Rampant asexual reproduction and limited dispersal in a mangrove population of the coral *Porites divaricata*

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Corals are critical to marine biodiversity. Reproduction and dispersal are key to their resilience, but rarely quantified in nature. Exploiting a unique system—a fully censused, longitudinally characterized, semi-isolated population inhabiting mangroves—we used 2bRAD sequencing to demonstrate that rampant asexual reproduction most likely via parthenogenesis and limited dispersal enable the persistence of a natural population of thin-finger coral (*Porites divaricata*). Unlike previous studies on coral dispersal, knowledge of colony age and location enabled us to identify plausible parent–offspring relationships within multiple clonal lineages and develop tightly constrained estimates of larval dispersal; the best-fitting model indicates dispersal is largely limited to a few metres from parent colonies. Our results explain why this species is adept at colonizing mangroves but suggest limited genetic diversity in mangrove populations and limited connectivity between mangroves and nearby reefs. As *P. divaricata* is gono-choristic, and parthenogenesis would be restricted to females (whereas fragmentation, which is presumably common in reef and seagrass habitats, is not), mangrove populations likely exhibit skewed sex ratios. These findings suggest that coral reproductive diversity can lead to distinctly different demographic outcomes in different habitats. Thus, coral conservation will require the protection of the entire coral habitat mosaic, and not just reefs.

1. Background

A major challenge in marine ecology is measuring reproduction and dispersal. These demographic processes have fundamental ecological, evolutionary and conservation impacts on marine species. Ecologically, these processes affect population dynamics by influencing the exchange of individuals among populations. Evolutionarily, they affect population divergence by influencing gene flow among populations. Knowledge of the underlying ecology and evolution, in turn, is critical to conservation management of the seascape [1,2]. Recently, direct measures of reproduction and dispersal, made using genetic parentage analyses, have started to emerge in some marine taxa such as coral reef fishes [3,4]. However, such data remain scarce for reef-building corals, which are an object of intense conservation concern given the precipitous decline in coral reef cover over recent decades and the uncertain future of the world’s coral reefs [5].

Coral demographic studies are complicated by the fact that the typical cnidarian can reproduce sexually and asexually, and may even have multiple distinct asexual reproductive strategies, such as budding, fragmentation or the production of asexual larvae [6,7]. Within a species, the relative contribution of alternative reproductive strategies is driven by variation in biotic or abiotic features of the environment [8–10]. While laboratory studies are useful for documenting how a species’ reproductive toolbox can respond to variation in...
particular environmental parameters [11,12], genomic analyses of population structure are increasingly being used to identify the signature of alternative reproductive strategies in populations across a seascape [7,13]. However, many coral species can survive across a range of distinct habitats, from fore reef, to back reef, to seagrass, and even mangrove forests, with each habitat representing a different ecological context potentially influencing reproduction and dispersal. Using genomic data alone, it is challenging to discriminate between alternative modes of asexual reproduction, e.g. production of ameiotic larvae versus polyp bail-out or fragmentation. However, it may be possible to speculate about the most likely reproductive mode(s) given the ecological context of the population.

A particularly important aspect of a coral’s life cycle is larval dispersal, as it represents the primary mechanism of ecological and evolutionary connectivity between populations, and as such, it can be critical to population recovery and rescue [14]. Larval dispersal in brooders is assumed to be limited and local, while in broadcast spawners larval dispersal is thought to be expansive [15]. Larval dispersal distances might also be strongly impacted by oceanographic currents and local features of the seascape, as has been predicted by models [16]. To date, most evidence of coral larval dispersal distances is based on indirect measures provided by genomic data (e.g. FSD spatial autocorrelation in genetic structure within sites) [17]. However, direct measurements of larval dispersal are possible using parentage analysis [4,18], although, to date, this approach has only rarely been applied to scleractinian corals [6,19]. This scarcity is likely attributable to the combination of the low likelihood of identifying parent-offspring pairs over the large spatial scales that coral populations often span, as well as extensive asexual reproduction.

Here, we characterize reproduction and dispersal in a mangrove population of the Caribbean thin-finger coral, Porites divaricata. Importantly, the unique features of our experimental system—a fully censused, longitudinally characterized, semi-isolated population of corals inhabiting mangrove prop-roots [20,21]—have allowed us to directly measure larval dispersal. Using 2bRAD sequencing across 136 geotagged corals spanning a 3-year range of ages, we show that the extensive recruitment previously documented in this population—i.e. the appearance of small, generally unbranched coral colonies [21]—results almost exclusively from asexual reproduction, and the asexual propagules generally disperse very short distances from their parent colonies (within a few metres). Given the constraints imposed by the habitat, we discuss how the most plausible mode of asexual reproduction that could produce such recruits would be the asexual production of larvae, which has been documented in a closely related species [22]. Importantly, the only previously known mode of asexual reproduction in this species, fragmentation, is not viable in the mangrove habitat, as negatively buoyant branch fragments will sink into the mud below the mangrove prop-roots, thus revealing that key life-history traits of corals can vary dramatically across habitats.

2. Results and discussion

(a) Generation of molecular markers
Using the reduced-representation genomic sequencing approach known as 2bRAD [23], we genotyped 136 colonies from a population of Porites divaricata growing on mangrove prop-roots in a relatively isolated mangrove channel located at Calabash Caye, Belize (figure 1). On an annual basis, in November–December of 2015–2018, this population was fully censused, and longitudinal monitoring of recruitment, growth, health and mortality of individual coral colonies was conducted. This longitudinal monitoring revealed a stable population where the loss of adult colonies was more than offset by larval recruitment [21]. We determined the genotypes of corals from four different annual cohorts that ranged in age from 0 to 3+ years and ranged in ecological volume from 0.22 to 11.817 cm$^3$ (table 1).

(b) Evidence of rampant asexual reproduction
Regardless of whether we used Prevosti distance based upon the single-nucleotide polymorphisms (SNPs) or Identity by State (IBS) based on genotype likelihoods calculated under a broad range of parameters (electronic supplementary material, table S1), the results are the same: this $P$. divaricata population reproduced predominantly asexually. Whether we mapped our reads to the assembled genome of $P$. lutea or to a de novo assembly of RAD tags, and regardless of which ANGSD parameters we used to call SNPs, we identified 10 clonal lineages (labelled A–J in figure 2; electronic supplementary material, figures S1 and S2) with almost identical membership (electronic supplementary material, table S2). For example, if we base our analysis on the highest confidence 118 SNPs (i.e. the ‘hard-called’ SNPs identified after mapping the sequencing reads to the $P$. lutea genome), 126 of 136 colonies (92.6%) belong to clonal groups A–J, which each comprise 2 to 39 individuals (figure 2). Mean pairwise distances between individuals within these clonal groups were 0.11% ± 0.004 s.e., less than or equal to the distance observed between technical replicates [24,25]. By contrast, the mean pairwise distance between individuals from different groups was 21.1% ± 0.045 s.e. The average relatedness between individuals that were not part of the same clone was generally equivalent to that of first cousins ($r_{ab} = 0.174 ± 0.04$ s.e.). The most closely related clones exhibited a relatedness equivalent to that of half-siblings ($r_{ab} = 0.31$, e.g. clones F and G, figure 2), while the most distant clone lines (e.g. cluster A relative to clusters B–J) exhibit a coefficient of relatedness $r_{ab} = 0.056$, similar to that exhibited by second cousins (figure 2).

Though a range of asexual reproductive strategies has been documented in corals, including colony fragmentation [26], polyp bail-out [11,27] and the asexual production of larvae [12,28,29], the unique ecological context of the mangrove population studied here implicates asexual production of planula larvae as the only plausible mode of clonal reproduction. Given that individuals are growing on prop-roots suspended over a mud/silt bottom, reproduction via colony fragmentation, which is thought to be a common mode of asexual reproduction in $P$. divaricata [30], would invariably result in mortality because colony fragments cannot re-adhere to the prop-roots, and those that fall to the bottom are quickly submerged in soft sediment, a phenomenon that we have repeatedly observed in the field (figure 1d). Similarly, isolated individual polyps produced by polyp bail-out, a mechanism of asexual reproduction that has never been reported in poritid corals, would be unable to colonize prop-roots and would fail to
the mud bottom because they are ‘slightly negatively buoyant’, with limited mobility [11,27]. By contrast, planulae are capable of directed swimming, searching behaviour and settlement, and asexual planulation has been reported in another Porites species [12]. Because P. divaricata is a gonochoric species that broods its larvae [30], we deduce that asexual larvae are produced through parthenogenesis and brooded by females.

(c) Evidence of limited dispersal

Previous studies have noted the dense clustering of individuals in this population and suggested that dispersal may be predominantly local [20,21]. Indeed, when we transposed the locations of corals living along the undulating banks of the channel to a common line (figure 2), most or all colonies within each clonal group are tightly clustered within a 50–100 m distance. However, a handful of individuals are hundreds of metres distant from their nearest clonemate (figure 2).

Unlike previous studies [20,21], here we have explicit knowledge of the underlying clonal relationships, allowing us to generate more tightly constrained estimates of dispersal. We first identified potential parent–offspring pairs within each clonal lineage (A–J) by assuming that new recruits identified

Table 1. Coral colonies sampled by age and size. All collections occurred in 2018.

<table>
<thead>
<tr>
<th>n</th>
<th>appeared in</th>
<th>age (years)</th>
<th>avg. ecological volume (cm³)</th>
<th>avg. branch tips</th>
</tr>
</thead>
<tbody>
<tr>
<td>55</td>
<td>2015 or prior</td>
<td>3+</td>
<td>1348.9 ± 356.5</td>
<td>16.1 ± 2.6</td>
</tr>
<tr>
<td>19</td>
<td>2016</td>
<td>2–3</td>
<td>803.0 ± 157.3</td>
<td>20.9 ± 5.1</td>
</tr>
<tr>
<td>30</td>
<td>2017</td>
<td>1–2</td>
<td>142.4 ± 50.3</td>
<td>5.2 ± 1.2</td>
</tr>
<tr>
<td>32</td>
<td>2018</td>
<td>0–1</td>
<td>10.7 ± 3.0</td>
<td>0.9 ± 0.3</td>
</tr>
</tbody>
</table>

Figure 1. Map of study site and representative coral colonies. (a) Turneffe Atoll, Belize, and (b) the mangrove channel study site at Calabash Caye. Coloured circles indicate the locations of sampled Porites divaricata colonies, with different colours representing individuals belonging to distinct clonal groups. White circles represent individuals not assigned to clones. To simplify dispersal simulations, the locations of coral colonies were orthogonally projected onto the white line shown in the figure (see examples of colonies projected onto the line at 350 m and 425 m). (c) A representative coral colony growing on a mangrove root, with soft sediment containing decaying mangrove leaves visible in the background. (d) A dying coral colony that fell from a mangrove root is partially buried in the sediment.
in 2016–2018 could have derived from any clonemate from a prior year (2015–2017), irrespective of colony size. We then calculated the distance between all possible parent–offspring pairs based on their GPS locations. From these distances, we determined the minimum, maximum, mean and median distance that each offspring could have dispersed from potential
parents. We then generated minimum, maximum, mean and median dispersal distance distributions for the population and fitted probability density functions to these distributions (figure 3). Across all 28 combinations of dispersal distances and probability density functions explored, the best-fitting model was the exponential distribution fitted to the minimum dispersal distances (figure 3a; electronic supplementary material, table S3). Under this model, the median dispersal distance is 1.1 m and the 99th percentile for dispersal is 89.2 m.

In addition to the distances between potential parent–offspring pairs, another measurement we used to compare the performance of alternative dispersal models was the distance between members of the same clone-cohort, i.e. clonemates that appear in the same year. We simulated annual dispersal events of larvae within each clonal lineage under the probability density functions that represented the best fit to the minimum (exponential), maximum (Weibull), mean (lognormal) and median (gamma) distance distributions and compared the observed versus expected distribution of distances between members of the same clone-cohort generated under each simulation. For all five clone lines for which we could obtain distances between members of the same cohort, the expected distribution generated under the minimum dispersal model most closely resembled the observed distribution (figure 4; electronic supplementary material, table S4).

Prior studies have also provided evidence of highly localized dispersal of larvae in brooding corals. For example, spatial autocorrelation analysis of within-site structuring in another brooding coral, Seriatopora hystrix, revealed that colonies within 20 m of each other were most similar genetically, suggesting that recruits typically settle within 20 m of parents [17]. Of note, the minimum dispersal model (figure 3a) generates a more leptokurtic distribution than the other models, especially the maximum dispersal model (figure 3b).

Leptokurtic dispersal distributions are common in nature [31–33], including marine taxa [3,4,34]. Dynamic models of coral dispersal that incorporated empirical measurements of larval period and settlement competence also produced leptokurtic distributions [35].

This study represents the first population genomic analysis of mangrove corals, and we find evidence that asexual reproduction predominates in the sampled population. This asexual reproduction, in conjunction with high survivorship and high growth rates of recruits, is critical to maintaining population viability [21]. Other coral populations have been found to reproduce predominantly asexually [13], particularly at the edge of their range or in ecologically marginal habitats [10]. Asexual reproduction allows for rapid population expansion and colonization of available substrate without requiring sexual partners, and it facilitates the spread of locally adapted genotypes in the absence of gene flow [7,13]. Despite these advantages, populations dominated by asexual reproduction may be more vulnerable to environmental change given their low genetic diversity, which may limit the rate of adaptive evolution, especially if these populations are relatively isolated [7,13].

Due to the unique ecological context of colonies in this mangrove habitat, we propose that asexual reproduction is most likely occurring through the production of parthenogenetic larvae, a reproductive mode that has not previously been documented in P. divaricata to our knowledge, although it has been reported in the closely related mounding coral, P. astreoides [12]. The production of asexual larvae could represent an adaptation to growing on a substrate suspended over a mud bottom. It is also possible that parthenogenesis is similarly common in populations of P. divaricata found in other settings, such as reefs. However, in habitats with suitable hard-bottomed substrate (e.g. reefs), fragmentation, which is the only previously documented mode of asexual reproduction in P. divaricata, may also be an important mode of asexual reproduction [30]. The ability to produce asexual larvae could facilitate colonization of mangroves by P. divaricata, making this species a case study for how possessing multiple mechanisms of asexual reproduction can facilitate niche expansion. Of note, if parthenogenetic larvae are produced solely by
Figure 4. Empirical (a–e) and simulated (f–j) distributions of the distances between siblings of the same clone for clonal groups A, B, C, D and G. Results from $k$ sample Anderson–Darling tests (AD) and Kolmogorov–Smirnov (KS) tests comparing observed and simulated distributions are shown. The test statistics and $p$-values for both versions (V1, V2) of the AD test are displayed (test statistic/ $p$-value). The lowest value of the AD statistic for each set of four simulations is displayed in bold red type, as are $p$-values of simulated distributions not significantly different from the observed distribution.
females, reliance on different modes of asexual reproduction in different habitats could lead to differing sex ratios in those habitats, e.g. female colonies may be more abundant in mangrove populations than in reef populations of *Porites divaricata*.

The preponderance of asexual reproduction in this particular population of *P. divaricata* could have multiple explanations. First, it may be typical of *P. divaricata*. Unfortunately, there is limited basis for comparison, as there is only one prior study of reproduction in a natural population of *P. divaricata* [30]. McDermond [30] suggested that asexual reproduction by fragmentation was the primary mechanism for population maintenance and growth of *P. divaricata* at Rodriguez Key in Florida, a shallow carbonate bank described as marginal habitat [30]. The conditions at Rodriguez Key could make larval settlement difficult while promoting fragmentation. Asexual reproduction could also be advantageous in a marginal habitat because of low mate availability and because it would facilitate the spread of locally adapted genotypes. Mate availability could be limiting sexual reproduction in Calabash Channel, if, as we suspect, the population is largely female colonies produced by parthenogenesis. It is also possible that asexual reproduction is causing a mangrove-adapted genotype or genotypes to spread. Indeed, a proclivity to reproduce via parthenogenesis could be an adaptation to living in the mangroves.

The best-supported model of dispersal suggests that asexually generated *P. divaricata* larvae typically disperse only a few metres from their parent colony and rarely disperse distances greater than 100 m. Such limited dispersal is presumed to be characteristic of brooding corals based on observations of the settlement competency of larvae in the laboratory [36], as well as population genetic structure of brooding corals in situ, e.g. [17]. However, few studies have provided direct estimates of coral dispersal. In earlier work, parentage analyses provided evidence for limited sperm dispersal among brooding corals, including the octocoral *Pseudopterogorgia elizabethae* [37] and the stony coral *Seriatopora hystrix* [38]. Limited sperm dispersal was also found in the stony coral *Porites damicornis*, which exhibits both brooding and broadcasting reproductive modes [28]. More recently, parentage analysis of larvae from the broadcast spawning fire coral *Millepora cf. platyphylla* also revealed limited dispersal with recruits often settling within 10 m of parent colonies, which is counter to the general expectation of increased dispersal in broadcast spawning species [6]. Unlike these previous studies, explicit knowledge of clone age and location enabled us to identify plausible parent-offspring relationships within multiple clonal lineages, providing an opportunity to compare field-based estimates of larval dispersal with alternative dispersal curves.

Occasional longer-distance dispersal may be occurring in the mangrove *P. divaricata* population studied here. Intermittent long-distance dispersal is thought to be possible in brooding corals because larvae are provisioned with photosynthetic symbionts. This energy source can extend the larval period and corals because larvae are provisioned with photosynthetic symbionts. These results represent the first data on reproduction and dispersal in a mangrove coral population. Recent studies suggest mangroves may be critical to the survival of many coral species by providing an ecological refuge during periods of extreme heat and ultraviolet radiation [42-44] and/or by promoting the evolution of resilient individuals [42,45]. Documenting the asexual production of larvae and their predominantly local dispersal helps to explain the 4-year stability of this population [21]. It also suggests that the movement of recruits between spatially isolated habitats, such as reef habitats and mangroves, might be more limited. Future work investigating dispersal patterns between this population and nearby *P. divaricata* populations in other habitat types can reveal potential patterns of gene flow as well as provide insight into the colonization history of this population. Additionally, future studies should investigate dispersal patterns in other mangrove-dwelling coral species with different life-history strategies and reproductive modes, while also sampling over larger spatial scales to estimate the potential for larvae to disperse between habitats. Further, methods developed here could be applied to other populations of reef-building corals, providing insights into modes of reproduction and how this might influence genetic diversity, which will facilitate optimization of coral reef restoration projects.

### 3. Materials and methods

#### (a) Study site

This study was conducted in a mangrove channel at Calabash Caye, located approximately 33 km off the coast of Belize within the Turneffe Atoll Marine Reserve (figure 1). Calabash Channel is a tidal channel fringed by red mangroves (*Rhizophora mangle*). Previous research revealed a dense population of *Porites divaricata* growing on the submerged prop-roots lining the channel [20]. Longitudinal monitoring of this population has shown it to be stable and slightly increasing in size over the monitoring period, with colonies spanning a wide range of sizes and ages [21]. The roots occupied by corals are suspended over a mud/silt bottom in approximately 1–2 m of water (figure 1c). During exhaustive field surveys, dense concentrations of coral colonies were located and tagged along three discontinuous sections of the shoreline (figure 1b). Other stretches of shoreline were largely devoid of *P. divaricata* colonies, with few scattered colonies found on the roots, in comparison to the dense clustering of colonies along the sampled shorelines. No colonies were ever observed in the two mangrove ponds despite repeated surveys (figure 1b). The population is located approximately 1.5 km from the nearest known reef population of *P. divaricata*.

#### (b) Field tissue sampling

In 2018, tissue samples were collected from 141 spatially discrete *P. divaricata* colonies growing on mangrove roots in Calabash Channel (figure 1b). Samples were collected from individuals known to have recruited into the population in (i) 2018, (ii) 2017, (iii) 2016 or (iv) 2015 and prior (table 1). Colonies sampled included larger, branched individuals and smaller, unbranched individuals (recruits). The smallest colonies were not collected to avoid lethal sampling. For branched individuals, 1–2 branch
fragments (approx. 2-3 cm) were collected from each discrete colony, and for encrusting individuals, 1-2 small fragments were chiselled from the edge of the colony. All fragments were immediately placed in 100% ETOH, and upon return to Calabash Caye Field Station were frozen until DNA isolation.

(c) 2bRAD sequencing
Genomic DNA was isolated using the Qiagen DNeasy Blood and Tissue sample kit [46]. Anonymous genomic loci were obtained for each individual using the 2bRAD sequencing approach designed by Wang et al. 2012 [23]. During library preparation, technical replicates were performed by producing two libraries from each of nine samples. Libraries were sequenced on the Illumina HiSeq 2500 platform (single end 50 bp) at The Tufts University Core Facility.

Raw reads were deduplicated, separated into individual bar-coded samples, trimmed of adapters and quality-filtered with cutadapt to remove both low-quality scores (less than 15) and short read lengths (less than 36 bp). The deduplicated, trimmed, filtered reads were deposited in the Short Read Archive at NCBI (https://www.ncbi.nlm.nih.gov/sra/PRJNA836675).

(d) Read mapping
The processed sequencing reads were mapped to the Porites lutea genome, the most closely related species to P. divaricata with a sequenced genome (downloaded at: https://plut.reefgenomics.org/download/) using the local alignment option of bowtie2 and the following parameters: a seed length of 16, a minimum alignment score of 16 + 1 * x, where x = read length, and a disposal of unaligned reads. This yielded an average mapping rate of 42%. Reads were also mapped to a de novo assembly produced from trimmed reads following the guidelines provided in the 2bRAD denovo github repository (https://github.com/z00n/2bRAD_denovo). This yielded an average mapping rate of 86%.

(e) Identification of single-nucleotide polymorphisms and genotyping
Single-nucleotide polymorphisms were identified by ANGSD [47]. Using the reads mapped to the P. lutea genome as well as the reads mapped to the de novo assembly, we identified SNPs using four different sets of ANGSD parameters, for a total of eight different approaches (electronic supplementary material, table S1). First, following Manzello et al. [48], we used default parameters for ANGSD, which include a minimum read depth of 1. Second, following a recent study by Sturm et al. [49], which also used a minimum read depth of 1, we specified the following conditions: remove tri-allelic SNPs; base quality score greater than 25; minimum p-value for deviation from Hardy Weinberg equilibrium = 10^{-5}; minimum p-value for a variable locus = 10^{-5}; minimum p-value for strand bias = 10^{-5}. Third, following a study by Fiser et al. [50], we required a minimum of five sequencing reads to score the genotype of an individual at each SNP and specified a minimum p-value for heterozygosity bias of 10^{-5}. In our final set of ANGSD parameters, we required a minimum of eight sequencing reads to score the genotype of an individual at each SNP and set the minimum p-value for a variable locus to 10^{-6}. Additionally, if a sample did not reach these thresholds for a particular SNP, and if the SNP could not be genotyped in at least 80% of individual samples, the genotype was scored as missing. We conducted the same four analyses to identify SNPs and call genotypes on samples mapped to the de novo assembly (electronic supplementary material, table S1).

(f) Identification of clones
Two different methods were used to identify clones from the eight ANGSD outputs: IBS and genetic distance. IBS is the proportion of times that two reads at the same locus are identical between individuals [48]. The resulting IBS matrices containing pairwise IBS distances between individuals were imported into R, clustered using the hclust() function using the ‘average’ agglomeration method of the R package stats v.4.0.1. Distance trees were then generated to visually assess the placement of individuals (electronic supplementary material, figure S1). The greatest IBS distance between technical replicates was used as the threshold for clonality. IBS is popular in previous 2bRAD studies because it is robust to variation in sequencing coverage among individuals [48]. The rationale for using IBS to identify clones is that clones are more likely to exhibit ‘identity by state’ than non-clones across a collection of SNPs. However, IBS was not expressly intended for identifying clones, and it can be positively misleading at loci where the clonemates are heterozygous, because, at such sites, despite sharing the identical genotype, IBS will be only 50% between clonemates. IBS would also be 50% between non-clonemates at a locus that differs in genotype, if one individual is heterozygous and the other is homozygous. For that reason, we also employed genetic distance (Prevosti’s) between individuals based upon their genotypes at SNPs, as in prior studies [51]. The R package poppr v.2.8.5 [24] was used to cluster genotypes and identify clones. Using the ANGSD vcf outputs with called genotypes, Prevosti’s absolute genetic distance was calculated between all pairs of individuals using the dist.diss() function [52]. The function mlg.filter() identified the presence of clones in the population by using the highest fraction of allelic differences observed between technical replicates as the threshold of clonality. A list of the individuals in the population collapsed into clonal groups was provided by the function mlg.id(). Distance trees were also generated by clustering individuals using the ‘average’ agglomeration method of the hclust() function (electronic supplementary material, figure S2).

All four SNP calling methods identified the same 10 clonal lineages with nearly identical clonal membership when mapped to either the P. lutea genome or de novo assembly of RAD tags, and when using IBS or genetic distances (electronic supplementary material, table S2). All remaining analyses on the ANGSD output were performed using the highest confidence 118 SNPs (i.e. the ‘hard-called’ SNPs identified after mapping the sequencing reads to the P. lutea genome). To confirm that these SNPs were sufficient to discriminate individuals present in this analysis, we produced a genotype accumulation curve using the R function genoaccum() (electronic supplementary material, figure S2).

(g) Determination of relatedness
We calculated the pairwise relatedness coefficient (r_{ab}) between all individuals with NgsRelate v.2 [55], using the estimated population allele frequencies and genotype likelihoods generated from the ANGSD output (using option -dogff 3) [56]. We estimated pairwise relatedness (r_{ab}) at each node in the distance tree by taking the average r_{ab} of all individuals descended from each node.
(h) Estimating dispersal distances
Within each clonal group, putative parents and offspring were identified based upon the year each colony first appeared during exhaustive surveys of the population conducted from 2015 to 2018 [21]. The geodesic distance—the shortest path between two points along earth’s surface—between each possible parent–offspring pair was then computed using Vincenty’s formulae [57] from the GPS coordinates obtained in the field [21]. The calculated distances did not account for differences in depth, e.g. two colonies with identical GPS coordinates but located at different depths would be scored as having distance equal to zero. The distribution of potential dispersal distances was estimated under four different assumptions: (i) minimum possible distance, (ii) maximum possible distance, (iii) median distance and (iv) mean distance between possible parent–offspring pairs. A density function was then fitted to each distribution based on Akaike’s information criterion using the package fitdistplus [58] (figure 3).

(i) Testing alternative dispersal models
To simplify the simulation of dispersal distances, a common line was used to represent the relative location of all colonies. The line was drawn through the points representing the GPS location of all colonies using least-squares regression. Each colony’s location was then transposed onto this line at the point representing the shortest distance from the line to the colony. For each observed dispersal event that occurred within a clonal group in a given year (2015, 2016 and 2017), the four alternative probability density functions representing the four dispersal distance models (exponential/minimum distance, Weibull/maximum distance, gamma/mean distance and lognormal/median distance; figure 3) were used to generate expected dispersal events along the line as follows. First, a parent colony was chosen at random in each clonal group from all potential parents in a given year. For a given dispersal curve (figure 3), a random larval dispersal distance was drawn and randomly multiplied by 1 or −1 to determine the direction of dispersal. This distance represented displacement of the larva from its parent along the line, i.e. the location of the new recruit. This process was repeated for each clonal group using each dispersal curve until the number of simulated dispersal events equalled the number of observed recruits in a given year. Each year, the number of potential parents increased as colonies from the year prior were presumed to be potential parents of larvae (table 1). After all the locations of the new recruits were generated, the distances between clone mates were calculated. Clone mates are defined as colonies in the same clonal group that recruited to the population in the same year. This entire process was repeated one hundred times for each alternative dispersal curve. Finally, the expected distances between clone mates were compared to the observed (i.e. empirical) distances between clone mates using alternative goodness of fit tests including k sample Anderson–Darling and two-sample Kolmogorov–Smirnov tests. A k sample Anderson–Darling test was conducted using the ad.test function from the R package epanechnikov v. 1.2-9 [59,60]. Two-sample Kolmogorov–Smirnov tests were also conducted using the ks.boot function in the R package Matching v. 4.9.11 [61]. A bootstrap p-value was obtained from 10,000 bootstraps. These simulations were only conducted for clonal groups that contained at least two or more clonemates within a cohort: Group A, Group B, Group C, Group D and Group I.

Ethics. The research was conducted under Aquatic Scientific Research Permit #0041-18 (2018) issued by the Belize Fisheries Department.

Data accessibility. The deduplicated, trimmed, filtered DNA sequencing reads generated in this study were deposited in the Short Read Archive at NCBI (https://www.ncbi.nlm.nih.gov/sra/?term=PRJNAS33675). The data are provided in the electronic supplementary material [62].

Authors’ contributions. K.S.L.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, writing—original draft and writing—review and editing; K.C.L.: investigation and writing—review and editing; P.M.B.: formal analysis and writing—review and editing; S.W.D.: investigation and writing—review and editing; C.C.D.: formal analysis and writing—review and editing; J.R.F.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, supervision, writing—original draft and writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. The authors declare that they have no competing interests.

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References


