

COMMENT

Visual discernment of sexual recruits is not feasible for *Acropora palmata*

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ABSTRACT: In fragmenting corals, estimation of population structure, extinction risk, and recovery potential requires accurate assessment of the relative contribution of sexual versus asexual reproduction. This yields an operational tendency for field ecologists to surmise levels of sexual recruitment from visual surveys, as in a recent study by Grober-Dunsmore et al. (2006; Mar Ecol Prog Ser 321:123–132). The recent development of microsatellite markers for threatened elkhorn coral *Acropora palmata* allowed us to test the accuracy of such visual assessments in 2 separate populations, showing them to be highly unreliable. Therefore, for clonal species that rely heavily on fragmentation, extreme caution is required in determining levels of sexual recruitment or recovery potential in the absence of molecular genetic screening.

KEY WORDS: Microsatellites · Clones · Acroporid coral · Caribbean Sea · Genetic screening · Monitoring

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Assessing population status of fragmenting corals

A recent study by Grober-Dunsmore et al. (2006) has provided a comprehensive characterization of the population status of threatened elkhorn coral *Acropora palmata* stands around St. John, US Virgin Islands. These data on patterns of colony abundance, size structure, and threats contribute to understanding species status, and for planning possible proactive recovery actions. Similar studies are needed throughout the species' range. However, we have concerns regarding the interpretation of the survey results by Grober-Dunsmore et al. (2006), specifically in inferring higher levels of sexual (than asexual) recruitment in the populations surveyed. They base this inference on change in size structure of the population, and the visual appearance of individual colonies. Moreover, Grober-Dunsmore et al. (2006, p. 128) state that '*Acropora palmata* populations

around the island of St. John show signs of recovery, as evidenced by the significant increase in total abundance of colonies and abundance of new recruits from 2001 to 2003'.

Population recovery for a clonal species cannot be inferred from surveys of colony abundance and size structure, because a population of few large colonies can suffer high rates of partial mortality, and thus result in the same local population (i.e. in terms of genotype richness) consisting of a much larger number of small colonies. More large and small colonies are necessary, but not sufficient, to achieve population recovery for a clonal species such as *Acropora palmata*, as genotypic diversity must be considered and the actual recruitment of new colonies (be they sexual or asexual) must be documented and distinguished from remnant colonies.

Ecologists and conservation managers share a need to understand the sexual versus asexual contribution to

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overall recruitment and population status of clonal species, particularly in threatened ones such as *Acropora palmata*. For most sessile marine invertebrates, sexual recruitment occurs via the settlement and metamorphosis of a small (often microscopic) pelagic larva. While some sessile invertebrates also produce larvae of asexual origin, asexual recruitment by fragmentation may be a dominant mode of reproduction for branching corals (Highsmith 1982). Scientific consensus (summarized in Bruckner 2003) is that reproduction in the Caribbean reef-building corals *A. palmata* and *A. cervicornis* follows this pattern: reproduction is predominantly asexual, because larvae of these species are nearly absent in studies on coral settlement using settlement plates (Bak & Engel 1979, Rogers et al. 1984, Quinn & Kojis 2005).

In recent years, there have been multiple field reports, often anecdotal (including our own), of new juvenile *Acropora palmata* appearing on reefs. These juveniles have been explicitly or implicitly attributed to larval recruitment (Jordan-Dahlgren 1992, Jaap 1998, Rogers et al. 2003, Grober-Dunsmore et al. 2006, M. W. Miller pers. obs.). In these examples, discrimination between sexual recruits (derived from planulae) and asexual recruits (reattached fragments) is based solely on growth position or morphology (Highsmith 1982, Rosesmythe 1984, M. W. Miller pers. obs.). Colonies with characteristics such as disc-shaped encrustation at the colony base, a colony base positioned on flat, smooth (i.e. no visible traces of a fragment) or a sloping or vertical substrate, symmetrical branch(es) yielding a bowl-shaped colony, or distant position from adjacent colonies are commonly assumed to be sexual recruits (e.g. Fig. 1A,B).

Field test of visual versus molecular screening

Molecular tools have only recently become available to distinguish genets of *Acropora palmata*. Colonies that share multilocus genotypes (ramets) are asexual offspring of a common 'mother colony' that settled as a sexual recruit at some time in the past. Sometimes, multilocus genotypes are observed only once in a population, indicating that the colony is either a recent sexual recruit or the lone survivor of a previously common genet. These tools allow us to test the hypothesis that local populations with many small colonies showing signs of sexual origin are indicative of high genotypic diversity (i.e. a high number of distinct multilocus genotypes), by comparing visual assessments of putative sexual recruits with results of genotyping. We have undertaken such a test by sampling 2 populations of *Acropora palmata*. These populations were composed of isolated colonies that were easily designated

as separate modules (i.e. not thickets) and had noticeably increased in abundance over the previous 2 years (authors' unpubl. data). These sites were Northwest Point in Navassa Island National Wildlife Refuge, situated in the Jamaica Channel off Haiti (Navassa, 18° 24.81' N, 75° 01.77' W), and Marker 3 Reef in Biscayne National Park, Florida, USA (BNP, 25° 22.41' N, 80° 09.62' W). The *A. palmata* population at BNP, particularly, displayed groups of similar-sized bowl-shaped individuals in at least 2 noticeable 'cohorts' with ~20 cm (Fig. 1B) and ~60 cm height (Fig. 1C), suggesting the possibility of distinct sexual recruitment events.

At each site, we placed a reference stake near the center of the *Acropora palmata* patch and used a transect tape and compass to map and sample all the colonies in a certain sector of the circle centered on that stake. At BNP we sampled all the colonies ($n = 64$) in a 75° sector between 225° and 300° bearing from the stake, extending to a distance of 45 m. Grober-Dunsmore et al. (2006) suggested that higher levels of genotypic diversity would be observed if sampling were 'restricted to small colonies' (p. 129). To address this concern, we searched this entire patch to locate the smallest colonies that we could find (2 to 15 cm diameter) occurring on base reef substrate (rather than on standing dead elkhorn skeletons) and sampled an additional 13 colonies (total $n = 77$). The Navassa population was sparser and we sampled all colonies ($n = 35$) in a 200° sector extending to 47 m distance from the reference stake.

Each colony was measured (length, width, and height) to the nearest 10 cm, photographed, and a small sample (<2 cm in length) was snipped from a branch tip or chiseled from the edge of the tissue in the case of unbranched crusts. At least 2 of the authors conservatively scored smaller colonies (<50 cm largest diameter) as to whether they appeared to be of sexual (i.e. larval) origin: these small colonies were presumed to be of asexual origin, unless they displayed obvious characteristics suggestive of larval origin (e.g. encrusting colonies, symmetrical encrusting base with no indication of an antecedent fragment, orientation of the substrate). Navassa colonies were scored from photographs, while BNP colonies were scored *in situ* at the time of sampling. The tissue samples were preserved and genotyped using the protocols and microsatellite markers described in Baums et al. (2005).

For mounding corals, population size frequency distributions can provide some reliable indication of demographic processes such as episodic recruitment events or patterns of juvenile mortality (Bak & Meesters 1998, Vermeij & Bak 2002). Therefore, we also constructed size frequency distributions for the

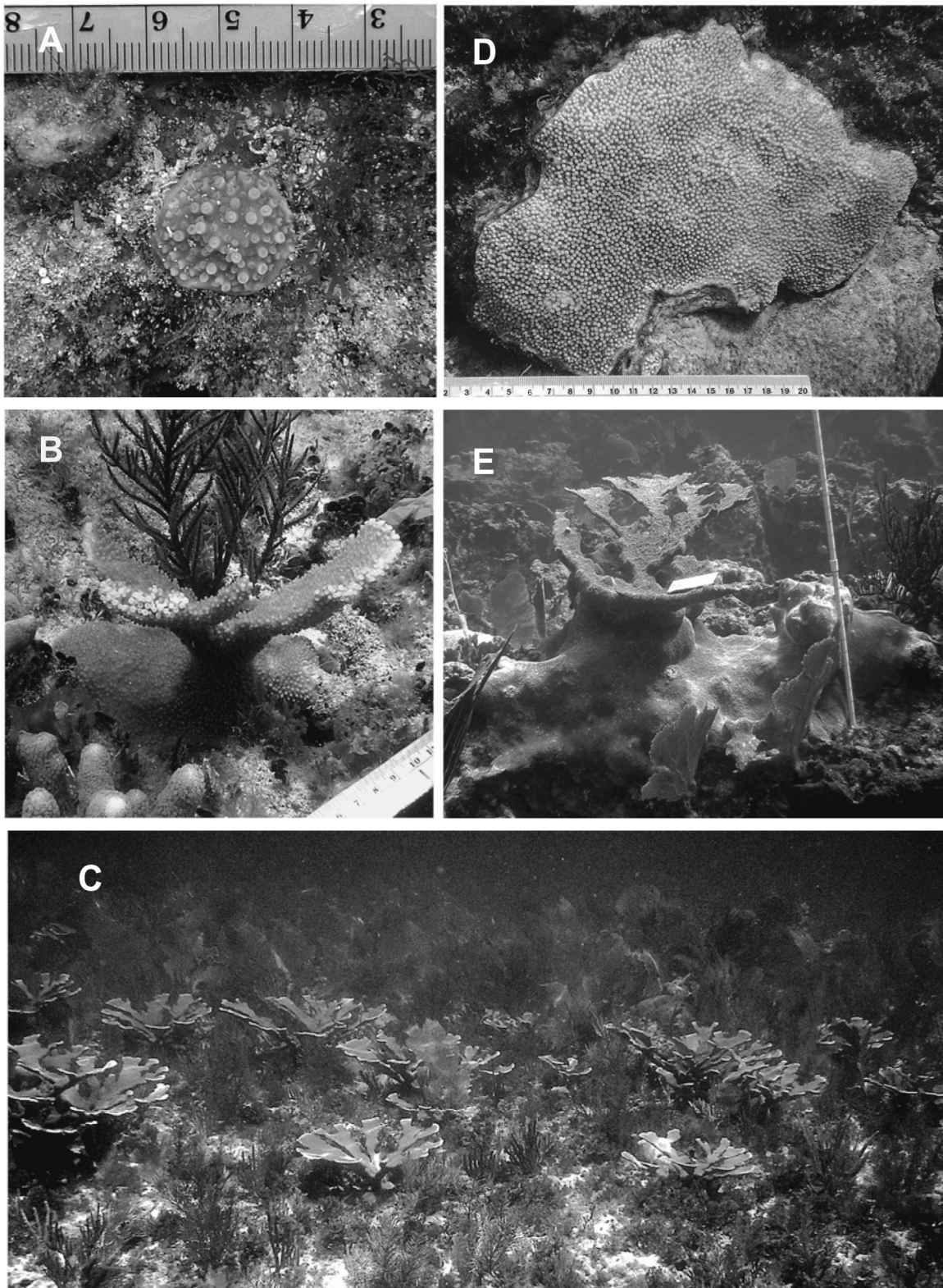


Fig. 1. *Acropora palmata*. Representative colonies sampled at: (A–C) Marker 3 Reef in Biscayne National Park (BNP), and (D,E) Northwest Point in Navassa. (C) Occurrence of many similar-sized and -shaped colonies suggests the presence of a cohort resulting from a single recruitment event; in fact, all colonies sampled at BNP were of a single genotype (asexual origin), while all colonies sampled at Navassa had distinct genotypes

sampled colonies (based on maximum colony diameter) to examine population structure. Only the original 64 colonies (the fully sampled segment of the population) were used in the size frequency distribution for the BNP site; the subsequently targeted small colonies were not included.

Both small crusts (2 to 15 cm, Fig. 1A,D) and larger 'bowl shaped' branching colonies (Fig. 1C) were observed at the 2 sites. Generally, small, 'single antler' colonies (still with substantial encrustation at the base) were observed only at BNP (Fig. 1B), while large, predominantly encrusting colonies were common at Navassa (Fig. 1E). Our visual assessment conservatively attributed about one-third of all colonies sampled at each site as being of larval (i.e. sexual) origin: 10 of 36 (27%) for Navassa, and 23 of 64 (36%) for BNP; higher proportions were estimated when considering only colonies <50 cm diameter (85% for Navassa, where most colonies are encrusting, and 42% for BNP). Also, the size frequency distributions of both sites were similar (Fig. 2, $p > 0.1$, Kolmogorov-Smirnov test). However, the 2 populations had radically different clonal structure. In the Navassa population, each colony sampled was a distinct genet (Baums et al. 2006), from which we infer each was of sexual origin (or a single asexual offspring of a sexually recruited colony which had since died). In contrast, the BNP population was monoclonal (Baums et al. 2006); all 77 colonies sampled, including crusts as small as 2 cm diameter, were genetically identical, and hence all colonies (or all but one) were asexual in origin.

Hence, our visual assessment provided a highly misleading estimate of the relative importance of sexual

recruitment, and thus of the genotypic diversity at these sites. The population size frequency distributions, being similar, also offered no hint that the population structure at the 2 sites could be radically distinct. Reliable assessment of current sexual recruitment in an existing population of *Acropora palmata*, let alone its contribution relative to fragmentation, is possible only via molecular genetic analysis of the clonal structure.

The microsatellite markers used for genotyping in this study have a probability of identity (i.e. estimated probability that 2 samples share identical multilocus genotypes by chance, rather than by descent) of 7.07×10^{-7} (Baums et al. 2005). Hence, our finding that all colonies at BNP were asexually derived is accompanied by a high degree of confidence. While fragmentation is known to be a dominant mode of reproduction in *Acropora palmata*, some additional, yet undescribed, mode of asexual propagation may account for the low genotypic richness at BNP.

Implications

These results dictate extreme caution in inferring *Acropora* spp. sexual recruitment from field observation of established juveniles. Williams & Miller (in press) have documented how fragmentation can yield small *A. cervicornis* without traces of their asexual origin. Similar processes have been observed in *A. palmata* (D. E. Williams unpubl. obs.). Although larvae are likely the only source of recruits in locations where *A. palmata* has been extirpated, local extirpations have not been well documented (Bruckner 2002). Even in locations where *A. palmata* has been drastically reduced in abundance, small remnant crusts may persist, so the sexual origin of small, apparently new, colonies should not be presumed.

Sexual recruitment can only be confidently identified in the field by the appearance of a small colony in a specific area of benthos where frequent monitoring previously documented absence. However, this requires finer spatial and temporal resolution of observations than is typical of most reef monitoring activities. We urge that molecular genotyping should be incorporated into assessment or recovery monitoring schemes for *Acropora palmata* and other fragmenting marine clonal invertebrates, in order to obtain a realistic understanding of population processes. Inference of population 'recovery status' based solely on abundance and size structure of clonal species (despite the appearance of many small colonies) should be avoided, as these parameters cannot give insight into genotypic diversity, which is a quintessential component of recovery.

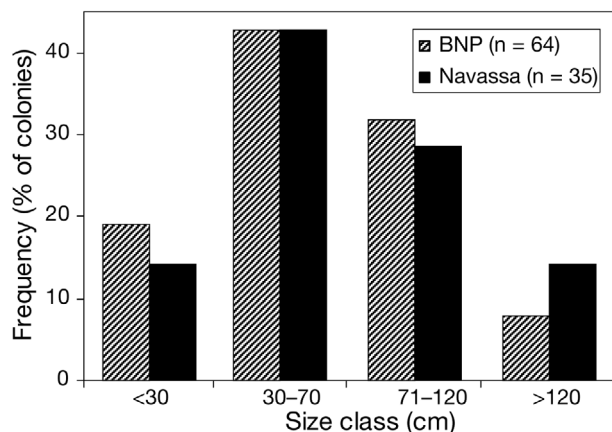


Fig. 2. *Acropora palmata*. Size-frequency distributions for colonies sampled at each site based on the largest diameter measurement for each colony, and binned as in Grober-Dunsmore et al. (2006). These 2 distributions do not differ statistically ($p > 0.1$)

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