



Signatures of speciation? Distribution and diversity of *Hypoplectrus* (Teleostei: Serranidae) colour morphotypes

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ABSTRACT

Aim To test historical and current influences on the distributions of sympatric colour morphotypes in the coral reef fish genus *Hypoplectrus*.

Location The Caribbean and surrounding tropical waters. These areas cover the entire distribution of the genus.

Methods A large and extensive database of *Hypoplectrus* sightings was used to establish the distribution of colour morphotypes and test a long-standing hypothesis regarding their origin. First, we considered the evidence for the previously proposed 'population centre' hypothesis, which suggests that current morphotype distributions reflect past conditions where these colour forms evolved in allopatry. Using morphotype sighting data, the existence of clusters in occurrence and density was tested. Second, we examined whether the observed patterns of morphotype co-occurrence deviate from random expectations using null model simulations, within subregions of the distribution of the genus, to infer ecological influences on distribution.

Results There is considerable variation in morphotype distribution, with even widespread morphotypes showing geographical clustering. There is also little evidence to suggest past or current geographical isolation, with only one of the 11 morphotypes (*Hypoplectrus chlorurus*) showing a density distribution that is consistent with the population centre hypothesis. Null model analyses show that variation in local morphotype co-occurrence is typically significantly lower than expected under random dispersal conditions.

Main conclusions Our results strongly suggest that morphotype co-occurrence is not random, but there is no evidence to suggest a past allopatric radiation in *Hypoplectrus* colour. Current distributions are likely to be driven by competitive interactions and/or habitat preferences. Our study highlights the value of the *Hypoplectrus* species complex as a system for the study of speciation in the marine environment, and implies that these closely related morphotypes have ecological relevance rather than being simple colour variants of a single polymorphic species.

Keywords

Caribbean, colour polymorphism, co-occurrence, coral reef, evolution, fish, hamlets, marine, sympatry.

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INTRODUCTION

Speciation, the evolutionary process by which new species arise, has long been a subject of interest to ecologists and evolutionary biologists alike, and the topic has attracted a great deal of attention in recent years (e.g. Fitzpatrick & Turelli, 2006; Rocha & Bowen, 2008; Seehausen *et al.*, 2008). Our understanding of spe-

ciation and the processes governing intra-specific evolutionary divergence has been traditionally informed by studies of terrestrial and freshwater systems (Gray & McKinnon, 2007). The possible reasons for this bias include familiarity, as often more is known about these systems, and practical issues, since studying marine taxa can be difficult and expensive (Gladstone & Davis, 2003). However, there are reasons to believe that the

mechanisms that are important for driving evolution in the marine environment may differ from those in other habitats. For example, opportunities for allopatric speciation appear to be limited in the vast expanses of continuous ocean and vicariance events are rare (Palumbi, 1992). Nevertheless, a diverse array of marine species exists and the origin of this diversity is currently unclear. Given these issues, natural study systems such as marine radiations are extremely valuable to evolutionary biologists, and studying the processes which have influenced the diversification of these systems is essential to improve our understanding of speciation in general.

An excellent example of a marine system that has been well studied in order to understand the mechanisms and processes of speciation is the Caribbean coral reef fish genus *Hypoplectrus* (the hamlets). These fish, often referred to as a species complex, represent a striking colour polymorphism, the origin and maintenance of which remains unclear in spite of decades of research (Thresher, 1978; Graves & Rosenblatt, 1980; Domeier, 1994; Aguilar-Perera, 2003; McCartney *et al.*, 2003; García-Machado *et al.*, 2004; Puebla *et al.*, 2007; Whiteman & Gage, 2007; Barreto & McCartney, 2008; Holt *et al.*, 2008). The hamlet group includes 10 described 'species' and many undescribed morphotypes (i.e. colour forms) that are distinguished solely by their coloration and geographical distributions. Since these described species have proven to be extremely difficult to distinguish genetically, even using high-resolution techniques (e.g. Barreto & McCartney, 2008), we refer to all colour forms as morphotypes throughout this paper. Most *Hypoplectrus* morphotypes have restricted distributions within the Caribbean basin but these distributions overlap substantially. As a result, most morphotypes are considered to be broadly sympatric, and many different morphotypes can be found on a single reef (Domeier, 1994).

The co-occurrence of hamlet morphotypes on reefs has prompted the suggestion that they are currently diverging in sympatry (Puebla *et al.*, 2007). Domeier (1994) suggested that *Hypoplectrus* morphotypes initially evolved under allopatric conditions created by falling sea levels during the last ice age. This work described the distributions of all known *Hypoplectrus* morphotypes, with the exception of the recently discovered Veracruz white (Holt *et al.*, 2008), combining field surveys in 34 locations with the results of museum surveys. While one morphotype, *Hypoplectrus puella*, appeared to be ubiquitous throughout the Caribbean region, all other morphotypes showed more restricted distributions. On the basis of these heterogeneities in distribution, Domeier (1994) suggested that 'population centres' (i.e. '[a] region where a particular morph is the most abundant relative to the rest of the range of that morph'), which represent centres of evolutionary origin, could be identified for all morphotypes. However, the empirical basis of population centres is unclear and there is little evidence of biogeographical population structure in the various molecular studies of *Hypoplectrus* (McCartney *et al.*, 2003; Ramón *et al.*, 2003; García-Machado *et al.*, 2004; Puebla *et al.*, 2008), which have all shown low levels of genetic isolation between geographically distant populations.

It is unknown why the distributions of the different morphotypes vary so greatly, and remarkable given that many can occur in sympatry, but this does suggest the possibility of competition and/or ecological differences between these colour forms. While it is clear that the colour forms of *Hypoplectrus* have some biological meaning, as they exhibit strong assortative mating (e.g. Puebla *et al.*, 2007), the ecological significance of these colour forms is less clear. Thresher (1978) suggested that *Hypoplectrus* morphotypes have evolved to become 'aggressive mimics' of non-predatory fish species due to competition for food resources. Some evidence for differences between the feeding strategies of *H. puella* and *Hypoplectrus unicolor* has been presented (Puebla *et al.*, 2007), but no ecological factor has yet been demonstrated to consistently differ between morphotypes across locations. Studies that have failed to find support for ecological (i.e. dietary) differentiation among colour morphs (Whiteman *et al.*, 2007; Holt *et al.*, 2008) also raise the possibility of inter-morphotype competition for resources. Co-occurrence patterns among species can be expected to deviate from random expectations under conditions such as differentiation of habitat preferences and interspecific competition, and the utility of non-random co-occurrence patterns for the detection of such conditions is well established (Gotelli, 2000). If *Hypoplectrus* morphotypes co-occur randomly then their existence is unlikely to be linked to any ecological factor, unless such a factor has no effect on habitat preferences and does not result in competitive exclusion. Alternatively, significant non-random co-occurrence would suggest that ecology does play an important role in spatial distribution of this polymorphism and that future research into *Hypoplectrus* divergence should focus on ecological phenomena such as habitat preferences and inter-morphotype competition.

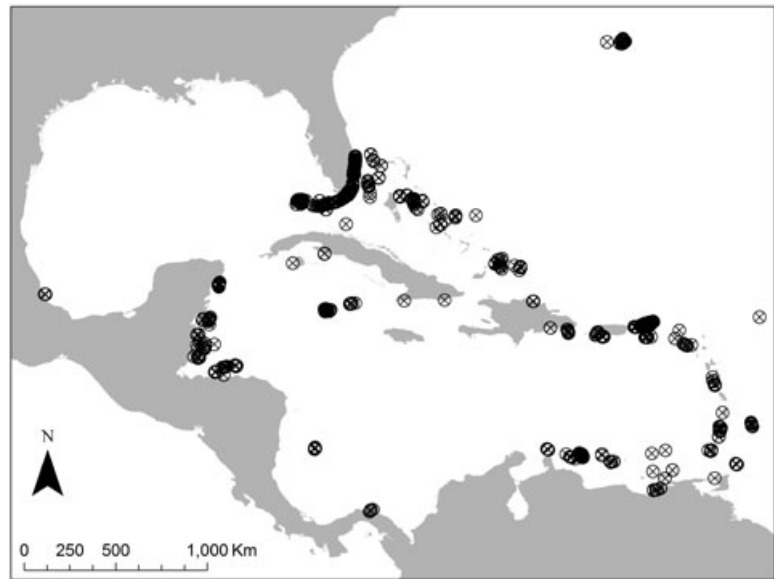
This study has two objectives. First, we use a large database of *Hypoplectrus* presence records to map colour morphotype distributions at the region-wide scale to evaluate evidence for past allopatric conditions or the influence of current ecological factors. We expected records to be geographically clustered for the majority of morphotypes, in accordance with existing descriptions of morphotype ranges (Domeier, 1994). By conducting the first formal test for the presence of clusters of high densities ('population centres') within distributions of each morphotype, we examine whether there is support for the past allopatric divergence hypothesis. Secondly, in order to assess the potential for ecological influences on morphotype distribution we use the same dataset to determine if morphotype co-occurrence can be explained by the random assortment of locally occurring morphotypes.

METHODS

Spatial data acquisition

Information on the distribution of hamlets and other Caribbean reef fishes was extracted from a database containing survey records submitted by hundreds of trained volunteer scuba divers as part of the REEF (Reef Environmental Education Founda-

Figure 1 A map of the Caribbean Sea showing the distribution of Reef Environmental Education Foundation (REEF) fish survey sites included in geographical analysis of distribution of the *Hypoplectrus* species complex ($n = 1027$ surveys).



tion) Fish Survey Project (<http://www.reef.org/programs/volunteersurvey>). At the time of this study, the REEF database consisted of more than 90,000 fish surveys carried out in the tropical western Atlantic since 1990.

REEF surveys were carried out using the roving diver technique (RDT), a visual survey method specifically designed for volunteer recorders. During roving surveys, divers swam freely throughout a site and recorded every observed fish species that could be positively identified. Data collection began as soon as the diver entered the water. To find as many species as possible, divers checked under ledges and overhangs and in the water column. At the end of each survey, each recorded species was assigned one of four abundance categories (Single, one individual observed; Few, 2–10 individuals; Many, 11–100 individuals; Abundant, >100 individuals), based on how many individuals were seen throughout the dive. GPS coordinates of the site location were also recorded.

REEF volunteers are categorized as either novice or expert and the status of each volunteer is recorded with each survey. To ensure maximum data reliability only the surveys completed by divers with expert status were used. Volunteers are awarded expert status after completing 35 fish surveys and passing a series of fish identification tests. In addition, only five surveys for each site were used, in order to standardize sampling effort across sites. These surveys were randomly selected from those available, and sites with fewer than five surveys were not included. A total of 1027 REEF survey sites provided the required number of expert-status surveys with *Hypoplectrus* sightings. These surveys were broadly distributed across the tropical western Atlantic covering the majority of the distribution of the genus (Fig. 1). However, it should be noted that some relatively small geographical areas are not covered, which is likely to affect our results for the rarest and most geographically restricted morphotypes such as *Hypoplectrus gummigutta* and *Hypoplectrus providencianus*. Surveys without hamlet sightings were not used as it was not possible to determine whether the

absence of data reflected true absence of these fish or simply that they were not positively identified by surveyors. Sightings recorded as 'hybrids' were not included in the co-occurrence analysis due to identification issues.

Analysis

Spatial analysis of morphotype distributions

Density scores for each hamlet morphotype at each site were calculated from the five randomly selected surveys as:

$$\text{Density score} = (S \times 1) + (F \times 2) + (M \times 10) + (A \times 100)$$

where S , F , M and A represent the number of surveys in which morphotype was scored as Single, Few, Many or Abundant, respectively. As the minimum threshold values for each abundance category are used in the calculation of density scores, and the range of each category is large, density scores can be considered underestimates of true abundance. Since only surveys where hamlets have been recorded were considered, density indices represent the minimum relative density of particular morphotypes at particular sites.

The geographical coordinates of each site and the density score for each morphotype were totalled for all five survey records and entered into ARCMAP (ARCGIS 9.2). To determine if morphotype density was spatially clustered, we used ARCMAP's Moran's I spatial statistic tool. Moran's I is commonly used to test for the presence of spatial autocorrelation in biological data (e.g. Dark, 2004; Ryan *et al.*, 2004; Beseres & Feller, 2007). It can range from -1 to $+1$, with positive scores representing clustering of values, negative scores dispersed values, and zero representing randomly distributed data. The statistical significance of each Moran's I score was determined with a Z score generated by a randomized null hypothesis computation.

We first tested for significant clustering of the presence/absence of each morphotype across the region. We then tested

for clustering of morphotype density within the distributions for each of the 11 morphotypes. It is thought that highly significant spatial clustering of density around a specific site reflects the existence of a population centre, which may be a remnant of past allopatric evolutionary origins (Domeier, 1994). The presence of multiple high-density sites within a morphotype distribution would suggest that either some of these sites are not evolutionary population centres or that populations of the same described morphotype may not have a common evolutionary origin.

To determine if density scores were clustered around single or multiple geographical locations, high-density hotspots were identified using the Getis–Ord G_i^* hotspot spatial analysis tool in ARCMAP 9.2. The G_i^* statistic is designed to detect local areas of high or low values (in our case, density scores), known as hotspots. Hotspots may be present even if the global Moran's I value indicates that density values are randomly distributed (Getis & Ord, 1992), in which case hotspots may be interpreted as being areas of random high density. The ARCMAP G_i^* tool calculates a Getis–Ord G_i^* statistic for each survey point as the sum of a particular feature for each point and its neighbouring points. The value of i equals the distance up to which another sampling point should be considered a neighbour. As sites throughout the *Hypoplectrus* distribution have the potential to influence each other via planktonic dispersal, all sites were deemed to be each other's potential neighbours but with the impact of a neighbour decreasing inversely with linear distance. The value for each site was calculated and compared with neutral random expectations to generate a Z score for each site. Z scores were used to classify sites as either significant hotspots (at the $P = 0.05, 0.01$ or 0.001 level) or 'normal' sites. Maps were produced representing the overall distribution of morphotype density scores, and high-density hotspots were plotted to determine if these sites were clustered in one or more locations.

Morphotype co-occurrence

To determine whether the co-occurrence of different morphotypes is simply the result of overlapping distributions, morphotype presence/absence matrices were constructed for a number of subregional areas. Rows in these matrices represented the morphotypes recorded in these subregions with columns representing sites where each morphotype was recorded. Eighteen subregions were defined within the overall *Hypoplectrus* dataset, which were identified by the two-digit codes used by REEF (<http://www.reef.org/data/twa/geog.htm>). Geographical variation in morphotype larval recruitment was expected to be relatively low within these subregions, which varied in size and shape but were all between 20,000 km² and 100,000 km². Only subregions which included 10 or more sites with hamlet sightings were considered.

Co-occurrence analysis is based on the assumption that if an area has equal probability of recruitment for all morphotypes present, then there should be a random assortment of morphotype occurrences across sites in the area. Species co-occurrence

indices have been compared with random expectations using null models in a variety of ecological datasets (Gotelli, 2000) to test hypotheses about interspecific competition (e.g. Luiselli, 2008). However, other factors such as habitat preferences (Luiselli *et al.*, 2007) and historical processes (Horner-Devine *et al.*, 2007) can also result in non-random species co-occurrence patterns. Therefore, care must be taken in interpreting significant non-random results. Furthermore, the choice of an appropriate null model is critical. If sites vary in their probability of being occupied by any species, i.e. vary in their capacity to accommodate multiple species, then the assumption of random co-occurrence must be modified. If there is good evidence to suggest that such variation does exist, then the random null model can be adapted to vary the probability of species occupancy between sites, based either on empirical data or on a relevant environmental factor (e.g. reef size). For hamlet morphotypes, it is currently unclear whether sites are equally likely to be occupied by multiple morphotypes. For this reason, we used two different approaches. First, we used the co-occurrence proportional (CP) null model, in which an unequal probability of occupancy was assumed, where the likelihood of a morphotype being randomly allocated to a site is proportional to the number of morphotypes found at that site in the empirical dataset. Second, we used the co-occurrence equal (CE) null model, which assumes that all sites have equal probability of morphotype occupancy.

All co-occurrence analyses were performed using EcoSIM version 7.72 (Gotelli & Entsminger, 2007). Each null model was run 5000 times to produce a null distribution of random results. We used the variance ratio co-occurrence index (V ratio) to compare the observed data with those produced by the null models. This co-occurrence index was chosen as it has been shown to 'behave well' (Gotelli, 2000) for the two null models used here, i.e. it does not tend to produce more type I or type II errors than would be expected by chance. The V ratio is a measure of variability in species richness per site. It is calculated from the presence/absence matrix as the ratio of the variance in number of species per site to variance in number of sites per species. Sites which were randomly assigned zero morphotypes were excluded from the V ratio calculations as sites without hamlet sightings were excluded at the onset from the empirical dataset. If inter-morphotype competition or niche partitioning exists, then the empirical V ratio should be significantly smaller than V ratios based on random expectations.

To test whether patterns of morphotype co-occurrence could be the result of geographical variation in dispersal, we also ran the analysis at an even smaller geographical scale than the subregions, using subdivisions of subregions corresponding to the areas defined by four-digit REEF codes. These subdivisions were typically less than 10,000 km². We tested the best sampled subdivision within each of the subregions. If non-random morphotype co-occurrence was driven solely by dispersal dynamics, we would expect the subdivision-level analyses to yield V ratios that are closer to random expectations than those produced by the analysis of subregions, since there should be less geographical variation in morphotype larvae available. As there were fewer

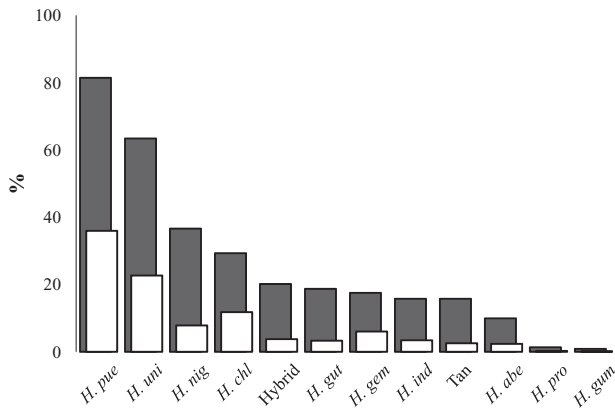


Figure 2 Percentage of total sightings due to each *Hypoplectrus* colour morphotype (white bars) and percentage of survey sites where sightings occurred for each colour morphotype (dark bars). In total, 9916 individuals recorded. Abbreviations: *H. abe* = *H. aberrans*, *H. chl* = *H. chlorurus*, *H. gem* = *H. gemma*, *H. gum* = *H. gummigutta*, *H. gut* = *H. guttavarius*, *H. ind* = *H. indigo*, *H. nig* = *H. nigricans*, *H. pro* = *H. providencianus*, *H. pue* = *H. puella*, *H. uni* = *H. unicolor*, Tan = tan hamlet.

sites in the subdivisions, the power of these analyses to detect significant differences between empirical results and random expectations was reduced.

RESULTS

Data overview

Records included sightings of all 10 morphotypes that have been described as species, as well as the not formally described tan hamlet morphotype (Williams *et al.*, 2006) and records of hybrid forms. Overall abundance, as estimated by the proportion of all sightings, varied considerably among morphotypes (Fig. 2). The proportion of sites occupied by each morphotype was also variable (Fig. 2), but the ranked order of morphotypes for site occupancy was slightly different from that for abundance.

Morphotype distributions

Overall clustering

Analyses of presence/absence for each of the 11 morphotypes produced positive Moran's *I* values, all but one of which were significantly ($P < 0.001$) higher than expected under random conditions. The single exception was *H. gummigutta*, for which the global distribution did not differ from random expectations (Fig. 3). Even morphotypes with extensive distributions such as *H. puella*, *Hypoplectrus nigricans* and *H. unicolor* show some geographical clustering. The non-significant result for *H. gummigutta* is likely to have been affected by the very small number of sites at which this morphotype has been sighted.

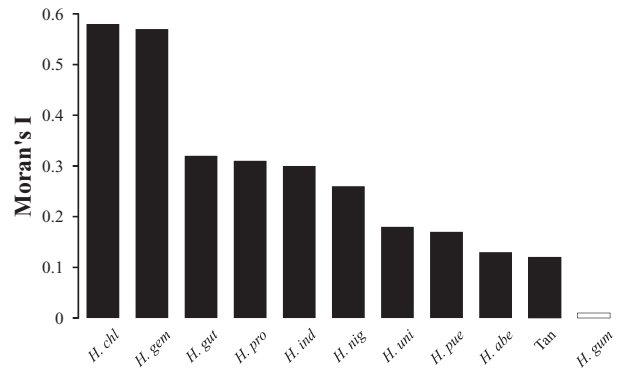


Figure 3 Overall Moran's *I* for *Hypoplectrus* morphotype presence/absence from 1027 Reef Environmental Education Foundation (REEF) fish survey sites across the distribution of the genus. Black: significantly ($P < 0.001$) higher than random expectations. White: not statistically significant from random expectations. Abbreviations: *H. abe* = *H. aberrans*, *H. chl* = *H. chlorurus*, *H. gem* = *H. gemma*, *H. gum* = *H. gummigutta*, *H. gut* = *H. guttavarius*, *H. ind* = *H. indigo*, *H. nig* = *H. nigricans*, *H. pro* = *H. providencianus*, *H. pue* = *H. puella*, *H. uni* = *H. unicolor*, Tan = tan hamlet.

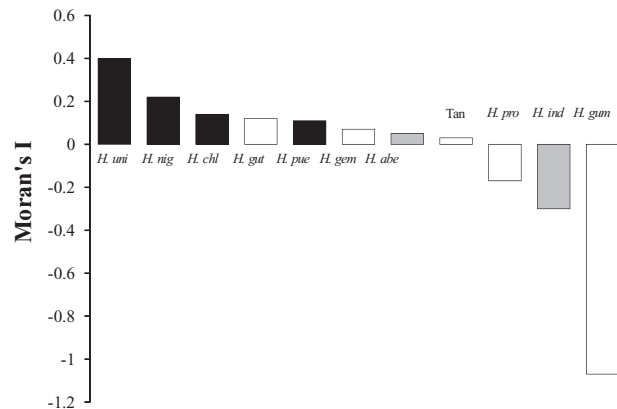


Figure 4 Moran's *I* for *Hypoplectrus* morphotype density from 1027 Reef Environmental Education Foundation (REEF) fish survey sites from throughout the distribution of each morphotype. Black: significantly higher than random expectations at the $P < 0.001$ level. Grey: significant at the $P < 0.05$ level. White: not statistically significant. Abbreviations: *H. abe* = *H. aberrans*, *H. chl* = *H. chlorurus*, *H. gem* = *H. gemma*, *H. gum* = *H. gummigutta*, *H. gut* = *H. guttavarius*, *H. ind* = *H. indigo*, *H. nig* = *H. nigricans*, *H. pro* = *H. providencianus*, *H. pue* = *H. puella*, *H. uni* = *H. unicolor*, Tan = tan hamlet.

Density clustering within morphotype distributions

Five morphotypes showed significantly positive Moran's *I* values (Fig. 4), indicating clustering of high-density sites within the distribution range of these morphotypes. One morphotype, *Hypoplectrus indigo*, yielded a significantly negative Moran's *I* (Fig. 4), suggesting that this morphotype is more uniformly dispersed than expected under random expectations. *Hypoplec-*

trus gummigutta showed a large negative value (Fig. 4), which was marginally non-significant ($P = 0.078$).

All morphotypes showed at least one high-density hotspot site and all but three morphotypes (*H. gummigutta*, *Hypoplectrus guttavarius* and *Hypoplectrus providencianus*) showed at least two hotspot sites (see Appendices S1 to S11 in Supporting Information). Of the five morphotypes that showed a significant Moran's I value, only one, *Hypoplectrus chlorurus*, produced a single cluster of high-density hotspots that does not overlap with similar clusters for other morphotypes. Five morphotypes (*Hypoplectrus aberrans*, *H. indigo*, *H. nigricans*, *H. puella* and the tan hamlet) showed clusters of high-density hotspots in multiple geographical locations. Some of these hotspot clusters for different morphotypes occur in the same locations.

Morphotype co-occurrence

All but one (the Central Bahamas) of the 18 subregions had V ratios that were significantly lower than the mean expected under the CP null model, where sites vary in their capacity to accommodate multiple morphotypes (Table 1a). In addition, all but one subregion (also the Central Bahamas) produced a V ratio lower than the mean expected under the CE null model (where all sites have equal likelihoods of morphotype richness scores), with the difference being significant in 12 cases.

The analysis of the smaller geographical subdivisions showed similar results, with all but one site (again in the Central Bahamas: the Exuma Islands) producing V ratios that were lower than expected under both null models (Table 1b). Fewer of these differences were statistically significant (10/18 cases for the CP null model, 9/18 for the CE null model) as might be expected given that fewer sites were included in these analyses. There were no significant differences between the V ratios produced from observed data in the larger subregion analysis (mean = 0.61 ± 0.17 SD) and those from the matching smaller subdivisions (mean = 0.59 ± 0.26 SD, Wilcoxon signed ranks test, $T = -0.41$, $P = 0.68$). The V ratios for observed data deviated from those produced by CP null models in approximately the same way for both geographical scales (mean $V_{CP} - V_R$, large-scale = 0.48 ± 0.15 SD, small-scale = 0.48 ± 0.25 SD; Wilcoxon signed ranks test, $T = 0.11$, $P = 0.91$). However, the differences between V ratios from empirical data and the CE null model were significantly larger in the small-scale analysis (mean $V_{CE} - V_R = 0.53 \pm 0.29$ SD) than the larger subregion analysis (mean difference = 0.36 ± 0.18 SD; Wilcoxon signed ranks test, $T = -2.33$, $P = 0.02$).

DISCUSSION

Hamlet colour morphotypes are distributed non-randomly. Within the overall range of the genus *Hypoplectrus*, 10 of the 11 morphotypes exhibited highly significant geographical clustering of presence records. However, the distributional patterns seen are not generally consistent with the evolutionary population centre hypothesis (Domeier, 1994) and present-day processes appear to influence hamlet distributions. In 17 of the 18

geographical subregions considered, variation in morphotype co-occurrence was significantly lower than expected under random conditions. This suggests that some form of biotic interaction or niche limitation might restrict the co-existence of some morphotypes.

Abundance and site occupancy

Abundance was highly variable between morphotypes, ranging from *H. puella*, which provided nearly 36% of all sightings, to *H. gummigutta*, which was only reported 14 times in total (Fig. 2). The extent of site occupancy (i.e. the proportion of sites with a given morphotype) also varied greatly between morphotypes but the ranked order for site occupancy did not exactly match the ranked order for abundance (Fig. 2). For example, *H. nigricans* was less abundant than *H. chlorurus* but occurred at far more sites. Therefore, variation in density exists between morphotypes at the sites where they occur.

As well as being abundant, *H. puella* is also the most widespread morphotype and, although it was not sighted everywhere (Fig. 2), it is distributed across the entire region (see Appendix S9). Following an analysis of demographic and genetic data, Puebla *et al.* (2008) suggested that *H. puella* may be the ancestral morphotype of the *Hypoplectrus* radiation. The results of this study do not provide any evidence for the existence of an ancestral form but, if any morphotype is ancestral, then the abundance and distribution of *H. puella* make this colour form the most likely candidate, a possibility first suggested by Thresher (1978).

The spatial patterns of presence/absence support the expectation that most morphotypes have distinct ranges within the overall *Hypoplectrus* distribution. Perhaps more surprising was the fact that even widespread morphotypes, such as *H. puella* and *H. unicolor*, which were assumed to be spread more or less evenly across the Caribbean (Domeier, 1994), show strong evidence of patchy distributions (Fig. 3). For example, although *H. puella* is distributed through the overall range of the genus, it was recorded at nearly 100% of sites only in the Bahamas and in Bermuda (see Appendix S9). All of the other morphotypes were completely unreported from various subregions but there seemed to be little spatial consistency among morphotypes, suggesting that morphotype ranges are not solely limited by biogeographical barriers.

The only morphotype which did not show significant overall clustering was *H. gummigutta* but this morphotype also had very few recorded sightings, which will have compromised our ability to detect clustering. However, sightings of *H. gummigutta* were spread across a large geographical area (see Appendix S4). This morphotype has been reported to occur at greater depths than other morphotypes (Fischer, 1980). This could reduce its likelihood of encounters with divers and result in its under-representation in the REEF dataset. Other rare morphotypes (i.e. *H. aberrans* and the tan hamlet) also showed low clustering values, which suggests that either these morphotypes can persist at low site occupancy rates or perhaps that they exist more commonly in habitats outside those normally visited by scuba divers.

Table 1 *V* ratios for *Hypoplectrus* morphotype co-occurrence within (a) large subregions and (b) smaller subdivisions of the overall distribution of the genus (see text for description of areas).

REEF code	Location	Morphs	Sites	VR	CP	CE
(a) Subregions						
33	SE Florida	7	89	0.78	1.10*	0.99
34	Florida Keys	7	162	0.82	1.11***	0.95
41	N Bahamas	6	25	0.50	1.08*	1.00*
42	Central Bahamas	8	44	1.08	1.17	0.97
44	Turks and Caicos	8	41	0.52	1.08***	0.99**
52	Cayman Islands	9	93	0.67	1.13***	0.98**
54	Mexican Caribbean	6	32	0.60	1.06*	0.95*
55	Belize	5	39	0.66	1.08*	0.99*
57	Honduras	6	25	0.63	1.08*	0.97
62	Dominican Republic	9	21	0.43	1.09**	1.00*
63	Puerto Rico	8	35	0.69	1.13*	0.97
64	US Virgin Islands	7	97	0.52	1.11***	0.98***
65	British Virgin Islands	7	45	0.49	1.11***	0.99**
71	Windward Lesser Antilles	7	27	0.54	1.03**	0.93*
73	Leeward Lesser Antilles	7	48	0.61	1.02**	0.93*
83	Columbia	9	17	0.25	1.02***	0.95**
84	Venezuela	9	24	0.56	1.10*	0.94
85	Netherlands Antilles	7	116	0.62	1.05***	0.96**
(b) Subdivisions						
3301	Jupiter Inlet to Key Biscayne	7	76	0.73	1.09**	0.99*
3410	Dry Tortugas	6	64	0.50	1.07***	0.98***
4101	Bimini	5	15	0.54	1.08	1.94
4209	Exuma Islands	6	19	1.38	1.09	1.89
4412	Grand Turk	9	14	0.35	1.08*	0.99*
5203	Grand Cayman Marine Park	8	34	0.46	1.08**	0.97**
5402	Isla Cozumel	6	22	0.69	1.08	0.96
5505	Glover's reef	5	14	0.85	1.06	1.00
5702	Roatan	5	12	0.35	1.02*	0.98
6203	Parque del Este	9	10	0.62	1.12	1.55
6302	SE Puerto Rico	7	16	0.70	1.10	0.93
6402	St Thomas	7	51	0.43	1.08***	0.98***
6501	Tortola	7	36	0.44	1.08***	0.99**
7112	Dominica	7	15	0.32	1.00*	0.96*
7305	Bequia	6	11	0.71	1.05	1.11
8321	San Andres Island	9	17	0.25	1.02***	0.95**
8402	Los Roques	6	10	0.67	1.06	0.94
8503	Bonaire	7	97	0.61	1.05***	0.96***

REEF, Reef Environmental Education Foundation; VR, *V* ratio for empirical data; CP, mean *V* ratio for randomly generated data in which the probability of morphotypes being assigned to sites was proportional to the number of morphotypes actually found at that site; CE, mean *V* ratio for randomly generated data in which the probability of morphotypes being assigned to sites was equal for all sites.

Asterisks indicate significant differences between randomly generated and observed mean *V*-values, with * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Five of the 11 morphotypes showed significant clustering of density within their own distribution (Fig. 4). However, these clusters do not suggest the existence of evolutionary population centres for these morphotypes because multiple, sometimes overlapping, high-density locations exist for most morphotypes. *Hypoplectrus chlorurus* is the only morphotype that shows a density distribution that is consistent with the population centre hypothesis, with all its hotspots clustered in a single geographical area, the southern Lesser Antilles. *Hypoplectrus unicolor* has many high-density hotspots, which are also all clustered in a

single location, the Florida Keys, but this area also contains high densities of *Hypoplectrus gemma*, which was only found in this region. *Hypoplectrus nigricans* and *H. puella* both have two clusters of hotspot sites and share a high-density cluster location in the US Virgin Islands. *Hypoplectrus aberrans* also shows two hotspot clusters, which are separated by more than 1500 km. The density of *H. indigo* was shown to be uniform throughout its distribution and therefore the high-density hotspots observed for this morphotype are likely to be due to random stochastic effects. The remaining morphotypes did not show any

significant non-random geographical density patterns. Thus, whilst the resolution of this analysis may be affected by the coarseness of the abundance categories used in REEF surveys (see Methods), our analysis provides no broad support for the allopatric population centre hypothesis.

Two main hypotheses can explain the existence of multiple high-density areas. First, some or all high-density locations could represent centres of recruitment rather than centres of origin, and high recruitment could be driven by dispersal or environmental settlement preferences. It seems likely that at least some hotspot areas are the result of high recruitment. For example, the *H. puella* hotspot cluster in Bermuda appears to be due to strong retention of locally spawned larvae (Schultz & Cowen, 1994). The second hypothesis is that some morphotypes may in fact not be monophyletic and that multiple high-density areas are the result of separate evolutionary centres of origin within the same colour form. Domeier (1994) suggested this scenario for *H. aberrans* based on variation in the colour pattern of this morphotype in different regions (which do not correspond with the hotspot cluster areas we identified for this morphotype). In the light of genetic connectivity analyses, Puebla *et al.* (2008) put forward the hypothesis that *H. nigricans* may have evolved repeatedly from *H. puella* in geographically separated areas. However, Puebla *et al.* (2008) also stated that their results could be explained by the differing population densities and distributions of these two morphotypes. Similar distribution patterns were also shown in this study, i.e. *H. nigricans* occurring at lower densities with a more patchy distribution than *H. puella* (see Appendices S7 & S9).

Overall there is little, if any, evidence from our distribution analysis to suggest that evolutionary population centres exist for hamlets. If *Hypoplectrus* morphotypes did indeed evolve under past allopatric conditions, then subsequent dispersal has diluted the effects of this historical isolation.

Morphotype co-occurrence

The *V* ratio analysis revealed that the local co-occurrence of *Hypoplectrus* morphotypes was typically significantly less variable than expected under random conditions. Furthermore, this difference does not seem to be driven by variation in the availability of morphotype larvae, as our analysis of smaller geographical areas produced very similar results. This suggests that morphotype richness within survey sites is not simply a random assortment of the morphotypes that have distributions that include the local area. Therefore, either competitive interactions do appear to exist between morphotypes and/or morphotypes may have distinct ecological niches. Habitat preferences have been suggested for some hamlet morphotypes (Fischer, 1980; Domeier, 1994); however, it is likely that hamlets also compete for the same resources. Generally speaking, hamlets are considered to be sympatric and to feed on similar sources (Whiteman *et al.*, 2007; Holt *et al.*, 2008).

The Central Bahamas and the subdivision of this area which was analysed (the Exuma Islands) were the only areas that produced *V* ratios that were not lower than the mean produced in

random models. If this part of the Caribbean should represent an anomaly for hamlet distribution patterns, then it is unclear why. It is possible that morphotype larval recruitment varies between the numerous small islands in the region.

While morphotype co-occurrence patterns were generally non-random across the spatial scales examined, it is possible that co-occurrence occurs more randomly at very small scales, e.g. within reef systems. This would be consistent with the growing evidence that dispersal in some coral reef fishes is more restricted than previously thought (Cowen & Sponaugle, 2009), which could generate variability in larval availability at small spatial scales. Future research based on specifically designed surveys could consider this possibility.

Comparisons with similar natural study systems

The value of teleost colour polymorphisms as natural systems for the study of evolutionary divergence is well established through the well-known and extensive work carried out with many freshwater cichlid groups. Colour has been a key feature in cichlid taxonomy and has been implicated in driving speciation (Smith & Kornfield, 2002). Within these systems, patterns of morphotype co-occurrence have been suggested as being consistent with sympatric speciation via sexual selection (Seehausen & van Alphen, 1999). Competitive interactions between male colour morphotypes are well documented (Korzan & Fernald, 2007) and competition has also been demonstrated between female morphotypes (Dijkstra *et al.*, 2009). These findings about cichlids may not provide direct insights into the *Hypoplectrus* system, where all individuals are hermaphroditic and planktonic larval dispersal has the potential to transport offspring far away from their parent's immediate environment. Future study should nevertheless consider the potential effects of sexual selection and aggressive interactions on morphotype co-occurrence.

Conclusions

Whilst geographical distributions vary considerably among *Hypoplectrus* morphotypes, there is no evidence to suggest that these patterns are the result of historical isolation and subsequent dispersal. However, this study does provide evidence that patterns of morphotype co-occurrence are non-random. This result suggests that on-going ecological processes may be associated with *Hypoplectrus* colour morphotype distribution patterns. Our study highlights the value of the *Hypoplectrus* species complex as a system for the study of speciation, and future studies of this group should focus on isolating the ecological factors that are influencing current distribution patterns of morphotypes and determining their effects on reproductive isolation between morphotypes.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Density distribution of *Hypoplectrus aberrans*.

Appendix S2. Density distribution of *Hypoplectrus chlorurus*.

Appendix S3. Density distribution of *Hypoplectrus gemma*.

Appendix S4. Density distribution of *Hypoplectrus gummigutta*.

Appendix S5. Density distribution of *Hypoplectrus guttavarius*.

Appendix S6. Density distribution of *Hypoplectrus indigo*.

Appendix S7. Density distribution of *Hypoplectrus nigricans*.

Appendix S8. Density distribution of *Hypoplectrus providencianus*.

Appendix S9. Density distribution of *Hypoplectrus puella*.

Appendix S10. Density distribution of *Hypoplectrus unicolor*.

Appendix S11. Density distribution of the tan hamlet.

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