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Ecological, genetic, and morphological differences among three *Pavona* (Cnidaria: Anthozoa) species from the Pacific coast of Panama

I. *P. varians*, *P. chiriqiensis*, and *P. frondifera*

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Abstract Ecological, genetic, and morphological differences among three Panamanian Pacific *Pavona* species with strongly developed collines (*Pavona varians*, *P. frondifera*, and *P. chiriquiensis*) were examined. Ecological factors included geographical distributions of species, habitat preferences, interspecific interactions, reproductive ecology, and tolerance to bleaching. Genetic differences were based on the electrophoretic analysis of ten allozyme loci. Morphological analyses consisted of tissue coloration, colony morphology, and measurements and counts of ten macro- and microskeletal characters. *P. varians*, present on reefs or in coral communities, is the most widely distributed and shows considerable morphological variation. *P. chiriquiensis*, a recently described species, encrusts basalt rock and has little morphological variation. *P. frondifera* is a reef dweller with a compact foliose morphology. Tissue coloration varies from light to dark brown in *P. varians*, from pink to brown in *P. frondifera*, and from brick red to brown or silvery in *Pavona chiriquiensis*. Also, the white to silvery polyp mouths of the latter species are a diagnostic feature that allows an easy identification in the field. Aggressive dominance during short-term interspecific interactions were as follows: *Pavona chiriquiensis* > *P. varians* > *P. frondifera*. *P. chiriquiensis* and *P. varians* showed contrasting responses to sea warming during the 1997–1998 El Niño Southern Oscillation. Whereas entire *P. chiriquiensis* bleached and died within 4 weeks of exposure to 30–31°C, colonies of *P. varians* did so only on their upper surfaces. The response of *P. frondifera* to elevated temperatures was not observed because it is mainly present in the Gulf of Panama where coral bleaching was absent in 1997–1998. The genetic data indicated that *P. chiriquiensis* differed strongly from both *P. varians* and *P. frondifera*, with Nei's unbiased genetic distances of 0.434 and 0.379, respectively. A fixed difference between *P. varians* and *P. frondifera*, and *P. chiriquiensis* exists at the triose phosphate isomerase (*TPI-2*) locus. A nearly fixed difference between *P. chiriquiensis* and *P. frondifera* and between *P. chiriquiensis* and *P. varians* was found at the hexokinase (*HK*) locus. *P. varians* differed slightly from *P. frondifera* with Nei's unbiased genetic distance of 0.068. No fixed difference was found between *P. varians* and *P. frondifera*. There were strong differences between *P. chiriquiensis* and *P. varians* in spawning times and gamete characteristics. Spawning in *P. varians* and *P. chiriquiensis* is 12 h out of phase. Also, eggs of the former species are white to beige and positively buoyant whereas those of the latter species are dark green and neutrally to negatively buoyant. No reproductive data are yet available for *P. frondifera*. Calicular diameters are significantly greater in *P. chiriquiensis* than in the other two species. In contrast, corallum thickness is greater in *P. varians* and *P. frondifera* than in *P. chiriquiensis*. Canonical discriminant function analysis readily separated the three species.

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Introduction

Pavona varians Verrill, 1864 (Verrill 1864); *P. chiriquiensis* Glynn, Maté, and Stemann, 2001 (Glynn et al. 2001b); and *P. frondifera* Lamarck, 1816 (Lamarck 1816) are similar members of the genus on the Pacific coast of Panama. In addition to their often small size, they differ from other Panamanian *Pavona* by their strong development of collines. *P. varians* and *P. frondifera* are distributed throughout the Indo-Pacific region

(Dana 1846; Dai and Lin 1992; Veron 1993). However, *P. chiriquiensis* is presumed to be endemic to the eastern tropical Pacific (Glynn et al. 2001b). In the eastern Pacific, *P. varians* is the most widespread species. It has been found at 10 of 11 (90.9%) major sites examined (Glynn and Ault 2000). The other two species are less common, with *P. chiriquiensis* present at 6 sites (54.5%) and *P. frondifera* present at just 2 sites (18.2%). In this region, *P. frondifera* is only found in Costa Rica (Cortés and Guzmán 1998) and Panama (Glynn and Ault 2000). All three species have been shown to form locally abundant populations, but their contributions to reef building are typically relatively low (Glynn 1974a, b, 1976; Glynn et al. 1982; Glynn and Wellington 1983; Guzmán and Cortés 1989; Vargas-Ángel 1996), particularly for *P. chiriquiensis*, which is usually found at inter-reef sites.

These three species of *Pavona* are considered the most taxonomically difficult *Pavona* in the Panamanian Pacific. *P. varians*, as the name implies, shows considerable morphological variation that may be closely related to variation in the environmental conditions resulting from the wide habitat preferences of the species (Glynn 1976, 1983; Veron and Pichon 1979). Extreme variation in morphology may even occur within the same colony (Glynn and Wellington 1983). These extremes in morphological variation may cause this species to be confused with *P. chiriquiensis* or *P. frondifera* in certain environments. *P. chiriquiensis* was considered for many years to be a morphological variant of *P. varians* and later was recognized as a separate species (Glynn et al. 2000). However, *P. chiriquiensis* has recently been described in terms of multiple differences in ecology, allozymes, and morphological characteristics that clearly set it apart from *P. varians* (Glynn et al. 2001b). Although *P. frondifera* has been recognized from the Pacific coast of Panama (Glynn and Maté 1997), it has been poorly studied. In many cases, the species status has been impossible to validate because of its morphological similarities with *P. varians*. Additionally, *P. frondifera* is a rare species that in Panama is restricted to only a few sites.

The recognition of species boundaries is a necessary step to understand the biogeographic and ecological distribution of organisms, as well as the mechanisms by which speciation occurs (McFadden 1999). Traditionally, scleractinian coral species have been classified primarily on the basis of their skeleton, since this is readily available in museum collections (Vaughan and Wells 1943; Potts et al. 1993). However, the high levels of polymorphism attributed to local environmental conditions or genetic differences among populations are among the most difficult problems confronting coral systematists (Budd 1993; Weil and Knowlton 1994). The use of non-skeletal characteristics to circumvent problems arising from this classical approach has figured prominently in the characterization of only a few scleractinian species (Lang 1984). Molecular studies have recently proved useful in delineating species boundaries, particularly in marine species with few informative morphological characteristics and high intraspecific phenotypic diversity (e.g. Avise 1974, 1994; Weil and Knowlton 1994; Williams 2000; Glynn et al. 2001b). In sympatry, fixed genetic differences are enough. But sometimes genetic differences are not fixed. Then, other characters are especially useful (e.g. *Montastraea*) when concordant. Multi-character approaches have been successfully used to separate problematic coral species, for example, two *Acropora* species on the Great Barrier Reef by Ayre et al. (1991), three *Montastraea* species from the Caribbean by Knowlton et al. (1992) and Weil and Knowlton (1994), four Pacific *Porites* species by Potts and Garthwaite (1991), and four Caribbean *Porites* species by Potts et al. (1993).

In this study, I examined the differences among three *Pavona* species with collines from the Pacific coast of Panama by means of a multi-character approach that united the ecological, molecular, and morphological characteristics. The ecological aspects of the study included species distributions, habitat preferences, interspecific interactions, reproductive ecology, and responses to the 1997–1998 El Niño sea-warming event. Allozyme electrophoresis was used to examine the genetic differences between species because of its high resolving power with closely related species (Avise 1974, 1994). The morphological analyses included colony form, tissue coloration, and the traditional macro- and micro-morphological characteristics of the coral skeleton.

Materials and methods

Collections and sample preparation

Samples of *Pavona varians*, *P. chiriquiensis*, and *P. frondifera* (Fig. 1A–C, respectively) were collected at several sites in both the non-upwelling Gulf of Chiriquí and the upwelling Gulf of Panama on the Pacific coast of Panama (Table 1, Fig. 2A–D). Forty-four sites, including reef and inter-reef habitats, were surveyed in both areas. During diving surveys information on habitat, depth, and colony coloration was recorded for sampled specimens. Large coral fragments (up to 735 cm^2) were collected when possible to allow for a morphological analysis after the required tissue for electrophoresis was taken. The size of fragments removed was adjusted to minimize colony death. Tissue samples for electrophoretic analysis were removed from the coral skeleton using flat-tip pliers or chisels, placed in cryovials, and mixed with five drops of Stoddart grinding buffer (Stoddart 1983) before being frozen in liquid nitrogen. The remainder of the coral skeleton was bleached in 10% sodium hypochlorite, rinsed, dried, catalogued, and stored until later morphological analyses. The identifications of *P. varians* and *P. frondifera* were performed according to the taxonomic treatments of Yabe et al. (1936); Veron and Pichon (1979); and Dai and Lin (1992), as well as the original descriptions by species authors (Lamarck 1816; Verrill 1864). The identification of *P. chiriquiensis* follows the species description (Glynn et al. 2001b). Most collections were made in early 1997, before the beginning of the El Niño event.

Interspecific tissue interactions

Field surveys were conducted to observe coral-to-coral interactions (Lang 1973) among *Pavona* species. Those *Pavona* species observed to interact in the field (*P. chiriquiensis* and *P. varians*) were brought to the laboratory for interaction studies. Fragments of two colonies

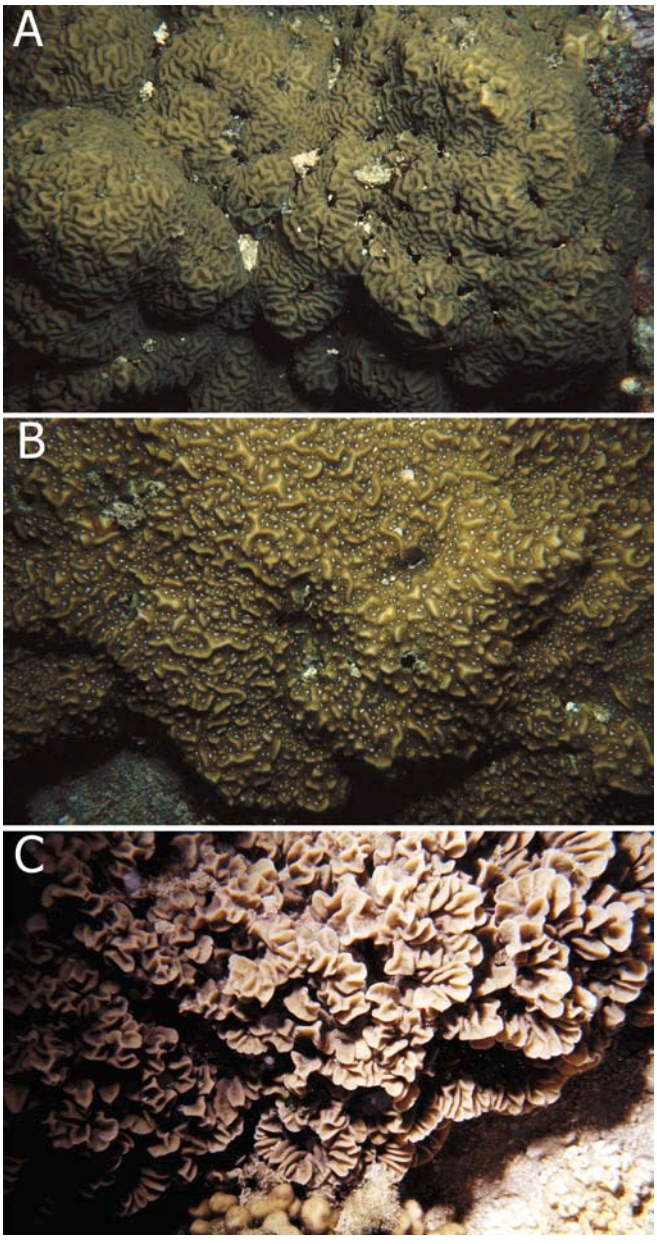

Fig. 1 Live in situ colonies of **A** *Pavona varians* (Uva Island, site 5, 3.2 m depth, May 1997); **B** *P. chiriquiensis* (Uva Island, site 5, 3 m depth, May 1997); and **C** *P. frondifera* (Saboga Island, site 1, 2.5 m depth, May 1999). Note *long ridges and valleys* and *uniform dark-brown coloration* of polyps and coenosarc in *P. varians* (**A**), *white oral discs and mouths*, *hydnophorae*, and *short collines* in *P. chiriquiensis* (**B**), and *long ridges and valleys*, *foliose morphology*, and *uniform light-brown coloration* of polyps and coenosarc in *P. frondifera* (**C**)

(from 2×2 cm to 10×15 cm) of the same or different species were placed in immediate contact so they barely touched. The contacts were observed daily for a maximum of 7 days and included observations on extension of mesenterial filaments, time of onset of the interaction, and extent of tissue mortality.

Reproductive ecology

The timing of spawning of *P. chiriquiensis* and *P. varians* was estimated from histological slides to occur around new and particularly full moons during the dry season that extends from mid-December to mid-May (Glynn et al. 2000). Detailed field and laboratory methodologies related to spawning observations and fertilization trials can be found in Glynn et al. (2000). No reproductive data are available for *P. frondifera*.

Coral bleaching and mortality

The 1997–1998 El Niño provided an opportunity to study the effects of anomalous sea warming on corals, particularly those species considered here. Warm waters from El Niño influenced only sites in the Gulf of Chiriquí. Sea temperatures were not unduly elevated in the upwelling Gulf of Panama. El Niño warming effects were monitored in *P. varians* and *P. chiriquiensis* in the Gulf of Chiriquí, but not in *P. frondifera* due to its rarity there. A flexible measuring tape calibrated in millimeters or a square wire mesh (2.5×2.5 cm) was used to measure the areas of normal, bleached, and dead tissues. Colonies were recorded as normal only when their natural brown coloration predominated. Bleached colonies exhibited loss of coloration (from pale to fully bleached). Dead areas were in most cases covered with filamentous algae. In some colonies it was possible to identify two or more categories. The percentage of each condition was recorded. In situ temperature recorders (Onset Computers) provided information on the timing of the two sea-warming events that led to the bleaching and mortality of corals (see Fig. 5 in Glynn et al. 2001a). The anomalous El Niño warming affected corals in two different periods, August 1997 and March 1998. Species affected by the first bleaching event were monitored monthly from September through December 1997. Effects from the second bleaching event and recovery were observed during March 1998, May 1999, and May 2000.

Electrophoretic analysis

Horizontal starch (SIGMA S-4501) gel electrophoresis was used to analyze seven enzymes coding for ten loci under two buffer systems. Enzymes, buffers, and running conditions are presented in Table 2. On the day of the electrophoresis run, a small portion of the tissue was placed on a grinding plate cell and macerated in three drops of Stoddart's buffer (Stoddart 1983). A piece of Miracloth filter (Calbiochem Inc.) was placed on top of the homogenate to reduce the amount of coral mucus that would adhere to the paper wicks (Whatman #3 filter paper), which were loaded onto starch gels and run at 4°C. After the electrophoretic run, gels were sectioned with a custom-designed "guitar-string" slicer. Zymograms were visualized using stain recipes modified by Williams (1992) and Weil and Weigt (1996) from Harris and Hopkinson (1976). Alleles were labeled alphabetically according to their mobility from fastest to slowest. For those enzymes having two loci, the loci were labeled numerically starting with the fastest migrating one. The BIOSYS-1 software package (Swofford and Selander 1989) was used to calculate gene frequencies and Nei's (1978) unbiased genetic distances (*D*) among samples and to perform an unweighted pair group method average (UPGMA) cluster analysis.

Morphometrics

Digital images of skeletal characters were generated using a Kodak DCS 420 digital camera fitted with a 1:1 SIGMA macro lens. Images were imported into the SigmaScanPro (SPSS, Inc.) image analysis package where linear measurements and counts were made on ten morphological characters (Table 3, Fig. 3). Characters were measured and counted on six corallites per colony, from which a colony mean was calculated. Ten colonies per locality were generally used in the analyses, except for those localities with fewer numbers of individuals (Table 1). The statistical packages Sigma Stat and SPSS (SPSS, Inc.) were used for all univariate and multivariate analyses, respectively. *Pavona* species groups were separated by discriminant canonical analysis that tested the hypothesis that the three groups were homogeneous.

Table 1 Locality, total number of colonies collected of each species (*n*), and depth ranges with reference to mean lower low water for each locality. See Fig. 2 for geographic location of sites by site number

Locality	Site	Species					
		Pavona chiriquiensis		*P. varians*		*P. frondifera*	
		n	Depth (m)	*n*	Depth (m)	*n*	Depth (m)
Gulf of Panama							
Saboga Island	1	1	6.7	58	5.0–9.0	40	3.5
Iguana Island	2	6	4.2	5	1.5–15.2	0	–
Gulf of Chiriquí							
Jicarita Island	3	6	4.5	2	4.5	0	–
Coiba Island	4	1	6.3	15	4.5–9.0	2	6.1
Uva Island	5	36	3.0–9.0	94	1.2–15.1	2	13.0
Secas Island	6	0	–	9	4.5–9.0	0	–
Silva de Afuera Island	7	5	16.7	5	15.1	0	–
Montuosa Island	8	7	6.6	9	5.1–16.7	0	–
Restinge Island	9	4	4.6	2	3.0	0	–

Results

Distribution, abundance, and habitat preference

All three *Pavona* species are found in both the Gulf of Panama (Fig. 2A–C) and the Gulf of Chiriquí (Fig. 2A, D). *P. varians* is the most widely distributed, being found at 46.7% of the sites (Fig. 4A). *P. chiriquiensis* and *P. frondifera* were found at 24.4% and 6.7% of the sites examined, respectively (Fig. 4B, C). Depth distributions demonstrate the overlapping occurrences of the three species (Table 1).

At Saboga Island (site 1, Fig. 2C) and Uva Island (site 5, Fig. 2D), *P. varians* is particularly abundant, with more than 100 colonies seen at each of these sites. The rocky shores of Uva Island and Jicarita Island (site 3, Fig. 2D) harbor the largest known populations of *P. chiriquiensis*.

P. varians has the broadest habitat preference of the three species. It lives free or attached to reef frameworks or to rocky substrates, in the rubble zone, and in soft bottom areas. It also occurs on open substrates, in crevices, caves, and overhangs. *P. chiriquiensis* typically encrusts basaltic outcrops with only four specimens being found in reef areas. *P. frondifera* has been found

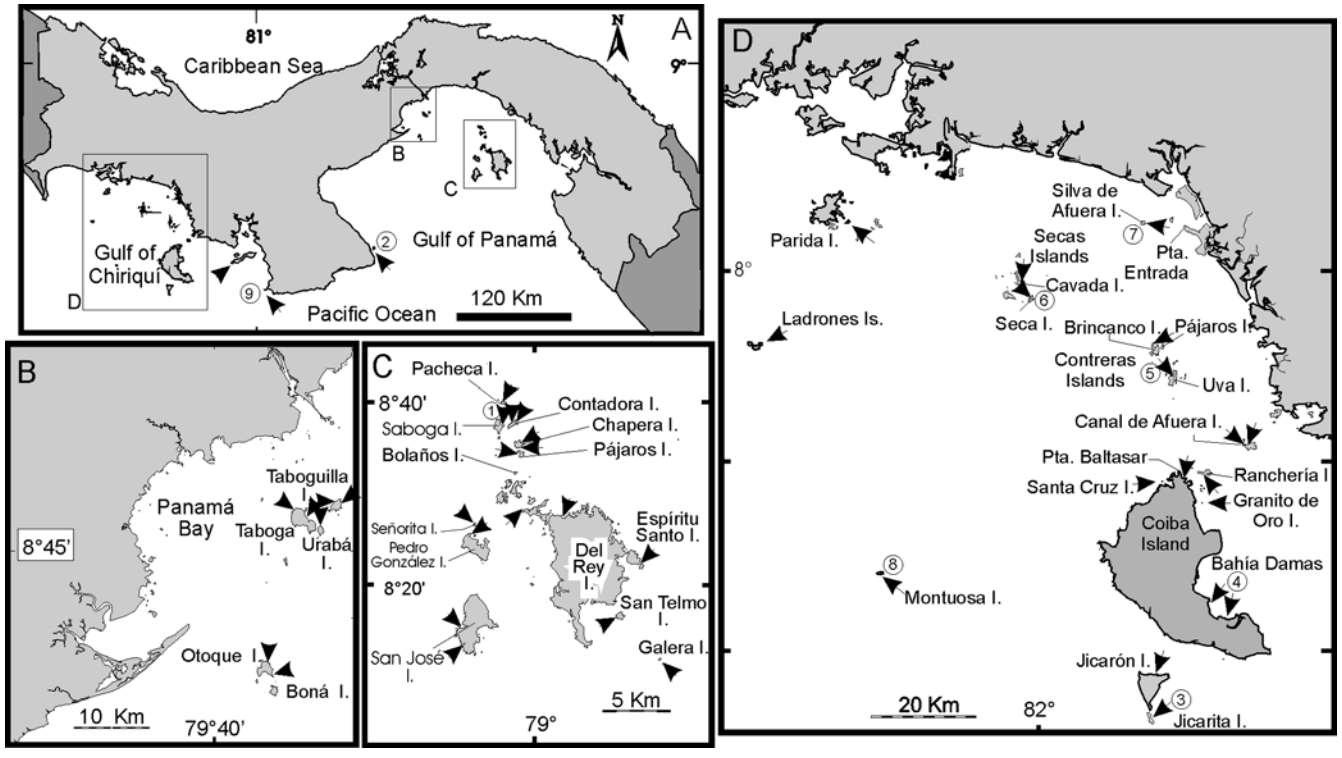

Fig. 2 **A** Map of Panama showing explored and collecting sites in detail; **B** Bay of Panama; **C** Pearl Islands; and **D** The central portion of the Gulf of Chiriquí. *Black arrows* point to the 44 sites explored during this study. Circled numbers in **A**, **C**, and **D** indicate collecting sites: *1* Saboga Island; *2* Iguana Island; *3* Jicarita Island; *4* Coiba Island; *5* Uva Island; *6* Secas Island; *7* Silva de Afuera Island; and *8* Montuosa Island

Table 2 Enzyme buffer systems employed in the electrophoretic analysis of ten putative loci in three *Pavona* species. *E.C.* Enzyme Commission

Enzyme	E.C. number	Number of loci	Buffer system
Glucose phosphate isomerase (*GPI*)	5.3.1.9	1	TC 8.0[a]
Glutamate dehydrogenase (*GTDH*)	1.4.1.2	2	TC 8.0
Hexokinase (*HK*)	2.7.1.1	1	LiOH 8-1-8.4[b]
Leucyl-proline peptidase (*LPP*)	3.4.11/13	1	LiOH 8-1-8.4
Leucyl-valine-peptidase (*LVP*)	3.4.11/13	2	LiOH 8-1-8.4
Phosphogluconate dehydrogenase (*PGDH*)	1.1.1.44	1	TC 8.0
Triose phosphate isomerase (*TPI*)	5.3.1.1	2	LiOH 8-1-8.4

[a] Tris citrate (TC 8.0), pH 8.0, 90 mA, 6–8 h (Selander et al. 1971)
[b] Tris citrate borate (LiOH), pH 8.4, 350 V, 4–6 h (Selander et al. 1971, modified by Harris and Hopkinson 1976)

Table 3 Macro- and micromorphometric measurements taken in three species of *Pavona* with strongly developed collines. See Fig. 3 for a graphic representation of most characters

Skeletal characters measured	Code	Description
Maximum calicular diameter	CD1	Maximum distance across the inside area of the corallite not including the walls
Minimum calicular diameter	CD2	Maximum distance across the inside area of the corallite not including the walls but perpendicular to the maximum calicular diameter
Main septa length	SL	Linear distance of the largest septa reaching the columella
Maximum columellar diameter	CL1	Maximum distance across the columella
Minimum columellar diameter	CL2	Maximum distance across the columella perpendicular to the columella maximum diameter
Colline width	CW	Linear distance across the flat top of the ridge
Number of septa	NS1	Count of the total number of septa
Number of septa reaching the columella	NS2	Count of the total number of septa that join the columella
Number of septocostae	SC	Count of colline septocostae in 1 mm
Corallum thickness	CT	Maximum distance from the base to the top of the corallum

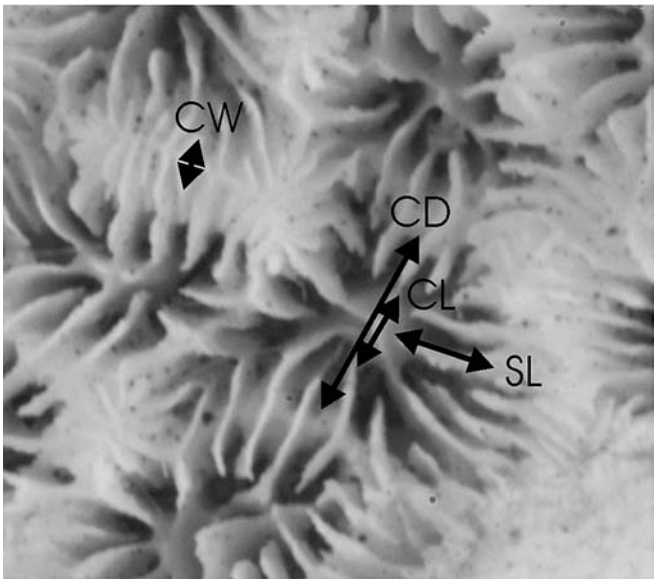

Fig. 3 Measurements made on individual corallites for morphometric analyses. *CD* Calicular diameter (measured as the maximum and minimum diameter); *CL* columella diameter (measured as the maximum and minimum diameter); *SL* septum length; and *CW* colline width

exclusively in reef rubble areas where it occurs mainly as attached colonies. Larger colonies of *P. frondifera* encrust substrata; smaller ones occur free or are lightly attached to the substrata.

P. varians may exist under weak to strong current regimes, but rarely in high-energy environments. *P. chiriquiensis* occurs on protected rock surfaces, not exposed to direct wave assault. *P. frondifera* occurs only in protected reef areas.

Polyp expansion and interspecific tissue interactions

None of the three *Pavona* species displays expanded polyps during the day except during periods of rapid water flow. In all species, polyps are usually extended at night and colony surfaces are ruffled.

Field contact interactions between colonies of *P. chiriquiensis* and *P. varians* at Uva Island ($n=1$) and at Jicarita Island ($n=2$) showed *P. varians* overgrowing *P. chiriquiensis* by overtopping with a free laminar edge. Laboratory contact experiments demonstrated unilateral extracoelenteric destructive effects by *P. chiriquiensis* (Table 4). Also, *P. varians* killed the tissues of *P. frondifera*. Stand-offs were evident between *P. chiriquiensis* and *P. varians*, as well as in all intraspecific contact trials (Table 4).

Coral bleaching and mortality

P. chiriquiensis was the most sensitive species to the 1997–1998 El Niño warming event. Uniform bleaching (100%) occurred in *P. chiriquiensis* approximately

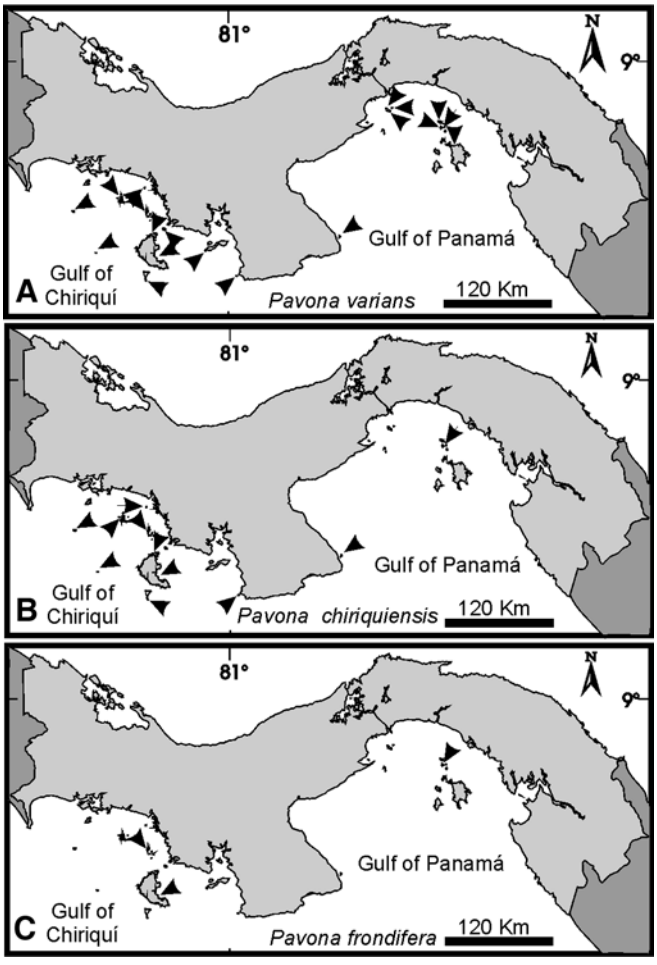

Fig. 4 Distributional maps of **A** *P. varians*; **B** *P. chiriquiensis*; and **C** *P. frondifera*. *Black arrows* indicate species presence at a site. See Fig. 2A–D and Table 1 for names of localities

3 weeks after the warming began. A month later, tissue mortality was 99%. However, once temperatures returned to ambient levels, surviving fragments had rapidly regained their normal coloration. This species recovered quickly. In contrast, *P. varians* underwent partial bleaching and mortality on its upper surfaces only. *P. varians* colonies with an encrusting growth form bleached in a similar way to *P. chiriquiensis*, but no mortality was evident. Mortality rates in *P. varians* were of the order of 1–15% of the live surface cover (see also Glynn et al. 2001a).

Reproductive ecology

Spawning in *P. varians* and *P. chiriquiensis* occurs in sympatry about 12 h apart during the crepuscular period on the same day. *P. varians* spawns 15 min to 2 h before sunrise whereas *P. chiriquiensis* spawns shortly after sunset. The white mouths of *P. chiriquiensis* become extremely distended prior to spawning. No evident change in mouth size was noted in *P. varians*. In *P. varians* and *P. chiriquiensis* sperm was released as a diffuse cloud. In *P. varians* the barely visible eggs, which are white to beige, are confined to mucus strings and rise slowly to the surface; in *P. chiriquiensis*, eggs are released as clouds, have a dark green color, and are neutrally to negatively buoyant (see Glynn et al. 2000).

Allozyme electrophoresis

All 40 *P. frondifera* colonies sampled at Saboga Island showed identical banding patterns and fixed heterozygosity at the *HK* locus. In *P. chiriquiensis*, there were 39 different multi-locus genotypes in 61 specimens examined (63.9%). In *P. varians*, there were 75 in 173 specimens examined (43.4%).

Eight enzymes were polymorphic (Table 5). The allozyme comparison of eight populations of *P. varians*, six populations of *P. chiriquiensis*, and three populations of *P. frondifera* indicated the distinctiveness of *P. chiriquiensis*. *P. chiriquiensis* differed strongly from both *P. varians* and *P. frondifera*, with Nei's unbiased genetic distances of 0.434 (6.40 m.y.a.) and 0.379 (5.57 m.y.a.), respectively. A fixed difference was found at the triose phosphate isomerase (*TPI-2*) locus (Table 5). Nearly fixed differences exist between *P. chiriquiensis* and the other two species at the hexokinase (*HK*), the phosphogluconate dehydrogenase (*PGDH*), and the glutamate dehydrogenase (*GTDH-2*) loci. *P. varians* differed slightly from *P. frondifera* with Nei's unbiased genetic

Table 4 Intra- and interspecific contact-induced mortality in three *Pavona* species as measured by visible tissue loss. Maximum tissue dissolution for all interactions was approximately 1×1 mm. No mortality of the dominant species was observed. Duration of interactions was 3–7 days

Pairing type	Number of pairs	% of pairs aggressive	% of stand-offs	Dominant species	Onset days
Interspecific					
P. chiriquiensis–P. varians	50	50	50	*P. chiriquiensis*	1
P. chiriquiensis–P. frondifera	10	100	0	*P. chiriquiensis*	1
P. varians–P. frondifera	10	100	0	*P. varians*	1
Intraspecific (fragments from different colonies)					
P. chiriquiensis–P. chiriquiensis	10	0	100	–	–
P. varians–P. varians	10	0	100	–	–
P. frondifera–P. frondifera	10	0	100	–	–

Table 5 Summary chart of sample size, number of genotypes, and gene frequencies for six populations of *P. chiriquiensis*, eight populations of *P. varians*, and three populations of *P. frondifera* collected on the Pacific coast of Panama. *1* Saboga Island; *2* Iguana Island; *3* Jicarita Island; *4* Coiba Island; *5* Uva Island; *6* Secas Island; *7* Silva de Afuera Island; and *8* Montuosa Island. See Fig. 2 for geographical reference. *LVP-1* and *TPI-1* are monomorphic; *n* number of individuals

	P. chiriquiensis						*P. varians*								*P. frondifera*		
Site	2	3	4	5	7	8	1	2	3	4	5	6	7	8	1	4	5
n	6	6	1	36	5	7	51	5	2	15	77	9	5	9	40	1	2
Genotypes	2	4	1	24	3	5	10	5	1	11	34	5	3	6	1	1	2
Locus/Allele																	
TPI-2																	
A	0	0	0	0.069	0	0	0	0	0	0	0	0	0	0	0	0	0
B	0.500	0.917	0.500	0.222	0.900	0.571	0	0	0	0	0	0	0	0	0	0	0
C	0.500	0.083	0.500	0.708	0.100	0.429	0	0	0	0	0	0	0	0	0	0	0
D	0	0	0	0	0	0	1.0	0.9000	1.0	0.933	0.935	0.944	1.0	1.0	1.0	1.0	0.750
E	0	0	0	0	0	0	0	0.100	0	0.067	0.065	0.056	0	0	0	0	0.250
HK																	
A	0	0	0	0	0	0	0	0	0	0.100	0	0	0	0.111	0.500	0	0
B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.500	0	0
C	0	0	0	0.028	0	0	0.500	0	0	0.467	0.864	0.667	0.800	0.500	0	0.500	0.500
D	0	0	0	0	0	0	0.250	0	1.0	0.367	0.093	0.222	0.200	0.389	0	0.500	0.500
E	0	0.083	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F	0	0	0	0.014	0	0	0	0	0	0	0	0	0	0	0	0	0
G	0	0	0	0.056	0	0	0	0	0	0	0	0	0	0	0	0	0
H	1.0	0	0.500	0.361	0.100	0.643	0.056	0.500	0	0	0.042	0.056	0	0	0	0	0
I	0	0.917	0.500	0.542	0.900	0.357	0.194	0.500	0	0.067	0	0.056	0	0	0	0	0
GPI																	
A	0	0	0	0.015	0	0	0.063	0	0	0	0	0	0	0	0	0	0
B	0.167	0	0	0.324	0.125	0.083	0.302	0	0	0	0.081	0.222	0.200	0	0	0	0
C	0	0	0	0.044	0	0	0.083	0	0	0.033	0.027	0.056	0	0.222	0	0	0
D	0	0	0	0.265	0.500	0.333	0	0	0	0.033	0	0	0	0	0	0	0
E	0.833	1.0	1.0	0.338	0.375	0.167	0.552	1.0	1.0	0.933	0.892	0.722	0.800	0.778	1.0	1.0	1.0
F	0	0	0	0.015	0	0.417	0	0	0	0	0	0	0	0	0	0	0
PGDH																	
A	0	0	0	0	0	0	0	0	0	0	0	0	0	0.222	0	0	0
B	0	0	0	0	0	0	0	0	0	0	0.066	0	0	0	0	0	1.0
C	1.0	1.0	1.0	0.956	1.0	0.917	0	0.100	0	0	0.098	0	0	0	0	0	0
D	0	0	0	0.015	0	0	0	0.200	0	0	0	0	0	0	0	0	0
E	0	0	0	0.029	0	0.083	1.0	0.700	1.0	0.967	0.836	1.0	1.0	0.778	1.0	1.0	0
F	0	0	0	0	0	0	0	0	0	0.033	0	0	0	0	0	0	0
GTDH-2																	
A	0	1.0	0	0.143	0	0	0	0	0	0	0	0	0	0	0	0	0
B	1.0	0	1.0	0.619	0.900	1.0	0	0.833	0	0	0.023	0	0	0	0	0	0
C	0	0	0	0.214	0.100	0	0.179	0.167	0	0.633	0.136	0.063	0.750	0	0	1.0	1.0
D	0	0	0	0.024	0	0	0	0	0	0	0	0	0	0	0	0	0
E	0	0	0	0	0	0	0.786	0	1.0	0.300	0.091	0.563	0.250	0.444	1.0	0	0
F	0	0	0	0	0	0	0.036	0	0	0.067	0.682	0.313	0	0.556	0	0	0
G	0	0	0	0	0	0	0	0	0	0	0.068	0.063	0	0	0	0	0
LVP-2																	
A	0	0	0	0	0	0	0	0.400	0	0	0	0	0	0.125	0	0	0
B	1.0	1.0	1.0	1.0	1.0	0.833	1.0	0.600	1.0	0.821	0.951	1.0	0	0.250	1.0	1.0	1.0
C	0	0	0	0	0	0.167	0	0	0	0.179	0	0	1.0	0.625	0	0	0
D	0	0	0	0	0	0	0	0	0	0	0.024	0	0	0	0	0	0
E	0	0	0	0	0	0	0	0	0	0	0.024	0	0	0	0	0	0
LPP																	
A	0	0.167	0	0.250	0	0	0.214	0	0.500	0	0	0	0	0	0	0	0
B	0	0	0	0	0	0	0	0.500	0	0.056	0.009	0	0	0.222	0	0	0
C	0.500	0.667	1.0	0.458	1.0	1.0	0.786	0.500	0.500	0.556	0.269	0	0	0.556	0	1.0	0
D	0	0	0	0	0	0	0	0	0	0	0.019	0	0	0	0	0	0
E	0.500	0.167	0	0.292	0	0	0	0	0	0.389	0.435	1.0	1.0	0.222	1.0	0	1.0
F	0	0	0	0	0	0	0	0	0	0	0.269	0	0	0	0	0	0
GTDH-1																	
A	0	0	0	0	0	0	0	0	0	0	0.031	0	0	0	0	0	0
B	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.969	1.0	1.0	1.0	1.0	1.0	1.0

distance of 0.068 (1 m.y.a.). No fixed differences were found between these species. The UPGMA phenogram shows all populations of *P. chiriquiensis* grouping together before joining the other two species (Fig. 5). *P. varians* and *P. frondifera* did not show independent clustering (Fig. 5).

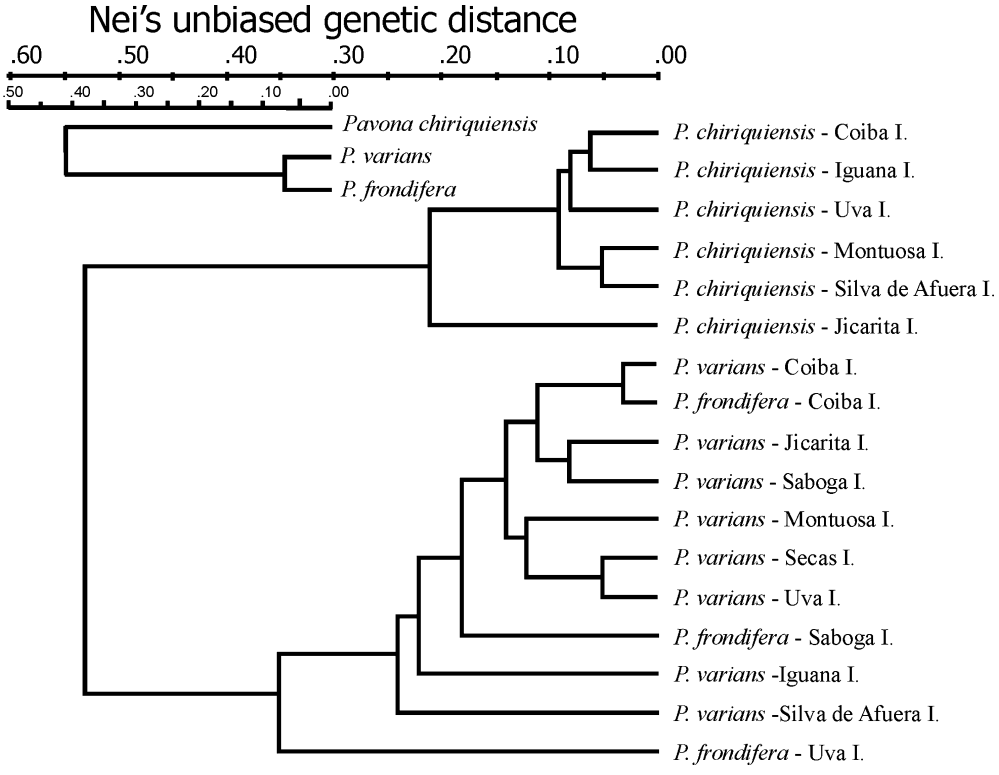

Fig. 5 Unweighted pair group method average phenogram using Nei's unbiased genetic distances (Nei 1978) summarizing the relationships among 17 populations of three *Pavona* species from the Pacific coast of Panama. See Fig. 2A–D for locations of collection sites. The *insert to the left* summarizes the species relationships once all clonemates have been removed from the analysis

Colony morphology, coloration, and morphometrics

Colony morphology in *P. varians* is highly variable. It can be (1) encrusting to slightly massive on basalt rock, (2) platy or laminar on the skirts of massive colonies, on coralline and rocky ledges, and in crevices, (3) massive in reef habitats, on rubble, and soft bottoms, or (4) with secondary bifacial folia on platy or massive colonies on soft bottoms. Two or more growth forms may be found on different parts of the same colony. *P. chiriquiensis* and *P. frondifera* showed little or no variation in colony morphology. *P. chiriquiensis* is always encrusting and deposits a thin veneer of skeleton over the substrate, mainly basaltic rock. Colonies of *P. frondifera* are always encrusting with a compact foliose morphology.

Encrusting colonies of *P. varians* and *P. chiriquiensis* follow the contour of the underlying substrate (from nearly horizontal to completely vertical). Slightly massive growth forms in *P. varians* may develop on portions of the encrusting colonies but rarely reach thicknesses greater than 10 cm. The platy growth forms of *P. varians* have a wedge appearance (i.e., thicker at the attachment point and gradually decreasing in thickness distally) and are always unifacial, and the calices are restricted to the upper surface. Massive colonies may be firmly attached to the substrata or completely free. Free colonies may occur as coralliths (Glynn 1974a), dome-shaped colonies, or other irregular shapes with tops showing partial or total mortality.

Most coralla of *P. varians* are < 30 cm in diameter, although some encrusting colonies may surpass 2.5 m. Colonies of *P. chiriquiensis* are generally small (< 0.5 m^2) but may rarely cover up to 10 m^2 of substrate. The largest observed colony of *P. frondifera* measured only 78 cm in length by 50 cm in width.

The polyps and coenosarc of *P. chiriquiensis* are light to dark brown, brick red to brown, or silvery blue and contrast with the bright white to silvery oral discs and tentacles (see also Glynn et al. 2001b). This contrast between the coenosarc and oral discs is a diagnostic feature of the species (Fig. 1B). Silvery blue and brick red colonies of *P. chiriquiensis* have been observed exclusively at Iguana Island (site 2, Fig. 2A) and at Silva de Afuera Island (site 7, Fig. 2D), respectively. Coenosarc, oral discs, and tentacles of both *P. varians* and *P. frondifera* are usually uniform in color (Fig. 1A, C). The color of *P. varians* varies from light to dark brown whereas the tissues of *P. frondifera* are pink to light or dark brown. Extremely dark colonies may be found in low-light habitats, such as crevices or the undersides of ledges.

Canonical variate 1 (CV1) accounted for 83.6% of the variance. A chi-square analysis of the Wilk's lambda showed the two canonical functions to have a significant discriminating power ($P < 0.001$). CV1 separated *P. chiriquiensis* from *P. varians* and *P. frondifera*. CV2 separated *P. frondifera* from *P. varians* and further separated *P. chiriquiensis* from *P. frondifera*. CV1 was most heavily weighted for maximum calicular diameter (CD1), corallum thickness (CT), and maximum columellar diameter (CL1). CV2 was most heavily weighted for main septa length (SL), number of septa (NS1), minimum calicular diameter (CD2), and number of septa reaching the columella (NS2; see Table 3 for a description of skeletal characters measured and counted).

Differences between two of the three *Pavona* species were found in eight of the ten skeletal characters (Table 6). However, no single morphological character was simultaneously significantly different between all three species. *P. chiriquiensis* had significantly larger calicular and columellar diameters, and thinner corallum thickness than *P. varians* and *P. frondifera* (Table 6). Columellar diameter was smaller and the total number of septa were significantly lower in *P. frondifera* (Table 6).

A discriminant canonical analysis of 19 populations of *Pavona* readily separated the three *Pavona* species in accordance with morphometric and genetic metrics (Fig. 6). The jackknife analysis indicated that 91.2% of all *P. varians*, 96.7% of *P. chiriquiensis*, and 90.0% of *P. frondifera* were correctly classified by the discriminant function.

Discussion

Consistent differences in habitat preferences, tissue coloration, skeletal morphology, allozyme patterns, and reproductive ecology have confirmed the separate specific status of *Pavona varians*, *P. frondifera*, and *P. chiriquiensis* (Table 7). The congruence of independent

Table 6 Morphometric comparisons of the three *Pavona* species. *Ph P. chiriquiensis*, *Pv P. varians*, *Pf P. frondifera*. *SNK* Student–Newman–Keuls. *NS* not significant. See Table 3 and Fig. 3 for a description of characters

Character	Code	*P. chiriquiensis* Mean ± SE	*P. varians* Mean ± SE	*P. frondifera* Mean ± SE	*P*	a posteriori results of SNK
Maximum calicular diameter	CD1	1.85 ± 0.05	1.31 ± 0.03	1.23 ± 0.04	***	Ph > Pv, Pf
Minimum calicular diameter	CD2	1.11 ± 0.04	1.50 ± 0.02	1.04 ± 0.03	***	Ph > Pv, Pf
Main septa length	SL	0.62 ± 0.02	0.47 ± 0.01	0.53 ± 0.02	***	Ph, Pf > Pv
Maximum columellar diameter	CL1	0.34 ± 0.03	0.26 ± 0.01	0.17 ± 0.01	***	Ph, Pv > Pf
Minimum columellar diameter	CL2	0.20 ± 0.02	0.16 ± 0.01	0.12 ± 0.01	***	Ph, Pv > Pf
Colline width	CW	0.90 ± 0.02	0.50 ± 0.03	0.56 ± 0.05	NS	–
Number of septa	NS1	20.02 ± 0.53	19.86 ± 0.62	15.96 ± 0.74	*	Ph, Pv > Pf
Number of septa reaching the columella	NS2	7.17 ± 0.16	7.88 ± 0.15	6.73 ± 0.28	***	Pv > Ph, Pf
Number of septocostae	SC	6.77 ± 0.50	7.08 ± 0.20	6.66 ± 0.80	NS	–
Corallum thickness	CT	15.14 ± 1.48	55.52 ± 3.64	92.27 ± 11.57	***	Pv, Pf > Ph

* $P < 0.05$; *** $P < 0.001$

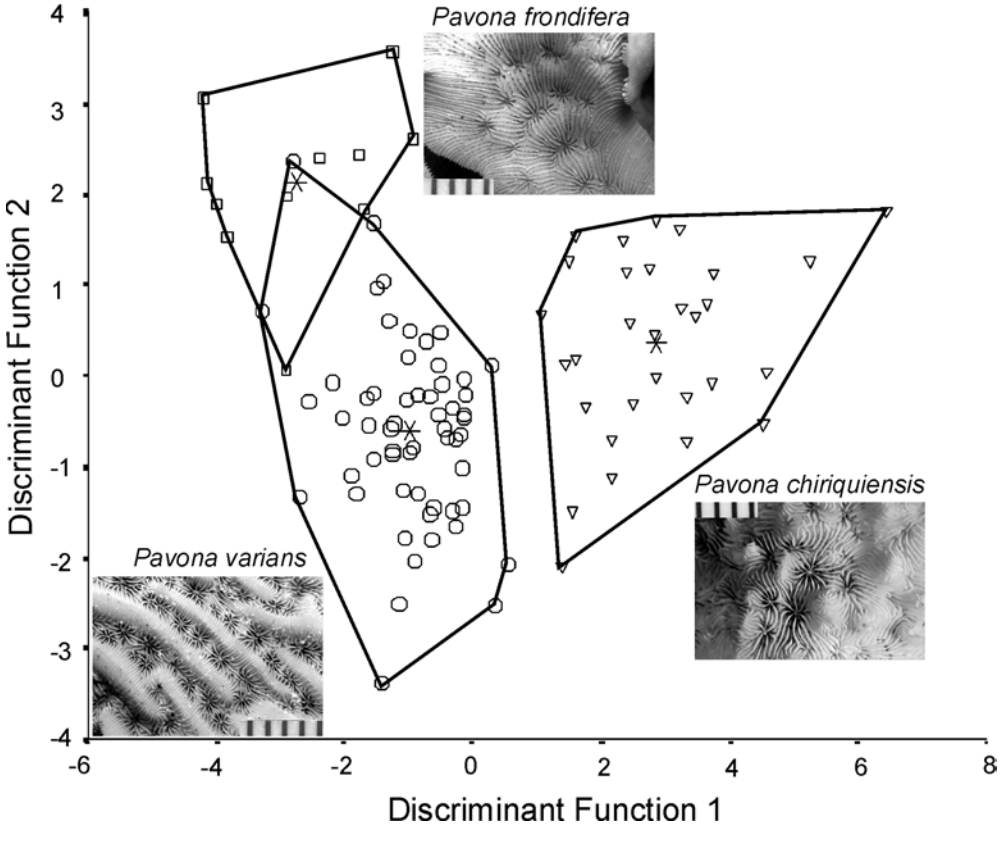

Fig. 6 Canonical discriminant function scores for *P. varians*, *P. chiriquiensis*, and *P. frondifera*. Note that *polygons* enclosing all individuals of the same species show little overlap except for *P. varians* and *P. frondifera*, which are the most similar genetically. *Stars* represent group centroids. *Corallite detail* is shown for all species (*Scales* in mm)

characters has been shown to be a compelling argument to support species separations in scleractinian corals (Knowlton et al. 1992; Szmant et al. 1997).

This study has also validated the use of traditional morphological analyses in the genus *Pavona*. However, members of this genus have posed some difficulties, as have members of the genus *Leptoseris* (see Dinesen 1980). Both genera belong to the family Agariciidae, which is characterized by thamnasteroid corallites (Wells 1956; Veron and Pichon 1979). These corallites lack a well-defined wall, making calicular measurements dependent upon one's designation of the corallite border. Calicular diameter can be more easily estimated in those corallites located within collines. However, these corallites in general have a larger number of septa than those in open areas because many septocostae are inserted within the corallite.

Macro-morphology is not a good character for distinguishing species boundaries among these three *Pavona* species. This is particularly true for *P. varians*, a species with high phenotypic plasticity. Even within a single colony it is possible to find three or four different growth forms that appear to reflect micro-habitat differences. In the western Pacific coral *Pavona cactus* Forskål, 1775 (Forskål 1775), there is a strong association between genotype and growth form (Ayre and Willis 1988). Although this type of association has not been investigated in Panamanian *P. varians*, this does not appear to be the case for this species, since multiple morphologies are found within a single colony. In contrast, *P. chiriquiensis* and *P. frondifera* appear to show little intraspecific variation, perhaps in part related to the narrower habitat preferences of these species. Skeletal plasticity similar to that observed in *P. varians* has also been observed in other coral species such as *Montastraea* spp. (Budd 1993), and *Pocillopora damicornis* (Veron and Pichon 1976).

An important result of this study is the agreement between the reproductive findings and the allozyme data. *Pavona varians* and *P. chiriquiensis* were shown to be reproductively isolated in sympatry, both in terms of timing of spawning and gamete characteristics (Glynn et al. 2000; this study). Also, the genetic data indicate that *P. chiriquiensis* is clearly distinct from both *P. varians* and *P. frondifera*, and that *P. varians* differs less strongly from *P. frondifera*. The levels of divergence observed between species (0.068–0.434) are consistent with that reported for congeneric pairs of invertebrates (Thorpe 1983), as well as for other coral species (Weil and Knowlton 1994).

Table 7 Species characteristics for Panamanian *P. varians*, *P. chiriquiensis*, and *P. frondifera*

Character	*P. varians*	*P. chiriquiensis*	*P. frondifera*
Growth morphology	Massive, encrusting, platy or laminar, rolling stone	Always encrusting, rarely with massive build-up; thin veneer of skeleton	Thick and compact branching, encrusting on substrate
Coloration	Light to dark brown; polyps, oral disc, tentacles, and coenosarc of uniform coloration	Light to dark brown, red-brick to brown, silvery blue to brown; mouths and tentacles always white to silvery	Pink to brown; polyps, oral disc, tentacles, and coenosarc of uniform coloration
Spawning characteristics	Dawn spawner, ~1 h before sunrise; eggs in mucus strings; clouds of sperm	Sunset spawner, ~1 h after sunset; clouds of eggs and sperm	Unknown
Gamete characteristics	White to beige eggs, 105.2 ± 2.3 μm; positively buoyant; eggs without zooxanthellae	Dark green eggs, 100.3 ± 2.4 μm; neutrally to negatively buoyant; eggs without zooxanthellae	Unknown
Distribution			
Gulf of Panama	Abundant to rare depending on site	Rare at the two sites found	Present at only one locality, a single clone
Gulf of Chiriquí	Common to rare depending on site	Abundant to rare depending on site	Extremely rare; found at just two sites
Other areas	Indo-west Pacific	Restricted to the eastern Pacific	Indo-west Pacific
Habitat	Reef, rocky substrata, soft sediments (coarse sand–muddy)	Almost exclusively on basalt; only four specimens found on reefs	Almost exclusively a reef species; two specimens found on soft bottom
Depth range	Shallow to deep, 1–16.7 m	Shallow to deep, 3–15.1 m	Mostly shallow, 3.5–13 m
Colline type	Short or long collines, uniform; collines one or many corallites in width	Short collines, mostly irregular; many hydnophorae; collines commonly one corallite in width, rarely more than two corallites in width	Generally long collines; collines in general one corallite in width
Corallite series	Most commonly > 4; may reach 17 in skirt platy growth forms	Commonly 2–4; in rare occasions may reach 5–7	Commonly > 5; may reach 16
Calice diameter (mean ± SE)	0.825–2.11 mm (1.37 ± 0.04)	1.25–2.32 mm (1.91 ± 0.07)	0.95–1.42 mm (1.25 ± 0.03)
Number of septa (mean)	13–36 (20)	10–28 (21)	14–18 (16)
Zooxanthella clade[a]	*C1*	*C1*	*C1*
Corallum thickness	27–110 mm	5–40 mm	45–157 mm

[a] From Baker (1999)

The use of electrophoretic data to identify precisely even completely reproductively isolated species may only be possible if the species have evolved some "fixed" allelic differences at one or more loci (Richardson et al. 1986). These reproductively isolated taxa will, in time, accumulate additional fixed genetic differences (Knowlton and Weigt 1997). The fixed and the two nearly fixed differences between *P. varians* and *P. chiriquiensis* confirm that these species are indeed reproductively isolated distinct species in the biological sense (Mayr 1970). It has been suggested that some reproductively isolated taxa may exhibit only small levels of genetic differentiation due to a recent origin, slow rates of molecular evolution, or large population sizes (McFadden 1999). This may be the case for *P. frondifera*, but additional studies are needed on its reproductive biology and other life-history characteristics.

The reproductive patterns observed in *P. varians* and *P. chiriquiensis* suggest likely mechanisms underlying the maintenance of species boundaries by extreme prezygotic barriers to successful fertilization (both temporal and gamete characteristics). Differences in reproductive timing or in gamete recognition systems that control fertilization have been recognized as the most likely barriers to reproduction among octocorals in the *Alcyonium coralloides* complex (McFadden 1999). Similarly, dilution (increasing with time), duration of gamete viability (decreasing with time), and egg–sperm contact time have been reported to reduce fertilization success in other marine organisms with external fertilization (Levitan et al. 1991; Oliver and Babcock 1992; Palumbi 1994; Szmant et al. 1997; Willis et al. 1997; Maté et al. 1998), thus minimizing interspecific hybridization. Knowlton et al. (1997) noted that a difference in spawning times of just 1–2 h was sufficient to maintain reproductive isolation between *Montastraea franksi* and *M. annularis* on the Caribbean coast of Panama. Thus, spawning in *P. varians* and *P. chiriquiensis*, being out of phase by more than 12 h, should prevent successful fertilization under field conditions.

Dawn spawners are rare in corals and may be restricted to the genus *Pavona*. *P. cactus* on the Great Barrier Reef (Marshall and Stephenson 1933) and *P. varians* in Panama (this study) are the only dawn spawners known to date. Even though *P. varians* and *P. chiriquiensis* spawn near extreme high-water stands, the eggs of *P. chiriquiensis* are negatively buoyant, possibly facilitating retention on the reef during peak spring tides (Babcock et al. 1986). However, the eggs of *P. varians* are positively buoyant, and thus likely to be dispersed off-reef. Thus, the characteristics of the gametes may possibly help explain the distributional patterns of both species.

Colonies of *P. cactus* with the same genotype have been shown to be separated by distances of up to 93 m (Ayre and Willis 1988). This lack of genetic variation is consistent with an asexual reproductive mode (Ayre and Willis 1988; McFadden 1999). Populations of species with asexual reproduction are often dominated locally by individuals belonging to one or a few clones (Wright 1969; Jain 1976; Suomaleinen et al. 1976; Baur and Klemm 1989). From the allozyme data of *P. frondifera* at Saboga Island (Gulf of Panama), the 40 colonies studied were genetically identical and thus probably asexually produced.

Allozyme patterns in *P. varians* and *P. chiriquiensis* also provide evidence of the presence for clonal reproduction within populations. Most of the collections for allozyme work were completed before the 1997–1998 El Niño event. At that time, adjacent colonies were considered to have recruited sexually on the basaltic substrata. However, six colonies monitored at Uva Island (Gulf of Chiriquí) before, during, and after the 1997–1998 El Niño event suggested that these adjacent colonies were surviving fragments of a larger colony that probably suffered partial mortality in previous disturbances. Colonies that ranged in size from 1.5 to 7 m in length suffered massive mortalities during the 1997–1998 El Niño and produced 10–115 isolated fragments each, some close to each other, some farther apart. Although this hypothesis of clonality by partial colony mortality may seem feasible for *P. chiriquiensis*, it is less likely for *P. varians*. Coral mortality in *P. varians* during the 1982–1983 and 1997–1998 El Niño events was low and limited to the upper surfaces and did not fragment larger colonies into smaller ones. The activities of predatory fishes that feed on boring *Lithophaga* bivalves may provide an alternative explanation for *P. varians*. Fish like *Pseudobalistes naufragium* Jordan and Starks and *Balistes polylepis* Steindachner have been observed to remove chunks of skeleton from *Pavona varians* in search of those bivalves (see Glynn et al. 1972, Fig. 12, p. 503). These broken fragments may survive and later grow larger colonies in relatively close proximity to their parent colony.

The high sensitivity of *P. chiriquiensis* to warm waters that accompanied the 1997–1998 El Niño is compared to that of fire corals in the genus *Millepora* (Glynn 1990; Glynn and Feingold 1992; Glynn et al. 2001a, b). However, the ability of *P. chiriquiensis* to recover from this disturbance surpasses that of the fire corals. Five years after the event, most of the colonies of *P. chiriquiensis* had increased considerably in size, approximating colony sizes before the disturbance (see Table 7 in Glynn et al. 2001a). *Millepora* colonies, however, had not yet recovered (Glynn et al. 2001a). Reef-building corals maintain an obligate association with endosymbiotic zooxanthellae. However, not until recently has the importance of this symbiosis, in terms of zooxanthellae diversity, been recognized as a possible determinant of coral zonation (Rowan and Knowlton 1995) and coral bleaching (Rowan et al. 1997). Whereas in the Caribbean coral genus *Montastraea* bleaching differences can be attributed to the zooxanthella genotypes they harbor, bleaching in Panamanian *Pavona* cannot be attributed to differences in the symbionts hosted since the species harbor the same type (clade *C1*) of symbiont (Baker 1999; Glynn et al. 2001a). Thus, zooxanthella genetic

diversity does not appear to impart ecological or taxonomic differences between these *Pavona* species.

Although tissue colors are considered the most common "live coral" trait mentioned by systematists (Lang 1984), in most species diagnoses this trait is omitted. Glynn et al. (2001b) showed that tissue coloration is a diagnostic field character for *P. chiriquiensis*. The validity of this character has been confirmed by the present study. All specimens observed in Panama had dark polyps and coenosarc, and contrasting bright white to silvery oral discs and tentacles. Other coral species descriptions that have included tissue coloration as a diagnostic character include those of *Scolymia* spp. (Lang 1971) and *Mycetophillia* spp. (Wells 1973).

In terms of species distributions, the case of *P. chiriquiensis* may be the most interesting one. The distribution of this species in Panama may suggest the preference for year-round warm habitats (it is common in the Gulf of Chiriquí but rare in the Gulf of Panama). However, this species is also abundant in the relatively cool waters of the Galápagos Islands (Glynn et al. 2001b). It is possible that the distributional pattern of this species may be related to the peak reproductive season from January to June (Glynn et al. 2000) when the Gulf of Panama experiences cold (16–25°C) seasonal upwelling (Glynn and Maté 1997). Although the low water temperatures may not be that critical for the survival of adult colonies, they may affect fecundity and larval survival, becoming a physical barrier for the expansion of the species into the Gulf of Panama. In the Galápagos Islands, the peak of the reproductive season occurs from January to May (Glynn et al. 2000), which spans the warmest months of the year there (Podestá and Glynn 1997). This may explain the high abundance of the species at several localities within the Galápagos Islands. Still, we do not know how the species got established there, nor why *P. varians* with its reproductive peak at the same time as *P. chiriquiensis* is abundant in the Gulf of Panama.

Corals can compete for space by direct and indirect means (Jackson and Hughes 1985). Direct interactions, such as those observed in the Caribbean coral genus *Scolymia* (see Lang 1973), normally involve an aggressive behavior wherein the dominant colony or species digests the tissues of the subordinate neighbor. In contrast, indirect interactions involve the growth of one colony above another, depriving it of light or food (Connell 1973). *P. varians* and *P. chiriquiensis* exhibited both types of interactions. During the direct interaction phase, *P. chiriquiensis* is able to digest rapidly the tissues of *P. varians* (within 1 day). In the longer term, however, *P. varians* is able indirectly to outcompete *P. chiriquiensis* by overtopping it. Similar reversals have been observed between *P. gigantea* Verrill, 1869 (Verrill 1869) and *Pocillopora damicornis* Linnaeus in Panama (see Wellington 1980). In the laboratory, *Pavona frondifera* is the subordinate species of the three herein tested. In conclusion, *P. varians*, *P. chiriquiensis*, and *P. frondifera* are three distinct species that show clear differences in almost all of the characters considered in this study.

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