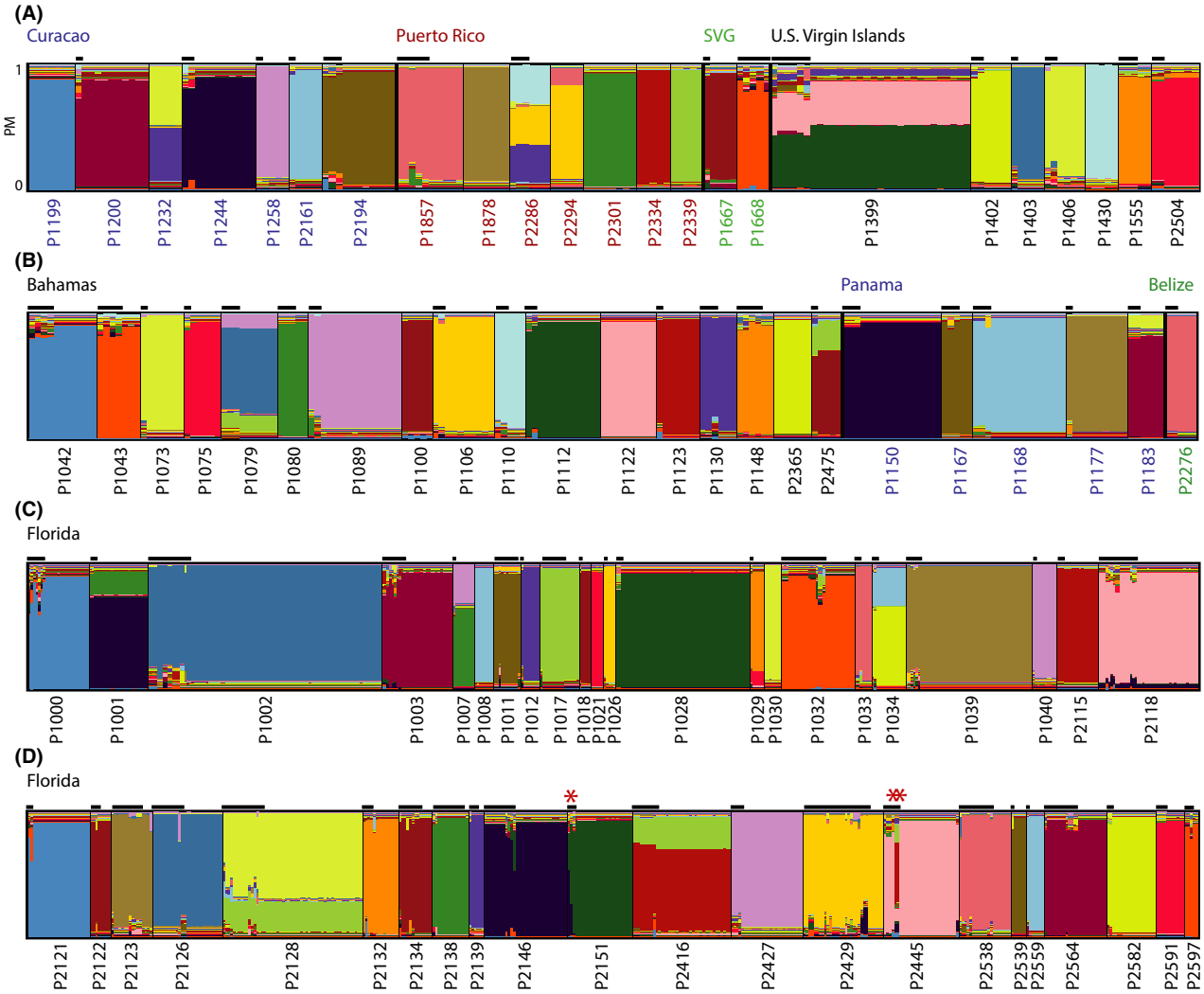

Fig. 4 Assignment of ramets to genets using Bayesian clustering analysis in *A. palmata*. Included were all genets with $n \geq 5$ ramets (Table 2). Black lines above graphs indicate samples that have mutations. An asterisk indicates colonies that have a < 40% assignment probability to the most closely related genet. These colonies are possible chimeras. Probability of membership to a given cluster (*y*-axis) is plotted for each sample (*x*-axis). Colours indicate cluster membership for each panel (A–D). Genets from the eastern Caribbean are shown in panel A, and genets from the western Caribbean are shown in panels B–D. Florida was split into two groups (C, D) because of the large number of genets from this region.

MLG. We chose clonal richness as an indicator for clonal diversity because other measures assume a constant ploidy level (most often diploidy, e.g. G_o/G_e) and were not designed for samples with somatic mutations.

Estimates of genet age using genetic divergence

The methods for calculating clonal age utilizing genetic divergence are described in Ally *et al.* (2008). In brief, there are two statistics, π_k and S_k, that describe genetic divergence within a clone (Slatkin 1996). We calculated the average number of pairwise differences per locus for the *k*th clone:

$$\pi_k = \frac{1}{\binom{n}{2}} \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} s_{ij},$$

where n is the number of sampled ramets and s_{ij} is the number of genetic differences between ramet i and j averaged across loci (Ally *et al.* 2008). We chose π_k to measure the level of genetic divergence because it has been shown to be more robust to deviations from a star-like phylogeny than S_k (the observed proportion of polymorphic loci) (Ally *et al.* 2008). Two demographic models were contrasted: one of constant ramet population size (as in the classic Wright–Fisher model), while

the second demographic model is one of population growth. The ramet population growth model was determined by plotting both π_k vs. $S_k/\sum_{i=1}^{n-1}(1/i)$, which should exhibit a 1:1 slope if a population has been constant in size, and π_k vs. $2S_k/n$ in which a 1:1 slope would be predicted for a clonal growth model. The fit of the models was determined by regression analysis obtained in SIGMAPLOT 10.0.

Further restrictions, to the sample set, were applied for clonal age estimates, with ramet size of $n \geq 5$ resulting in $n = 90$ genets used in this analysis (Table 2). While most colonies were sampled once, we captured the allelic variation within a genet by restricting age calculations to those genets with at least five ramets. We still may have missed some somatic mutations at these loci leading to an underestimation of the minimum genet age. Note that ramets lacking mutations but belonging to a genet that had other ramets with mutations (ramet number 5 or greater) were included (Table 2). If the genet had at least five ramets but no ramets had mutations, then microsatellite divergence, and therefore, age could not be calculated.

There are currently no direct estimates for microsatellite mutation rates in *A. palmata*. We assumed the same mutation rate for all samples, but we were uncertain about that rate. Hence, we used a range by setting a maximum and a minimum. The upper bound for the mutation rate (relatively fast mutation rate) implies that a shorter amount of time has passed to accumulate the observed variation relative to the lower bound of the estimate (relatively slow mutation rate). Genet P1028 from Elbow reef in Florida had the smallest microsatellite divergence rate. This genet had 55 ramets, among which the largest single colony was 270 × 170 × 70 cm ($L \times W \times H$). The branch extension rate was measured directly on three ramets of this genet (P1028) during January–July 2006. A small beaded cable tie was deployed on each of three branches of each ramet as a benchmark. The length of the branch tip from this benchmark was measured in situ over this 6-month period, averaged over branches and ramets and converted to an annualized rate of linear branch extension equal to 4.441 (±2.64 cm SD) cm/year. The annual increment in colony diameter was assumed to be twice the branch extension rate, 8.882 cm/year. The maximum measured diameter of a ramet of this genet was 270 cm so the colony must have been growing for at least 30.4 years. This results in a maximum mutation rate of 1.195^{-04} per locus per year.

We turned to the geological record to establish a minimum mutation rate. Carbon-14 dates from cores taken at Looe Key in Florida put the start-up of *A. palmata* reef growth at the base of present-day shallow spur and reef zone at around 6500 ybp (Lidz *et al.* 1985). Our clone with the highest π_k value is from Looe Key in Florida (Table S2, Supporting information), thus assumed to be the oldest, and the minimum mutation rate can be calculated by setting this clone at a maximum age of 6500 years. This results in a minimum mutation rate of 1.542^{-05} per locus per year. This is likely the maximal value of the minimum mutation rate because reef growth may not have been continuous at Looe Key.

Results

Identification of mutation type (somatic vs. chimera)

Of the 90 genets with at least five ramets (comprising 1294 samples), there were only three samples in two genets (two samples in genet P2445 from Looe Key, Florida and one sample in genet P2151 from Molasses Reef, Florida) that differed by more than 60% in their major cluster assignment from other ramets of the genet (Fig. 4). Therefore, the majority of samples (98%) showing three alleles were determined to be the result of somatic mutations rather than chimerism (Fig. 4).

Somatic mutations

Genets with at least two ramets were included in the mutation-step analysis. Of the 3352 samples genotyped, 1387 ramets of 147 genets satisfied this requirement across the Caribbean and Florida. We found 342 unique mutational changes across the five microsatellite loci (Table 3). Of the 342 somatic mutations, 305 involved a one-step increase ($n = 150$) or decrease ($n = 155$), with an additional 14 one-step mutations in which direction could not be determined due to the mutated allele size being equidistant from each parental allele (e.g. 163/169 parental genotype with mutated allele 166). This resulted in 93% of the mutations being either a one-step increase or decrease further supporting the explanation of somatic mutation for the 3rd alleles. The remaining 22 mutations were the result of either multistep changes or, in one case, involved the loss of heterozygosity.

An important factor contributing to a microsatellite mutation rate is the repeat length; the more repeat units, the greater the opportunity for replication slippage. The five loci used here had repeat lengths from 10 to 28 trinucleotide repeats (Fig. 3A). As expected, with increasing repeat length the number of UMs observed at a locus increased linearly (Fig. 3A). [This result has also been confirmed in experiments with trinucleotides in humans where the mutation rate for 28–31 repeat lengths was more than four times that seen for 20–22 repeat lengths (Zhang *et al.* 1994).] When considering all loci together, and designating allele 1 as the

smaller allele in an individual and allele 2 as the larger, there were more mutations found in allele 2 (213) than allele 1 (97) (Fig. 3B; excluding the 14 mutations in which the mutated allele could not be determined, 17 mutations in homozygotes and the one mutation determined to be a loss of heterozygosity).

Most colonies within our collection were only sampled once; however, 11 colonies from Florida were resampled in 2011 and 2014 at 2–8 locations within the colony (these samples were not included in any other analysis; Table S3, Supporting information). There were five colonies from Sand Island and Molasses reefs in Florida that had no mutations when initially sampled from 2005 to 2009 and reanalysis in 2011 and 2014 also showed no mutations (average $n = 4.6$ samples per colony). One colony from Sand Island had multiple alleles at locus 166 of 149/173/176 bp in 2007. The same three alleles were found in the additional sampling throughout the colony ($n = 4$) in 2011. In two colonies, multiple alleles were not recovered when resampled ($n = 8$). In three colonies intracolonial variation was observed: in one case, a mutation was found in only half the samples from one colony. In the other two colonies, a new mutation was recovered in some samples, with the original mutation(s) varying throughout replicate samples (Table S2; Fig. S4, Supporting information). Thus, sampling a colony once may cause an underestimation of mutational load due to intracolonial variation in some colonies (Table S2, Supporting information).

Clonal Richness vs. mosaicism

Clonal richness ranged from 0 to 1 and is directly proportional to the number of sexual recruits. The proportion of nonmosaic genotypes (i.e. those with only biallelic loci) increased with increasing genotypic diversity of the *A. palmata* stand (Fig. 5A) considering a total sample size of 3352 from 13 regions. However, we were concerned that this result may be due to a greater power of detection in genets with more ramets. Therefore, we limited our analysis to colonies that were sampled on three spatial scales (5, 10 and 15 m radii) using

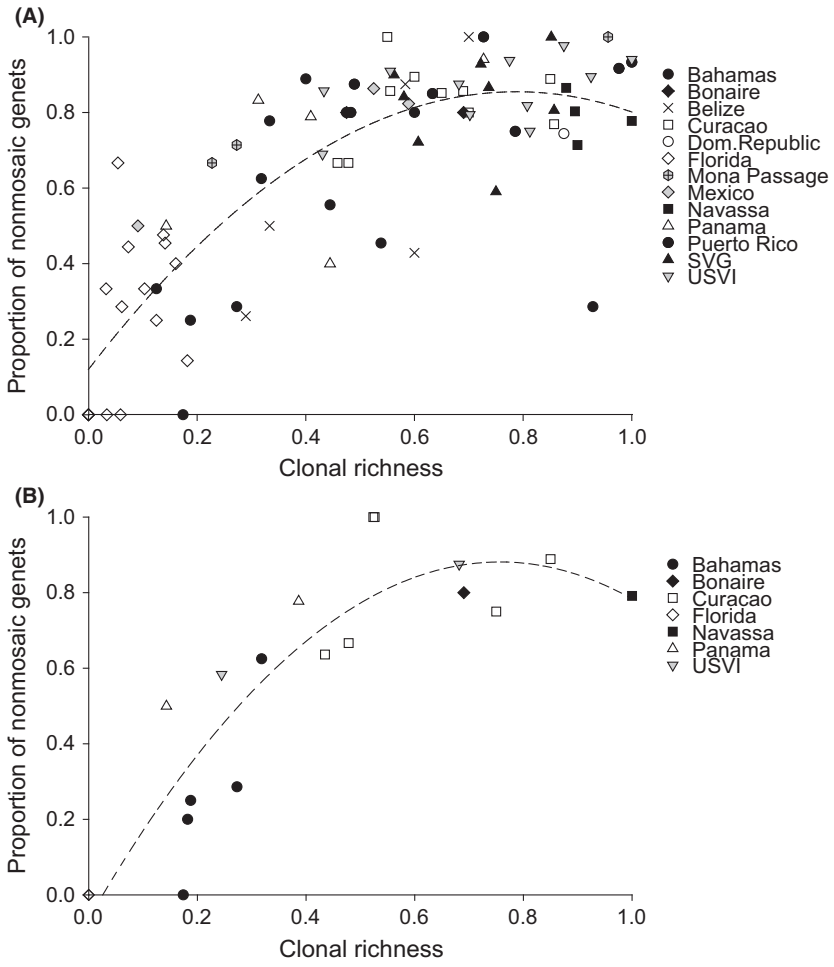

Fig. 5 The proportion of nonmosaic genets per reef as a function of clonal richness at each reef. (A) Total sample size of 3352 colonies from 86 reefs within 13 different regions across the Caribbean with $n \geq 10$ colonies per reef. Exponential Rise to Maximum, Single, 2 Parameter equation: $f = 0.88 \times (1-\exp(-3.94 \times x))$ (adjusted $R^2 = 0.65$). (B) Including only colonies that were sampled on three spatial scales (5-, 10- and 15-m-radius plots) using a random sampling procedure (described in Baums *et al.* 2005a) for a total of 486 total samples from seven regions. Exponential Rise to Maximum, Single, 2 Parameter equation: $f = 1.02 \times (1-\exp(-2.48 \times x))$ (adjusted $R^2 = 0.76$).

a random sampling procedure (Baums *et al.* 2006a) to detect both common and rare genets, resulting in 486 total samples from seven regions. Again the proportion of nonmosaic genotypes increased with increasing genotypic diversity when only considering reefs sampled with similar sampling effort (Fig. 5B). Therefore, mosaicism appeared to be more common on reefs dominated by asexual reproduction than those dominated by sexual recruitment.

A previous study showed that genotypic richness was greater and more homogeneous in the eastern (US Virgin Islands, St. Vincent and the Grenadines, Bonaire and Curaçao) than the western province (Florida, Bahamas, Panama and Mexico) with the exclusion of Navassa (Baums *et al.* 2006b). When comparing the proportion of nonmosaic genotypes per reef between western (also including Belize, the Dominican Republic, Mona and Navassa) and eastern populations, the east had significantly more nonmosaic genets than the west (Mann–Whitney *U*-test, east $n = 38$, west $n = 48$, $P < 0.001$).

Growth models

The regression of π_k vs. $S_k/\sum_{i=1}^{n-1}(1/i)$ (Fig. 6A) for the western population had a slope of 1.03 ± 0.10 SE ($F_{1,66} = 98.09$, $P < 0.0001$, adj. $R^2 = 0.59$) and was not significantly different from the value expected [1:1 relationship of π_k vs. $S_k/\sum_{i=1}^{n-1}(1/i)$] if genet size were approximately constant over time with continuous ramet turnover (ANCOVA, $P = 0.47$), whereas the regression of π_k vs. $2S_k/n$ (Fig. 6B) for the western population had a slope of 1.19 ± 0.22 SE ($F_{1,66} = 29.06$, $P < 0.0001$, adj. $R^2 = 0.30$) and was significantly different from the value expected (1:1 relationship of π_k vs. $2S_k/n$) if the genet had been spatially expanding continuously since larval settlement (ANCOVA, $P < 0.0001$).

The regression of π_k vs. $2S_k/n$ (Fig. 6C) for the eastern population had a slope of 1.07 ± 0.11 SE ($F_{1,14} = 95.47$, $P < 0.0001$, adj. $R^2 = 0.86$) and was significantly different from the value expected (1:1 relationship of π_k vs. $S_k/\sum_{i=1}^{n-1}(1/i)$) (Fig. 6D) if genet size were approximately constant over time with continuous ramet turnover (ANCOVA, $P < 0.01$). The regression of π_k vs. $S_k/\sum_{i=1}^{n-1}(1/i)$ for the eastern population had a slope of 0.82 ± 0.11 SE ($F_{1,14} = 54.37$, $P < 0.0001$, adj. $R^2 = 0.78$) and was not significantly different from the value expected (1:1 relationship of π_k vs. $2S_k/n$) if the genet had been spatially expanding continuously since larval settlement (ANCOVA, $P = 0.17$).

Microsatellite divergence estimate of age

Estimated age calculations in the western Caribbean reefs ranged from 30 to 838 years old (y/o) from the maximum mutation rate and 236 to 6500 y/o from the minimum mutation rate. Both the youngest genet and the oldest genet were from reefs in Florida (Elbow and Looe Key; Table 4). Genets in the eastern Caribbean were from 76–627 y/o to 590–4865 y/o. An age

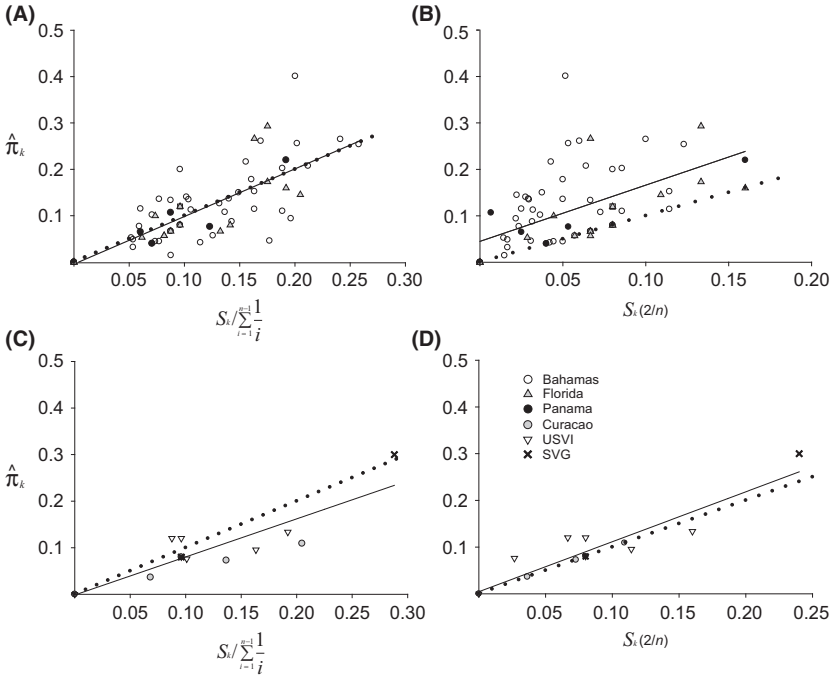

Fig. 6 A comparison of two growth models for the western (panels A and B) and eastern (panels C and D) Caribbean. The western Caribbean population included Florida, Bahamas, Panama and Belize. The eastern Caribbean population included Curacao, US Virgin Islands (USVI) and St. Vincent and the Grenadines (SVG). Panel (A, C): In a constant population model with continuous ramet turnover, the slope of π_k vs. $S_k/\sum_{i=1}^{n-1}(1/i)$ would exhibit a 1:1 relationship (dotted line). Panel (B, D): In a population that is growing in size, the slope of π_k vs. $2S_k/n$ should exhibit a 1:1 relationship (dotted line). See text for statistical analysis.

Table 4 Calculated age of *Acropora palmata* genets from throughout the Caribbean and northwest Atlantic

Region	Reef	Clonal ID	N	π_k	Oldest age (years)	Youngest age (years)	Within a 5% CI around growth model
Bahamas	Black Bouy	P1100	5	0.000	<254	<30	Yes
	BockCay	P1106	10	0.080	1397	167	Yes
		P1110	5	0.160	2794	335	Yes
	Charlies Beach	P1089	15	0.053	931	112	Yes
	Great Iguana	P1042	11	0.145	2540	304	Yes
		P1043	7	0.267	4657	558	No
	Halls Pond	P1130	6	0.173	3027	363	Yes
	Little Darby	P1112	12	0.067	1164	139	Yes
	Middle Beach	P1079	9	0.100	1746	209	No
		P1080	5	0.120	2095	251	Yes
	Nairn Cay	P2365	6	0.000	<254	<30	Yes
	Perry Shallow	P1073	7	0.057	998	120	Yes
		P1075	6	0.067	1164	139	Yes
		P2475	5	0.080	1397	167	Yes
		P1148	6	0.293	5122	613	No
		P1123	7	0.057	998	120	Yes
		P1122	9	0.000	<254	<30	Yes
Belize	GSTF12	P2276	5	0.120	2095	251	Yes
Curacao	Blue Bay	P2161	5	0.080	1397	167	Yes
		P1200	11	0.036	635	76	Yes
	East Point	P1258	5	0.080	1397	167	Yes
		P1244	11	0.073	1270	152	Yes
	Sea Aquarium	P1199	7	0.000	<254	<30	Yes
		P1232	5	0.000	<254	<30	Yes
		P2194	11	0.109	1905	228	Yes
Florida	Boomerang	P1040	10	0.040	698	84	Yes
	Carrysfort	P2115	17	0.092	1609	193	No
		P2118	41	0.137	2385	286	No
		P2121	24	0.049	848	102	Yes
		P2591	11	0.102	1778	213	No
	Elbow	P1028	55	0.015	254	30	Yes
		P1029	6	0.067	1164	139	Yes
		P1030	7	0.000	<254	<30	Yes
		P1033	7	0.152	2661	319	Yes
		P1032	30	0.256	4469	535	No
		P2122	8	0.136	2370	284	No
		P2123	16	0.265	4628	554	No
		P2126	27	0.135	2357	282	No
	French	P2539	6	0.067	1164	139	Yes
		P2538	20	0.261	4559	546	No
		P2128	54	0.126	2206	264	No
		P2564	24	0.178	3113	373	No
	Grecian Rocks	P2582	19	0.042	735	88	Yes
		P1034	14	0.057	998	120	Yes
	Horseshoe	P1000	25	0.113	1967	236	No
		P2559	7	0.114	1996	239	Yes
	Key Largo DR	P2132	14	0.202	3531	423	No
		P2134	13	0.254	4433	531	No
		P2138	14	0.110	1919	230	Yes
		P2139	6	0.133	2328	279	No
		P2597	5	0.200	3492	418	No
	Little Grecian	P1026	5	0.080	1397	167	Yes
		P1001	24	0.032	557	67	Yes
	Looe Key	P2427	28	0.052	915	110	Yes
		P2429	31	0.401	7000	838	No

Table 4 *Continued*

Region	Reef	Clonal ID	*N*	π_k	Oldest age (years)	Youngest age (years)	Within a 5% CI around growth model
		P2445	29	0.140	2452	294	No
	Marker 3	P1039	52	0.046	801	96	Yes
	Molasses	P2151	25	0.207	3621	434	No
		P2146	32	0.150	2619	314	No
	RockKey	P1018	5	0.080	1397	167	Yes
		P1017	16	0.115	2008	241	No
	Sand Island	P1007	9	0.044	776	93	Yes
		P1002	96	0.094	1641	196	No
		P1003	29	0.216	3776	452	No
		P1021	5	0.000	<254	<30	Yes
	Triangle	P2416	38	0.087	1525	183	No
	Western Sambo	P1012	8	0.044	776	93	Yes
		P1011	11	0.108	1881	225	Yes
		P1008	8	0.000	<254	<30	Yes
Panama	Bastimentos I	P1150	16	0.065	1135	136	Yes
	Bocas Del Drago	P1168	15	0.076	1330	159	Yes
		P1167	5	0.220	3842	460	No
	Tobobe West I	P1183	6	0.107	1863	223	No
	Wild Cayne	P1177	10	0.040	698	84	Yes
Puerto Rico	Cayo Ron	P2286	6	0.173	3027	363	Yes
		P2294	5	0.000	<254	<30	Yes
		P2301	8	0.000	<254	<30	Yes
	La Cordillera	P2334	5	0.000	<254	<30	Yes
		P2339	5	0.000	<254	<30	Yes
	San Cristobal	P1857	10	0.204	3570	428	Yes
		P1878	7	0.000	<254	<30	Yes
SVG	Mustique	P1667	5	0.080	1397	167	Yes
		P1668	5	0.300	5239	627	Yes
USVI	Grounding VI	P1430	5	0.000	<254	<30	Yes
	Hawksnest Bay	P1399	30	0.076	1325	159	Yes
		P1403	5	0.080	1397	167	Yes
		P1402	6	0.120	2095	251	Yes
		P1406	6	0.133	2328	279	Yes
	Salt Pond	P1555	5	0.120	2095	251	Yes
	Tague Bay	P2504	7	0.095	1663	199	Yes

N is the number of ramets; π_k is microsatellite divergence; CI, confidence interval; SVG, St. Vincent and the Grenadines; USVI, US Virgin Islands.

comparison between the eastern and western populations, including only genets with somatic mutations (west $n = 61$, east $n = 15$) yielded no significant differences (Kruskal–Wallis test, $P > 0.05$).

Discussion

Determination of genet age distribution in coral populations is important for understanding demographic changes in response to environmental perturbation and ultimately for understanding the evolutionary potential of these foundation species. *A. palmata*, the now endangered but previously dominant shallow reef-builder in the Caribbean, lends itself to somatic mutation analyses because of the importance of asexual reproduction via fragmentation resulting in genets with many members. Here, we show that some *A. palmata* genets are apparently of substantial age (Table 4). This was surprising, as previously only cold-water corals were found to be >1000 y/o (Table 1).

The Quaternary fossil record of *A. palmata* assemblages suggests that their habitat tolerances and preferences have remained relatively constant through time and space (Goreau 1959; Shinn 1963; Gischler 2015). Consequently, the distribution of *A. palmata* on shallow-water reefs has persisted through repeated glacial–interglacial cycles. Thus, at scales from decades to millennia, the persistence of *A. palmata* and the assemblages they comprise was enabled by their capacity to incrementally track favourable environments that have shifted spatially over

time (Precht and Aronson personal correspondence). These geological data point to the possibility of potentially millennial-age (or older) genets within modern-day populations of *A. palmata*.

We stress that absolute genet ages derived from somatic mutations as presented here have to be interpreted cautiously. Because direct measurements of microsatellite mutation rates in corals are not available and probably will not be for some time, we used other evidence to bracket minimum and maximum mutation rates. We assigned the highest mutation rate to the genet with the smallest microsatellite divergence rate among clone members and measured the growth rate of the largest colony. Growth rates of *A. palmata* can vary with season, latitude and reef location, and the measured linear extension rate of 4.44 cm/year of this colony was somewhat slower than published growth rate measurements of 6–9 cm/year from Florida and across the Caribbean (Gladfelter *et al.* 1978; Lirman 2000; Bak *et al.* 2009). We set the minimum mutation rate to the genet with the largest microsatellite divergence rate among clone members and asked how long this genet could have existed in this location (Looe Key, Florida). By turning to the published fossil record, we ascertained that *A. palmata* colonies at this location could not have been more than 6500 years old (Lidz *et al.* 1985). While it is perhaps unlikely that this genet is 6500 years old because *A. palmata* presence at this location may not have been continuous over this time frame, it is a maximal estimate. The resulting mutation rates (1.195^{-04}–1.542^{-05} per locus per year) fall within reported microsatellite mutation rates from 10^{-2} to 10^{-6} per sexual generation (Kruglyak *et al.* 1998; Shimoda *et al.* 1999; Ellegren 2000; Hoekert *et al.* 2002; O'Connell & Ritland 2004; Peery *et al.* 2012) when adjusted to generational times of acroporids (4–8 years; Wallace 1985). An analysis of environmental markers in extant *A. palmata* skeletons could substantiate genet age estimates (however, the oldest portion of the genet may no longer exist).

Despite the uncertainties surrounding absolute genet age determination, relative genet age comparisons across the range of *A. palmata* should still be valid and are presented here for the first time.

Range-edge populations and dominance of asexual reproduction

Sessile organisms capable of asexual reproduction are often largely clonal at the edge of the species' range, both in terrestrial and marine ecosystems (Eckert 2002; Baums 2008). Populations at the range margins of the marine angiosperm *Zostera marina* had clonal richness values of <0.2 and sexual reproduction was rare or absent (Reusch & Boström 2011). The marginal *A. palmata* population of Florida averaged 3.7 UMs per MLG, whereas eastern, lower latitude populations such as Bonaire, Curacao and USVI ranged from 1.2 to 1.3 UMs per MLG, $n = 1387$ (Table 3). This would mean that the Florida genets are older. Nevertheless, when considering only the large clonal stands the ages were not significantly different between the eastern and western populations (Table 4) suggesting a more or less similar historical presence of *A. palmata* in both populations but a higher frequency of sexual renewal in the East.

Mosaicism due to somatic copy number variations

At first glance, the appearance of three alleles per locus in *A. palmata* MLGs is puzzling. One explanation is gene or genome duplication (Wang *et al.* 2009; Richards & Oppen 2012). However, several lines of evidence argue against this interpretation. Preliminary assembly of two lanes of genomic sequencing data (Illumina) showed no evidence of genome duplication (I. Baums personal observations). Additionally, a chromosomal spread analysis of *A. palmata* larvae revealed a count of $n = 24$ (Fig. S5, Supporting information), a diploid state. The basic scleractinian chromosome number is $x = 14$ and $x = 12$ (Kenyon 1997). Inherited, duplicated genomic regions are also unlikely. In the latter case, all five microsatellite loci would have to be located in duplicated regions as all five loci show triallelic genotypes, albeit usually only one locus was mutated in any given sample: for genets with $n \geq 5$ ramets, 15.56% had zero mutated loci, 58.89% had one mutated locus, 20% had two mutated loci and 5.56% had three mutated loci. Four of the five loci amplify a similar range of allele sizes in the Caribbean sister species, *A. cervicornis*. Fossil records date back 6.6 (Budd & Johnson 1999) and 2.6–3.6 (McNeill *et al.* 1997) million years, respectively, for *A. cervicornis* and *A. palmata*. Thus, the duplication events would have to have occurred before the speciation event because triallelic genotypes were found in both species across the entire Caribbean range. Such duplicated genomic regions would have been mutating separately for several million years making it unlikely that the majority of mutations are just one mutation step away as observed here.

Genomic instability is a mechanism of ageing with somatic copy number variations (CNV) prevalent in many human cancers (Shlien & Malkin 2009) and somatic CNVs increase with age in human blood cell genomes (Forsberg *et al.* 2012). We posit that *A. palmata* genomes accumulate somatic duplications with age, resulting in multiple copies of the microsatellite loci available for replication slippage (Fig. 7). This led to some ramets having up to four alleles at a single locus.

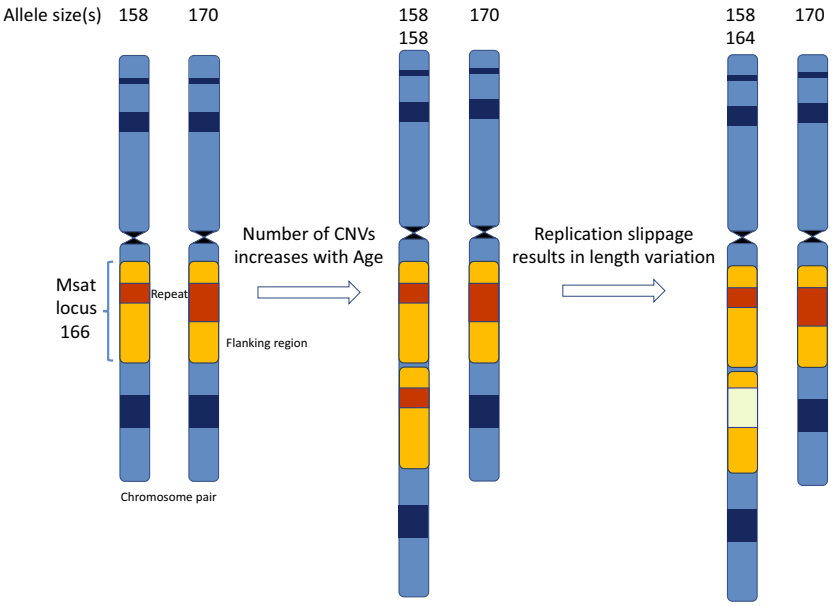

Fig. 7 Diagram depicting how duplication of a microsatellite (msat) locus (yellow) leads to copy number variation (CNV) on chromosomes (blue) in a diploid species. Once a locus is duplicated, the microsatellite repeats (orange/white) may mutate through slippage of the DNA polymerase during mitotic replication leading to the detection of three alleles in electropherograms. With time, alleles on both chromosomes may duplicate and mutate leading to detection of four alleles per samples (not shown). Allele sizes are given in base pairs. Diagram not to scale.

Recovery of triallelic genotypes was robust to repeated DNA extractions, and repeated PCRs, and has been observed in other coral species (Wang *et al.* 2009) and the marine angiosperm *Zostera marina* (Reusch & Boström 2011). Baums *et al.* (2005a) found triploid larvae in some experimental crosses, ranging from 7% to 36% of the larvae genotyped. Larvae did survive to 90 h post fertilization but it is unknown if they would settle and grow into reproductive adults. The most likely explanation for the triploid status was having a second maternal allele, either due to retention of a polar body, self-fertilization or mitotic parthenogenesis. Multiple alleles (3–5) were detected in 15% of Pacific Acroporids at a single locus due to inherited gene duplication; in this study, all alleles in the example chromatogram were greater than a one-mutation-step difference (130, 140, 150, 162 bp, Richards & Oppen 2012). Interestingly, predominately sexually reproducing coral species on the Great Barrier Reef show somatic mutation in the form of two alleles per locus (presumably generated by a single slippage event without duplication) rather than three alleles (Schweinsberg *et al.* 2015). This leads us to hypothesize that highly fragmenting coral species such as *A. palmata* accumulate somatic CNVs over the long lifetime of the genet.

Independent evidence for or against somatic CNV would have to come from fluorescent in situ hybridization (FISH; Langersafer *et al.* 1982) or through controlled crosses of gametes from a triallelic genet and a genet without mutations within the five microsatellite loci, if there is not a sequestered germline.

A triploid (or tetraploid) state at a microsatellite locus could also stem from the mutation of cells that are able to proliferate, such as stem-like cells (Reyes-Bermudez & Miller 2009), resulting in two (or more) diploid cell lineages found throughout the colony.

Mosaicism vs. chimerism

Genetic diversity within a colony could stem from the fusion of two or more larvae or juvenile corals, producing a chimera (Fig. 1). Such fusion in early life stages has been observed in scleractinian corals and is generally attributed to an immature immune system that is not yet able to distinguish between self and nonself (Frank *et al.* 1997; Permata & Hidaka 2005; Puill-Stephan *et al.* 2009). However, the prevalence of chimerism in adult colonies in the genus *Acropora* is generally low (2–5%; Schweinsberg *et al.* 2015). Retrieval of genotypes that vary at several loci among branches from one colony may indicate chimerism (Fig. 1). A colony was classified as a chimera if it differed by more than 60% in its major cluster assignment probability from other members of its genet as defined by Schweinsberg *et al.* (2015). Only 0.2% of samples from the 90 genets (n = 1296) were classified as possible chimeras, thus making mosaicism the more likely explanation for most of the observed intracolony genetic variation.

Evolutionary and ecological consequences of genet longevity

The presence of large, potentially centennial-aged genets within a population begs questions with regard to their history as well as their adaptive potential over the coming decades of rapid environmental change. It is

likely that the environmental conditions in most shallow coastal habitats over the lifespan of these very old genets were quite different from today, which implies (i) that these old genets possess a great degree of plasticity enabling them to persist throughout these environmental variations (Barshis *et al.* 2013) and/or (ii) that they have in fact 'migrated' among nearby coastal habitats over the centuries. For example, it is possible that our current observation of a very old clone is in a distinct location from where it originally recruited with fragments 'migrating' upslope thereby tracking slow holocene sea level change (Gischler 2015).

Alternatively, the General-Purpose Genotype model (Baker 1965) explains the ubiquity of clonal organisms by their ability to retain the most competent genotypes over time, favouring the absence of sexual reproduction once an optimal genotype is found. For example, Van Doninck *et al.* (2002) showed much higher ecological tolerances of a ubiquitous asexual ostracod in comparison with additional species that were asexual and narrowly distributed or that had mixed reproductive modes. If *A. palmata* genets have persisted over hundreds to thousands of years, it implies persistence through substantial environmental changes and possibly gives hope that they can survive additional anticipated climate change. The overall recent declines of *A. palmata* including declines of certain moderate-sized clones in particular (Banks *et al.* 2010) suggest there is a limit to this tolerance, which may be exceeded soon.

However, *A. palmata* is not entirely asexual and there is also the possibility that a preponderance of large, old genets is not necessarily adaptive. Potts (1984) suggested that because of corals' extreme longevity, many species (or populations) have not had the opportunity, as current coastal habitats became habitable, to complete adequate sexual generations to reach evolutionary equilibrium. Because fecundity of corals increases with genet size (senescence notwithstanding), there may be a tendency for large old clones to dominate the gene pool and diminish the chances for newer genets, possibly even those better adapted to current environmental conditions, to expand. If this is true, it implies that the presence of large old clones (possibly of general-purpose genotypes) may impair the rapid adaptation needed for persistence under climate change.

The occurrence of somatic mutations raises the question of whether they can be the target of selection and rapid adaptation. Mosaicism is thought to be favoured in plants because it offers an advantage in the Red Queen race against pests and parasites by increasing the standing genetic diversity that prevents the evolution of specific metabolic pathways that could be used to overcome the defences of the plant (Valen 1974; Gill *et al.* 1995). Mutations in the soma are available for immediate selection pressure from the environment as they compete with other wild-type and mutated lineages within the organism. The selection of somatic cell lineages, termed intra-organismal selection (also called somatic, diplontic or cell-lineage selection; see Buss 1983; Hughes 1989; Otto & Hastings 1998; Clarke 2011) may have the potential for rapid evolutionary change in a modular organism by allowing within-organism gene frequency changes within a single generation (Klekowski & Kazarinova-Fukshansky 1984). Through the displacement of the wild-type lineage, the mutation of regenerating cells can be considered evolution as they are potentially heritable in clonal Cnidaria through both sexual and asexual routes. Alternatively, the coexistence of multiples lineages within an organism may result in intra-organismal competition or cell parasitism leading to the decrease of overall fitness (Michod & Roze 1999; Pineda-Krch & Lehtilä 2004). A theoretical population model suggested that strong negative selection against intra-individual mutations keeps changes of allele frequencies due to somatic mutations very low (Orive 2001).

Currently, empirical confirmation of somatic selection is limited. However, there are many organisms that have been evolving in the absence of sex including rotifers (Welch & Meselson 2000), *Artemia* (Perez *et al.* 1994) and salamanders in the genus *Ambystoma* (Hedges *et al.* 1992) [see Van Oppen *et al.* (2011) for a review on somatic mutations as fuel for adaptation in invertebrates]. Somatic selection has also been demonstrated experimentally in plants (Breese *et al.* 1965; Whitham & Slobodchikoff 1981; Monro & Poore 2009). Somatic mutations may be widespread in corals (Levitan *et al.* 2011; Schweinsberg *et al.* 2015) and within mosaic *Acropora hyacinthus* colonies it was shown that transfer of intercolonial genetic variation to the next generation via gametes is possible (Schweinsberg *et al.* 2014) albeit this was not the case in *Orbicella* (Barfield *et al.* 2016).

The ability of the coral host to respond to a changing environment occurs not only through genetic adaptation but also through acclimatization by varying phenotypic responses. It has recently become apparent that some environmentally induced nongenetic or epigenetic changes are also heritable through a process known as transgenerational acclimatization (van Oppen *et al.* 2015). Epigenetic changes include histone modifications, DNA methylation, chromatin remodelling and gene regulatory mechanisms involving small noncoding RNAs (Danchin *et al.* 2011). A recent study in the clonal tree poplar showed the persistent influence of geographic origin on the ability to respond to stress within a common garden experiment. The older the clone (longer clones of the same genet lived in different environmental conditions), the more divergent the transcriptomic

response was to drought and the greater the variation in genome methylation patterns (Raj *et al.* 2011). Although not directly linked to epigenetic changes, the pacific coral *Acropora hyacinthus* (cryptic species E) was able to acclimatize to new microenvironments by increasing bleaching resistance, as measured through transcriptomic responses and chlorophyll A changes, without altering their abundances of symbiont type (Palumbi *et al.* 2014). This imprinted 'memory' of past stress responses could have profound implications for asexually reproducing corals in that ramets distributed across a reef could have divergent epigenetic 'memories' due to varying environmental conditions such as water flow, light and pathogen exposure. In addition, epigenetic changes along with somatic mutations have the ability to be passed on to the next generation in organisms without segregated germlines.

The current paucity of clonal age estimates impairs our understanding of the ecology and evolution of marine foundation fauna. These estimates are difficult to come by because size and age are not related in colonial, asexually reproducing organisms. Significant asexual colony reproduction occurs in at least nine coral genera, and thus, the decoupling of size and genet age is a widespread phenomenon in corals (Table S1, Supporting information). Alternative methods to estimating genet age include the use of somatic mutations but without direct mutation rate measurements, the uncertainty of the age estimates is considerable. Regardless, when applied to a fragmenting Caribbean coral, the results point towards genet ages that rival those of the most ancient organisms on earth alive today. This raises questions about their adaptive potential to a rapidly changing climate. Does their past ability to survive environmental change predict future success? The answer will come from experimental studies combined with demographic and theoretical models.

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M.D. and I.B. designed the study and wrote the manuscript with key input from M.M. and W.P. M.D. analysed and interpreted the data. Funding was provided and samples were collected by I.B. and the Caribbean Acropora Research Group.

Data accessibility

Multilocus genotypes are available at Dryad: http://dx.doi.org/10.5061/dryad.f6600.

Appendix
Caribbean *Acropora* Research Group

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Supporting information

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

Table S1 Contribution of asexual reproduction to local population structure is wide-spread in corals.

Table S2 Microsatellite allele calls for genet P2429 collected from Looe Key Florida on 14 July 2009.

Table S3 Eleven *Acropora palmata* colonies from Florida were sampled and genotyped repeatedly over space and time.

Fig. S1 Photo time series illustrating how a fragment (red box) of *Acropora palmata* reattaches and grows into a new colony.

Fig. S2 Histogram of pairwise differences (Nei's genetic distance) for all 3352 *Acroproa palmata* samples.

Fig. S3 Example electropherograms of locus 166 from five *Acropora palmata* samples of genet P2429 collected from Looe Key, Florida.

Fig. S4 Assignment of ramets to genets using Bayesian clustering analysis in *Acropora palmata*.

Fig. S5 Somatic chromosome counts ($n = 24$) for *Acropora palmata* based on metaphase spreads of colchicine-treated embryonic cells.