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# Population genetic analyses of *Hypoplectrus* coral reef fishes provide evidence that local processes are operating during the early stages of marine adaptive radiations

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## Abstract

**Large-scale, spatially explicit models of adaptive radiation suggest that the spatial genetic structure within a species sampled early in the evolutionary history of an adaptive radiation might be higher than the genetic differentiation between different species formed during the same radiation over all locations. Here we test this hypothesis with a spatial population genetic analysis of *Hypoplectrus* coral reef fishes (Serranidae), one of the few potential cases of a recent adaptive radiation documented in the marine realm. Microsatellite analyses of *Hypoplectrus puella* (barred hamlet) and *Hypoplectrus nigricans* (black hamlet) from Belize, Panama and Barbados validate the population genetic predictions at the regional scale for *H. nigricans* despite the potential for high levels of gene flow between populations resulting from the 3-week planktonic larval phase of *Hypoplectrus*. The results are different for *H. puella*, which is characterized by significantly lower levels of spatial genetic structure than *H. nigricans*. An extensive field survey of *Hypoplectrus* population densities complemented by individual-based simulations shows that the higher abundance and more continuous distribution of *H. puella* could account for the reduced spatial genetic structure within this species. The genetic and demographic data are also consistent with the hypothesis that *H. puella* might represent the ancestral form of the *Hypoplectrus* radiation, and that *H. nigricans* might have evolved repeatedly from *H. puella* through ecological speciation. Altogether, spatial genetic analysis within and between *Hypoplectrus* species indicate that local processes can operate at a regional scale within recent marine adaptive radiations.**

*Keywords*: coral reef fishes, distribution and abundance, hamlets, marine evolutionary radiations, population genetics, speciation

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## Introduction

Empirical studies of recent adaptive radiations have provided valuable insights into the process of speciation during the early stages of diversification (Schluter 2000), and have stimulated the development of testable predictions in the quantitative theory of speciation (Gavrilets 2004). For example, the genetically based, spatially explicit model of adaptive radiation developed by Gavrilets & Vose (2005) predicts empirical patterns such as the 'area effect', where larger areas allow for more intensive diversification.

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Another set of predictions generated by this model, illustrated in Figure 5 of Gavrilets & Vose (2005), concerns the levels of genetic structure at neutral markers within and between species. Specifically, it is predicted that the initial phase of adaptive radiation will be marked by more pronounced spatial genetic structure within species ($F_{SC}$ in Gavrilets & Vose 2005) than between species when considered over all locations ($F_{CT}$ in Gavrilets & Vose 2005). It is also predicted that the spatial genetic structure within species will be similar to the spatial genetic structure over populations of all species formed during the same radiation ($F_{ST}$ in Gavrilets & Vose 2005). These two predictions might appear counter-intuitive, particularly in marine systems where gene flow mediated by planktonic

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larvae typically results in low levels of genetic structure within biogeographical regions (Ward *et al.* 1994; Palumbi 1995; Shulman & Bermingham 1995; Planes 1998; Bohonak 1999; but see, e.g. Taylor & Hellberg 2003 for a counter-example). Comparative population genetic analyses of a recent marine radiation at a regional scale would provide one opportunity to test these predictions.

*Hypoplectrus* coral reef fishes (Serranidae) represent one of the few potential cases of a recent adaptive radiation documented in the marine realm (Thresher 1978; Fischer 1980; Graves & Rosenblatt 1980; Domeier 1994; McCartney *et al.* 2003; Ramon *et al.* 2003; García-Machado *et al.* 2004; Puebla *et al.* 2007). These fish, commonly referred to as hamlets, constitute a remarkable system for investigating the relationship of spatial genetic structure within species to genetic divergence between closely related sympatric species. At least 11 colour morphs are recognized, most of which are described as species (e.g. Poey 1852; Acero & Garzón-Ferreira 1994), with as many as eight species observed on a single reef (Fischer 1980). With high fidelity, *Hypoplectrus* species mate assortatively with regard to colour pattern (Fischer 1980; Domeier 1994; Puebla *et al.* 2007). The geographical distribution of the different *Hypoplectrus* species varies dramatically throughout their Caribbean range (Domeier 1994), with some species widely distributed and common, others narrowly distributed and common, and others widely distributed and uncommon, suggesting the interaction of local and regional processes notwithstanding the 3-week, largely passive planktonic larval phase of hamlets (Domeier 1994).

Microsatellite analyses revealed small but highly significant genetic differences between sympatric species (Puebla *et al.* 2007), yet the different hamlet species do not sort into distinct mtDNA haplotype clades, suggesting recent diversification (McCartney *et al.* 2003; Ramon *et al.* 2003; García-Machado *et al.* 2004). Randall & Randall (1960), followed by Thresher (1978), have posited aggressive mimicry as an ecological mechanism responsible, in part, for adaptive radiation in *Hypoplectrus*. According to this hypothesis, predatory hamlets mimic the colour patterns of nonpredatory coral reef fish models and gain a fitness advantage owing to their increased success in the approach and attack of prey. We have recently provided the first empirical example of aggressive mimicry in hamlets (Puebla *et al.* 2007), thus supporting the premise that selection to mimic a range of nonpredatory reef fish models might be one cause of the recent adaptive radiation of hamlets.

Here, we use microsatellite analyses of *Hypoplectrus puella* (barred hamlet) and *Hypoplectrus nigricans* (black hamlet) from Belize, Panama and Barbados to test whether the spatial genetic structure within species sampled early in the evolutionary history of an adaptive radiation can be higher than the genetic differentiation between species overall locations, as predicted by Gavrilets & Vose (2005). We complement genetic analyses with an extensive field survey of *Hypoplectrus* population densities and individual-based simulations to explore how the combined effect of geographical distribution and relative abundance can potentially influence levels of spatial population genetic structure within *H. puella* and *H. nigricans*.

## Methods

We focused our analyses on *Hypoplectrus puella* and *Hypoplectrus nigricans* because these morphs are abundant and ubiquitous throughout the Caribbean (Doemeier 1994), thus permitting comparative analysis at a regional scale. Transect surveys at our three study sites in Belize, Panama and Barbados showed that only these two *Hypoplectrus* species were present concurrently at the three sites.

### *Sampling and genotyping*

Collecting, export, and import permits were obtained prior to fieldwork. *H. puella* and *H. nigricans* samples were collected with microspears while SCUBA-diving over coral reefs at depths ranging between 1 m and 30 m. Given the clear phenotypic difference between *H. puella* and *H. nigricans* (Fig. 2), samples could be confidently identified as belonging to either species on the basis of colour pattern. On rare occasions (0.6% and 0.5% of all *H. puella*–*H. nigricans* individuals from our transects and collection, respectively), individuals with intermediate colour patterns (i.e. black with vertical bars) were observed, suggesting ongoing gene flow between the two species. Only individuals whose identification was unequivocal were considered in the analyses. Samples were collected in the vicinity of Carrie Bow Cay (Belize) in July 2004, in Bocas del Toro (Panama) in March 2004 and along the west coast of Barbados in June 2005 (Fig. 1). In order to assess the potential effect of temporal genetic variation, an additional *H. puella* sample was collected in Bocas del Toro in March 2005.

Gill tissue samples were preserved in salt-saturated DMSO for genetic analyses and each fish was accessioned and stored as voucher specimen in the Neotropical Fish Collection (Bermingham *et al.* 1997) at the Smithsonian Tropical Research Institute in Panama. Specimens, DNA samples and photographs of all fish considered in this study are available upon request.

Total DNA was extracted using column DNeasy Tissue Kits (QIAGEN) and genotyped at 10 microsatellite loci (McCartney *et al.* 2003; Puebla *et al.* 2007). A detailed description of the 10 microsatellite markers and associated polymerase chain reaction (PCR) cycling conditions is provided in Puebla *et al.* (2007). Amplified fragments were separated by capillary electrophoresis on an ABI PRISM

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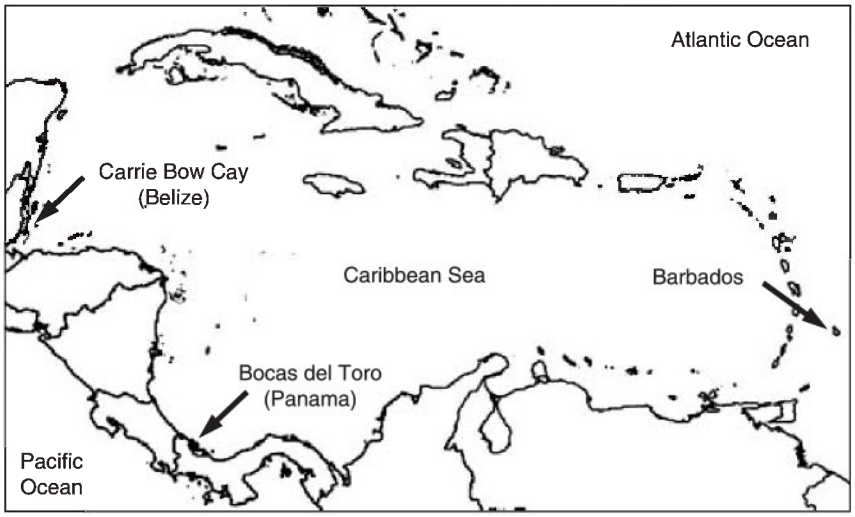


**Fig. 1** The three study sites considered in the present study. Field work was conducted in the vicinity of Carrie Bow Cay (Belize), Bocas del Toro (Panama) and along the west coast of Barbados.

3130xl automated genetic analyser (Applied Biosystems) with 500 LIZ size standard and analysed with the software GENEMAPPER (Applied Biosystems).

### *Genetic data analysis*

Linkage disequilibrium between all pairs of loci was tested over all samples with a total of 4500 genotype randomizations using the log-likelihood ratio *G*-statistic as implemented in FSTAT version 2.9.3 (Goudet 1995). Number of alleles, allelic richness (El Mousadik & Petit 1996), expected heterozygosity (Nei 1987), observed heterozygosity and $F_{IS}$ (Weir & Cockerham 1984) were estimated per locus and site. Hardy–Weinberg equilibrium within species was tested by site for each locus with 1000 allele randomizations per test, using $F_{IS}$ as test statistic. Allelic richness was also estimated over all sites for *H. puella* and *H. nigricans* independently, and a one-sided randomization test was performed to test whether allelic richness was significantly higher in *H. puella* than in *H. nigricans* as implemented in FSTAT with 10 000 randomizations.

Global $F_{ST}$ among the six samples was estimated over all loci following Weir & Cockerham (1984) with FSTAT. The same procedure was used to estimate (i) genetic differentiation between *H. puella* and *H. nigricans* over all sites, (ii) spatial genetic structure over all sites within *H. puella* and *H. nigricans* independently, and (iii) pairwise genetic differentiation between sites within *H. puella* and *H. nigricans* independently. Genetic differentiation over all loci was tested for significant departure from zero following the same scheme with *G*-tests (Goudet *et al.* 1996) as implemented in FSTAT. Testing procedures involved 10 000 among-sample genotype permutations and sequential Bonferroni corrections for multiple, pairwise tests (Rice 1989). Lastly, a one-sided randomization test was performed with FSTAT to test whether the spatial $F_{ST}$ estimate within *H. puella* was significantly higher than within *H. nigricans* (10 000 randomizations).

$F_{ST}$ estimates between sympatric *H. puella* and *H. nigricans* are reported in Puebla *et al.* (2007). Here, the Bayesian clustering method developed by Pritchard *et al.* (2000) was used to test whether *H. puella* and *H. nigricans* constitute distinct genotypic clusters in sympatry and whether the degree of clustering between these two species varies spatially. STRUCTURE version 2.0 (Pritchard *et al.* 2000) was used with admixture and correlated allele frequencies models (Falush *et al.* 2003). Each run consisted of $2 * 10^6$ burn-in steps followed by $10^6$ MCMC steps. The analysis was performed for each site independently with the number of presumed clusters ($K$) set to 1–5, and with all samples pooled together with $K$ set to 1–10. Ten runs were performed for each value of $K$ and the analysis was repeated within each identified cluster to insure that no subclustering is missed. Estimates of the posterior probability of the data Pr($X|K$) (Pritchard *et al.* 2000) and $\Delta K$ (Evanno *et al.* 2005) were used as model choice criteria to infer the number of genetic clusters present in the data set.

### *Population densities*

In an effort to complement genetic analyses with demographic data, *H. puella* and *H. nigricans* densities were assessed in the field. Two divers surveyed nonoverlapping 100 * 4 m transects at depths ranging from 3 m to 25 m, with each diver counting all *H. puella* and *H. nigricans* adults observed within 2 m on each side of a 100 m transect tape. A total of 94 000 m$^2$ of coral reef from 15 sites were surveyed, including our three study sites (Belize, $n = 29$ transects; Panama, $n = 40$ and Barbados, $n = 21$), four sites along the Caribbean coast of Honduras (Utila, $n = 12$; Guanaja, $n = 16$; Cayos Becerros, $n = 15$; and Cayos de Media Luna, $n = 12$) and eight sites along the Kuna Yala

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coast in Panama (El Porvenir, $n = 9$; Cayos Holandeses, $n = 12$; Islas Puyadas, $n = 12$; Cayos Ratones, $n = 12$; Cayos Ingleses, $n = 12$; Achutupu, $n = 12$; Isla Dupak, $n = 9$ and Islas Sasardi, $n = 12$).

A two-sided Wilcoxon signed ranks test was performed to test whether *H. puella* and *H. nigricans* densities were significantly different, with each data pair consisting of *H. puella* and *H. nigricans* densities from one transect. This test was performed with all the data (235 transects), as well as exclusively with the Belize, Bocas del Toro and Barbados data (90 transects) matching our microsatellite analyses.

### *Simulations*

We developed a simple individual-based simulation model to explore whether the observed differences in distribution and abundance between *H. puella* and *H. nigricans* could account for the observed difference in spatial genetic differentiation between the two species. The lower abundance of *H. nigricans* and its less continuous distribution in the Caribbean are both expected to increase spatial genetic differentiation. A one-dimensional lattice model of population structure was adopted, the lattice consisting of demes regularly spaced along a circle with a distance $d$ between adjacent demes. All demes had the same, constant effective population size of $N_e$ diploid simultaneous hermaphrodites (hamlets are simultaneous hermaphrodites). Generations were discrete and all individuals mated randomly at each generation, the next generation being a random sample from the pool of zygotes. Zygotes dispersal was implemented as a Laplace distribution, where the proportion of zygotes produced at position $x$ dispersing to position $y$ is given by $P(x, y) = (\alpha/2)\, e^{-\alpha|x-y|}$. This distribution is an exponentially decreasing and symmetrical function of distance, with $1/\alpha$ as the mean dispersal distance. The Laplace dispersal function is more leptokurtic than the normal distribution and allows more long-distance dispersal. It has also been used in one-dimensional models of marine dispersal (Botsford *et al.* 2001; Palumbi 2003). Microsatellite loci were modelled with 20 alleles, which corresponds to the average number of alleles observed at the 10 microsatellite loci analysed (Supplementary Table S1). A *K*-alleles model of mutation was adopted with alleles mutating into any of the other $k - 1$ alleles with probability $\mu/k - 1$, where $\mu$ is the mutation rate.

The combined effect of distribution and abundance on genetic differentiation was explored by varying the distance between demes $d$ and the effective population size $N_e$. The distance between demes varied between one and five times the mean dispersal distance and the effective population size between 100 and 500. The total number of demes in the lattice was set to 100 and 3 demes were sampled by randomly selecting 50 individuals, corresponding to our sampling of 50 individuals per species in Belize, Panama and Barbados. Simulations were run for 1000 generations with equal initial allele frequencies of 0.05. Ten unlinked loci were simulated by using results from 10 simulations for each set of parameters. The mutation rate $\mu$ was set to $5 * 10^{-4}$ per meiosis, which is representative of microsatellite loci (Ellegren 2000). Global $F_{ST}$ and 95% confidence intervals over all samples and loci were estimated following the same procedure used for the analysis of our actual genetic data (see above).

## Results

### *Basic statistics*

A total of 331 individuals were collected and genotyped at the 10 microsatellite loci, with less than 1.5% missing data overall (failed or poor amplification of a locus for a given individual) and an average of 47.3 individuals per sample (min = 35, max = 50, Table S1, Supplementary material). Number of alleles, allelic richness, expected and observed heterozygosity, $F_{IS}$ and proportion of randomizations that generated $F_{IS}$ estimates larger and lower than observed are presented in Table S1. There was no evidence of linkage disequilibrium between any pair of loci, and all loci were at Hardy–Weinberg equilibrium within all samples at the 0.05 level after sequential Bonferroni correction for multiple tests. Without corrections for multiple tests, five of the 140 tests performed (10 loci × 7 samples, heterozygote deficit and excess) were significant at the 0.05 level (four heterozygote deficits and one excess).

### *Genetic structure within and between* H. puella *and* H. nigricans

The $F_{ST}$ estimate between the two *H. puella* samples from Bocas del Toro collected in March 2004 and March 2005 was 0.001 and was not significantly different from zero (see Puebla *et al.* 2007). Therefore, temporal genetic variation between 2004 and 2005 is not expected to affect our conclusions. In order to keep sample sizes even (see Table S1), we only considered the 2004 *H. puella* sample from Bocas del Toro for our analyses, yet our results are robust to using the 2004, 2005 or both samples pooled together (data not shown).

The global $F_{ST}$ estimate among the six samples was 0.027 (95% confidence interval, 0.018–0.035) and the $F_{ST}$ estimate between *H. puella* and *H. nigricans* over all sites was 0.010 (95% confidence interval, 0.006–0.014), both highly significant ($P < 0.001$). $F_{ST}$ estimates between sites and allelic richness for *H. puella* and *H. nigricans* independently are presented in Table 1. Both *H. puella* and *H. nigricans* presented highly significant spatial genetic structure, overall and considering all pairwise comparisons ($P < 0.001$). Spatial genetic differentiation was significantly lower for

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**Table 1** Differences in spatial genetic structure (Weir & Cockerham 1984), allelic richness (El Mousadik & Petit 1996), abundance and distribution between *Hypoplectrus puella* (barred hamlet) and *Hypoplectrus nigricans* (black hamlet) in the wider Caribbean. Genetic parameters estimated on the basis of 50 samples per species, per site genotyped at 10 microsatellite loci, densities on the basis of 235 SCUBA transects covering a total of 94 000 m² of reef

| | *H. puella* | *H. nigricans* |
|---|---|---|
| $F_{ST}$ estimate between Belize, Panama and Barbados (95% CI) | 0.006*** (0.003–0.011) | 0.047*** (0.030–0.064) |
| $F_{ST}$ estimate between Belize and Panama (95% CI) | 0.007** (0.001–0.013) | 0.022*** (0.010–0.037) |
| $F_{ST}$ estimate between Belize and Barbados (95% CI) | 0.004** (0.001–0.007) | 0.063*** (0.034–0.090) |
| $F_{ST}$ estimate between Panama and Barbados (95% CI) | 0.008*** (0.002–0.018) | 0.059*** (0.034–0.084) |
| Allelic richness in Belize, Panama and Barbados | 6.70 | 5.87 |
| Mean density/100 m² in Belize, Panama and Barbados ± std. error | 1.74 ± 0.15 | 0.97 ± 0.10 |
| Mean density/100 m² at 15 sites in the Caribbean ± std. error | 1.40 ± 0.08 | 0.53 ± 0.05 |
| Number of occurences in the Caribbean (Domeier 1994) | 30 | 16 |
| Percent of hamlets in the Caribbean (Domeier *et al.* 1994) | 30.3 | 15.5 |

CI: confidence interval. **, ***: significant genetic structure at the 0.01 and 0.001 level respectively.

*H. puella* (global $F_{ST}$ estimate = 0.006) than for *H. nigricans* (global $F_{ST}$ estimate = 0.047, randomization test $P$ value = 0.0489), and the difference in spatial genetic differentiation between the two species was consistent across pairwise comparisons. Overall, allelic richness was significantly higher in *H. puella* (6.70) than in *H. nigricans* (5.87, randomization test $P$ value = 0.0358).

### *Clustering analyses*

Mean Pr ($K = 2$) from the 10 replicated analyses of the Barbados data was 0.701, larger than for any other value of $K$ (between 0.000 and 0.200). Similarly, $\Delta K$ was larger for $K = 2$ (4.675) than for any other value of $K$ (between 1.408 and 1.493). The pattern of clustering was consistently similar to the one illustrated in Fig. 2A and no subclustering was evidenced when the analysis was repeated within *H. puella* and *H. nigricans* independently (data not shown). Altogether, these results indicate that *H. puella* and *H. nigricans* from Barbados constitute two genotypic clusters in sympatry.

Mean Pr ($K = 2$) in Belize was 0.302, which was also larger than for any other value of $K$ (between 0.100 and 0.219). Nevertheless, this difference was less pronounced than in Barbados, $\Delta K$ presented no marked peak (all values ranged between 1.183 and 1.851) and no pattern of clustering emerged (Fig. 2C).

Sympatric *H. puella* and *H. nigricans* genotypes from Panama were characterized by an intermediate pattern of clustering. Mean Pr ($K = 2$) was 0.501 while this probability ranged between 0.000 and 0.275 for the other values of $K$ tested. $\Delta K$ did not present a marked peak (all values ranged between 0.914 and 1.146), yet a pattern of clustering emerged (Fig. 2B). Thus, clustering analyses in Belize, Barbados and Panama reveal considerable spatial variability in levels of genetic differentiation between sympatric *H. puella* and *H. nigricans* (Fig. 2).

When all samples where pooled together mean Pr ($K = 2$) was 0.600 while this probability ranged between 0.000 and 0.200 for the other values of $K$ tested. $\Delta K$ was also higher for $K = 2$ (4.550) than for any other value of $K$ (between 1.191 and 2.226), confirming the existence of two genotypic clusters. The pattern of clustering indicated that one group consisted of *H. nigricans* from Barbados and the other of the rest of the data (Fig. S1). A pattern of subclustering was nevertheless apparent within this last group, with two subclusters corresponding approximately to *H. puella* and *H. nigricans*, and this pattern was confirmed when the analysis was run within this group (data not shown). Altogether, these results indicate that *H. nigricans* from Barbados are genetically distinct from *H. nigricans* from Belize and Panama.

### *Population densities*

Estimates of *H. puella* and *H. nigricans* densities from our 15 Caribbean sites were 1.40 ± 0.08 and 0.53 ± 0.05 adults per 100 m² of reef, respectively (Table 1), and the difference in density between *H. puella* and *H. nigricans* was highly significant (Wilcoxon signed ranks test $P$ value < 0.001). This was the case whether all 15 sites were included in the analysis or only Barbados, Bocas del Toro and Belize.

### *Potential effects of distribution and abundance on genetic differentiation*

Results from the simulations are summarized in Fig. 3. Genetic differentiation between samples increased with decreasing effective population size $N_e$ and with distance between demes $d$. The combined effect of distribution and abundance on genetic differentiation resulted in a nonlinear response of genetic differentiation (Fig. 3), potentially leading to disproportionate effects of combined

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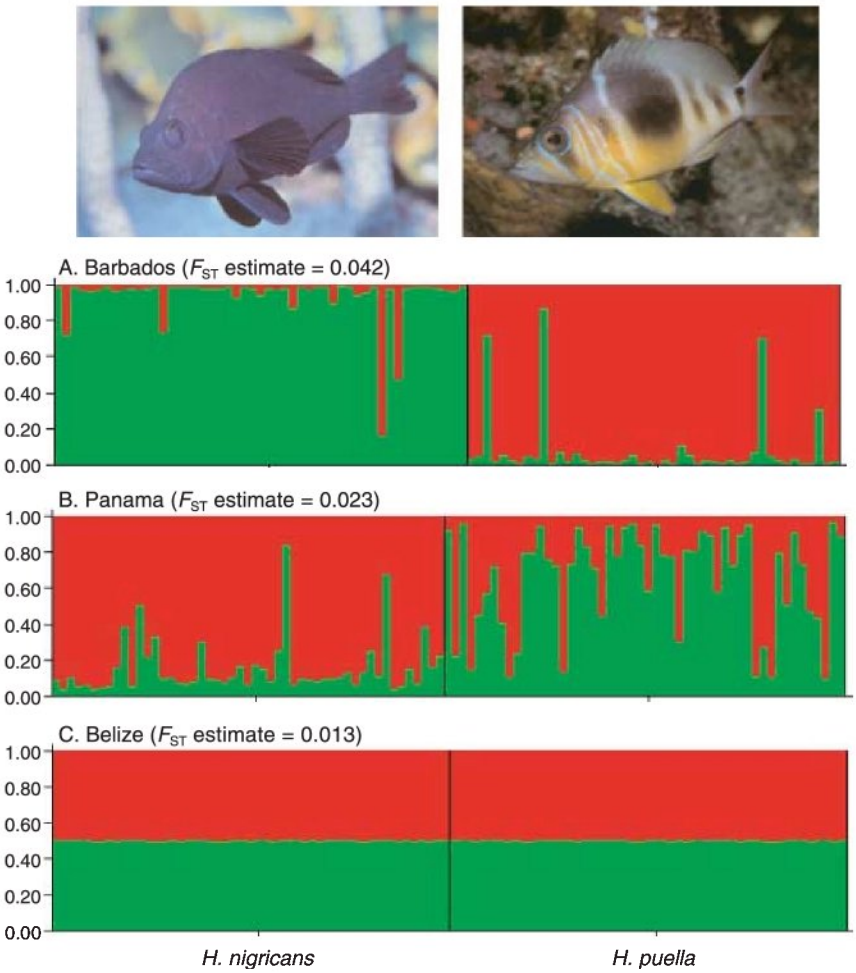


**Fig. 2** Graphical summary of clustering analysis of sympatric *Hypoplectrus nigricans* (black hamlet, left) and *Hypoplectrus puella* (barred hamlet, right) genotypes from Belize, Panama and Barbados. Genotypes from 50 individuals per species, per site scored at 10 microsatellite loci. Each individual is represented by a vertical line broken into two segments representing the estimated proportion of the individual's genome originating from each inferred cluster. Two clusters were clearly evidenced in Barbados, constituted at 94% and 98% of *H. puella* and *H. nigricans* genotypes, respectively. Sympatric clusters were less clearly defined in Panama, and not detected at all in Belize. These analyses illustrate the spatial variation in levels of genetic differentiation between sympatric *H. puella* and *H. nigricans*. $F_{ST}$ estimates from Puebla *et al.* (2007), all highly significant. Photographs with permission from Reef Fish Identification, New World Publications, © 2002, Paul Humann.

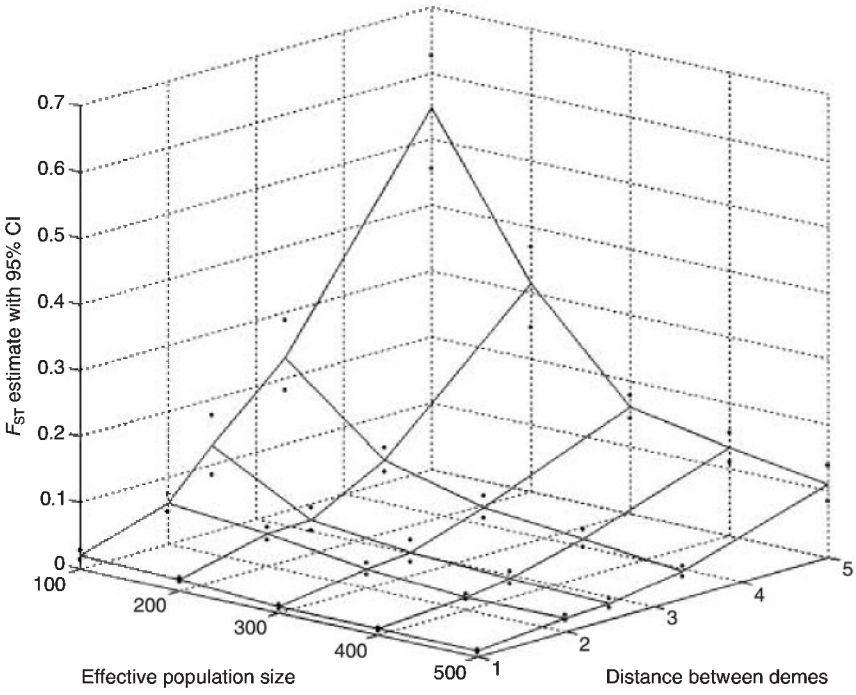


**Fig. 3** Combined effect of distribution (distance between adjacent demes *d*) and abundance (effective population size $N_e$) on spatial genetic differentiation ($F_{ST}$ estimate with 95% confidence interval). Individual-based simulations show that assuming dispersal limitation in a lattice model of population structure, the combined effect of the observed differences in distribution and abundance between *Hypoplectrus puella* and *Hypoplectrus nigricans* (Table 1) can potentially account for the observed difference in spatial genetic differentiation between these two species (Table 1). $F_{ST}$ estimated following Weir & Cockerham (1984) on the basis of three samples of 50 genotypes each consisting of 10 simulated microsatellite loci.

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changes in population size and distribution. For example, the combined effect of a twofold change in population size and interdeme distance (e.g. increasing $N_e$ from 200 to 400 and decreasing $d$ from 2 to 1 mean dispersal distance) generated a > fivefold decrease in $F_{ST}$ estimate, from 0.049 (95% CI 0.039–0.059, $N_e = 200$, $d = 2$) to 0.008 (95% CI 0.006–0.010, $N_e = 400$, $d = 1$).

## Discussion

### *Genetic differentiation within and between species*

Our genetic analyses reveal that the spatial genetic structure within *Hypoplectrus nigricans* ($F_{ST}$ estimate = 0.047) is higher than the genetic differentiation between *Hypoplectrus puella* and *H. nigricans* over all locations ($F_{ST}$ estimate = 0.010). This difference appears to be significant given that the 95% confidence intervals of the two $F_{ST}$ estimates do not overlap (nor do the 99% confidence intervals overlap, data not shown). This result is consistent with the spatially explicit model of adaptive radiation developed by Gavrilets & Vose (2005), which predicts that early in the evolutionary history of an adaptive radiation, the spatial genetic structure within species would be higher than the genetic differentiation between species over all locations at neutral markers. The spatial genetic structure within *H. nigricans* is also higher than the genetic structure over all samples ($F_{ST}$ estimate = 0.027), yet this difference does not appear to be significant as the confidence intervals of the $F_{ST}$ estimates overlap. This is also the case when the within-species comparisons are removed from the estimate of genetic structure over all samples by considering only the pairwise $F_{ST}$ estimates between populations of different species (mean $F_{ST}$ estimate = 0.028, SE = 0.005, $n = 9$ pairwise comparisons). This result is also consistent with Gavrilets & Vose (2005), who predicted that the genetic structure among populations of one species involved in an adaptive radiation will be similar to the genetic structure over populations of different species formed during the same radiation.

Although these patterns of genetic structure within and between species might appear counter-intuitive, they can be explained by the occurrence of gene flow between sympatric species that reduces genetic differentiation at neutral markers (Gavrilets & Vose 2005). This is consistent with spawning observations of *Hypoplectrus* in the wild, which suggest ongoing gene flow between species, notably between *H. puella* and *H. nigricans* (Puebla *et al.* 2007).

Nonetheless, the spatial genetic structure within *H. puella* is significantly lower than the spatial genetic structure within *H. nigricans*, and appears incompatible with the model of adaptive radiation proposed by Gavrilets & Vose (2005). The spatial genetic structure within *H. puella* ($F_{ST}$ estimate = 0.006) is lower than the genetic differentiation between *H. puella* and *H. nigricans* over all locations. It is also lower than the genetic structure over all samples, and this difference appears to be significant as the 95% confidence intervals of the two $F_{ST}$ estimates do not overlap (nor do the 99% confidence intervals overlap, data not shown). The difference in spatial genetic structure between *H. puella* and *H. nigricans* is striking given that the two species were collected concurrently at the same sites, and were analysed with similar sample sizes at the same microsatellite loci.

### *Lower genetic structure within* H. puella*: possible causes*

The higher spatial genetic structure within *H. nigricans* parallels spatial variation in body size, morphology and colouration reported within this species. Aguilar-Perera (2004) observed that *H. nigricans* from Puerto Rico are grayish with transparent pectoral fins and long, pointed pelvic fins, whereas *H. nigricans* from Mexico and Belize are smaller, darker, with dark pectoral fins and short, rounded pelvic fins. A detailed phenotypic analysis of our samples is beyond the scope of this study, but it is worth noting that we observed some striking differences between *H. nigricans* from different sites as well. Individuals that we collected in Barbados were generally large (SL = 10.9 ± 0.7 cm), brownish (including the colour of the pectoral fins) with long and pointed pelvic fins, whereas individuals from Panama tended to be smaller (SL = 7.6 ± 0.7 cm), pale black with transparent pectoral fins and short, rounded pelvic fins. The *H. nigricans* that we collected in Belize (SL = 7.5 ± 0.8 cm) matched the description of Belize fish provided by Aguilar-Perera (2004). Nevertheless, variation in colour pattern has also been reported for *H. puella* (Thresher 1978), and size differences were observed between the *H. puella* samples that we collected from the different sites (SL = 9.9 ± 0.8 cm in Barbados, 7.5 ± 0.7 cm in Panama and 7.9 ± 0.8 cm in Belize) as well. Thus, it seems that there is no straightforward relation between spatial phenotypic and genotypic variation in this case, which motivated additional analyses aimed at understanding the difference in population genetic structure between *H. puella* and *H. nigricans*.

One hypothesis would be that the lower spatial genetic structure within *H. puella* is due to genetic drift. *H. puella* seems to be about twice as abundant and ubiquitous as *H. nigricans* in the tropical western Atlantic (Table 1). This demographic difference between *H. puella* and *H. nigricans* appears to be robust as it is based on field surveys performed at 33 sites in the Caribbean (Doemeier 1994) and 235 SCUBA transects covering a total of 94 000 m$^2$ of coral reefs at 15 sites in the tropical western Atlantic (this study). The demographic difference between the two species could explain the significantly higher allelic richness observed within *H. puella* than within *H. nigricans*, and

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individual-based simulations show that distribution and abundance can have synergistic and disproportionate effects on genetic differentiation (Fig. 3). This last result is consistent with analyses of simpler, analytically tractable models of population structure such as the stepping stone model (Kimura & Weiss 1964) consisting of a circular array of $d$ demes with migration rate $m/2$ between adjacent demes. Slatkin (1991) showed that considering a pair of samples from two demes separated by $i$ steps, $F_{ST}(i) \approx 1/(1 + 8N_e m/i)$ at a single neutral locus with two alleles, assuming $i \ll d$ and low mutation rates. The combined effect of $N_e$ and $i$ on $F_{ST}(i)$ results in a nonlinear response of genetic differentiation similar to the one represented in Fig. 3. Our numerical approach differs somewhat from Slatkin's analysis as the distance between samples was kept constant in order to explore the relative importance of distribution and abundance while controlling for the effect of the sampling scheme. Moreover, our simulations show that the synergistic effect of distribution and abundance on genetic differentiation holds when long-distance dispersal (i.e. between nonadjacent demes) is considered. Although effective population sizes and dispersal patterns considered in our simulations are crude simplifications, they suggest that the difference in distribution and abundance between *H. puella* and *H. nigricans* can account for the observed differences in spatial genetic differentiation between these two species.

Another hypothesis for the difference in spatial genetic structure between *H. puella* and *H. nigricans* would be that *H. nigricans* evolved from *H. puella* through ecological speciation independently in Barbados, Panama and Belize. Randall & Randall (1960) and Thresher (1978) posited aggressive mimicry as an ecological mechanism underlying the origin of *H. nigricans*, and we have recently demonstrated the potential for sympatric speciation in *Hypoplectrus* based on our studies of colour-based assortative mating and disruptive selection on colour pattern (Puebla *et al.* 2007). Moreover, Thresher (1978) speculated that the ancestral *Hypoplectrus* morph was probably close to present-day *H. puella*. This species does not appear to be an aggressive mimic as no putative model has been identified. Furthermore, the Caribbean-wide survey performed by Domeier (1994) suggests that *H. puella* is the most abundant and ubiquitous *Hypoplectrus* species in the tropical western Atlantic, and our data reinforces this finding across the sites described here. We show in addition that *H. puella* have a significantly higher allelic richness than *H. nigricans*. Together, these observations are consistent with the idea put forward by Thresher (1978) that *H. puella* might represent the ancestral form. *H. nigricans* could therefore have evolved from *H. puella* through ecological speciation independently in Barbados, Panama and Belize, providing an explanation for the different levels of genetic differentiation between sympatric *H. puella* and *H. nigricans* at the three locations (Fig. 2), the increased genetic structure within *H. nigricans* (Table 1), and the fact that *H. nigricans* from Barbados are genetically distinct from *H. nigricans* from Belize and Panama (Fig. S1).

### *Evidence that local processes are operating*

Distinguishing between genetic drift and repeated sympatric speciation is not simple as these two scenarios are not mutually exclusive in open systems. Independent origins of *H. nigricans* through repeated sympatric speciation in Barbados, Panama and Belize could have been followed by limited gene flow between sites or, conversely, genetic drift within species could have been exacerbated by local evolutionary processes in Barbados, Panama and Belize. Nonetheless, the two hypotheses share one attribute: they both involve local processes. Both repeated sympatric speciation and genetic drift imply limited gene flow and subsequent differentiation between locations. This is clearly the case in the spatially explicit model of adaptive radiation developed by Gavrilets & Vose (2005), where migration occurs between adjacent patches only. Our results demonstrate that both *H. puella* and *H. nigricans* manifest highly significant genetic differences between sites, and the absence of significant genetic difference between the two *H. puella* samples collected in consecutive years in Bocas del Toro shows that our methodology does not detect significant genetic differences between samples when none are anticipated. Thus, the spatial genetic structure evidenced within *H. puella* and *H. nigricans* does not appear to be an artefact of the microsatellite analyses. The occurrence of highly significant spatial genetic structure within *H. puella* and *H. nigricans* shows that these two species are not panmictic at the Caribbean scale despite the potential for gene flow provided by their 3-week planktonic larval phase (Domeier 1994). In this respect, it should be noted that our three study sites (Fig. 1) are located in different connectivity regions identified by Cowen *et al.* (2006) on the basis of their biophysical model of the Caribbean region, namely the western Caribbean (Belize), the Panama-Colombian Gyre subregion (Panama) and the eastern Caribbean (Barbados). Thus, restricted dispersal between these three locations might be explained by the occurrence of major biogeographical breaks separating these regions (Cowen *et al.* 2006).

Furthermore, the spatial variation in levels of clustering we report between sympatric *H. puella* and *H. nigricans* in Barbados, Panama and Belize (Fig. 2) shows that local evolutionary processes are operating within the *Hypoplectrus* radiation. This variation in levels of clustering parallels spatial variation in $F_{ST}$ estimates and in the number of loci presenting significant differences between sympatric *H. puella* and *H. nigricans* (Puebla *et al.* 2007). The spatial variation in genetic differentiation between sympatric

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*H. puella* and *H. nigricans* appears to be significant given that the 95% confidence intervals of the $F_{ST}$ estimates from Belize and Barbados do not overlap (Puebla *et al.* 2007). Note that *H. puella* and *H. nigricans* from Belize present low but highly significant genetic differences at the population level (Puebla *et al.* 2007). Thus, the absence of clustering illustrated in Fig. 2(C) probably reflects a lack of power rather than a total absence of genetic differences between *H. puella* and *H. nigricans* from Belize. This is further suggested by microsatellite analyses of human populations showing how tens to hundreds of loci can be required to identify genetic clusters at the individual level when genetic differentiation is low (Rosenberg *et al.* 2002). It is also worth noting that sympatric *H. nigricans* and *H. puella* present the strongest levels of clustering in Barbados, which is probably the smallest and most isolated population owing to the size and location of the island. In contrast, the same species show the lowest levels of clustering in Belize, probably the largest population considering the size of the Belize Barrier Reef. These observations suggest that the spatial difference in levels of genetic differentiation between sympatric *H. nigricans* and *H. puella* could be associated with differences in population sizes, as small populations might sometimes respond faster to selection (Wade & Goodnight 1998; Gavrilets & Gibson 2002). This should not be confounded with the 'area effect' (Gavrilets & Vose 2005), where larger areas allow for more intensive diversification. The *Hypoplectrus* radiation appears to be consistent with this other prediction of the Gavrilets & Vose (2005) model of adaptive radiation as seven hamlet species were observed in the extensive Belize Barrier Reef vs. only four species in the spatially restricted Barbados reefs (Puebla *et al.* 2007).

## Conclusions and perspectives

This study demonstrates that several predictions generated by the genetically based, spatially explicit model of adaptive radiation developed by Gavrilets & Vose (2005) are verified in the *Hypoplectrus* radiation. The specific biology underlying different adaptive radiations appears nevertheless to limit the generality of predictions. We provided two examples with the demographic difference between *Hypoplectrus puella* and *Hypoplectrus nigricans*, and the possibility that one of the species included in our analyses (*H. puella*) might be the ancestral form of the *Hypoplectrus* radiation. The available data suggest that two fundamental aspects of this model, namely ongoing gene flow between sympatric species and dispersal limitation, are at work in the *Hypoplectrus* radiation. Altogether, spatial genetic analysis within and between *Hypoplectrus* species show that local processes can operate within recent marine adaptive radiations despite the potential for high levels of gene flow between populations provided by occurrence of long planktonic larval stages. Marine adaptive radiations might therefore consist of an assemblage of spatially semi-isolated breeding groups (Fig. 4B) rather than one large, panmictic entity (Fig. 4A).

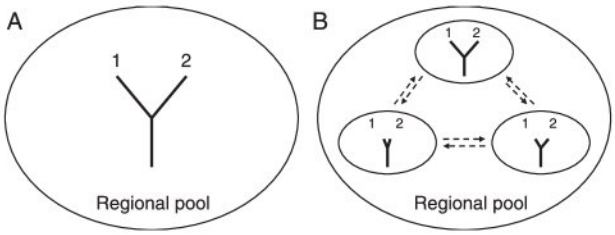


**Fig. 4** Two perspectives on recent marine adaptive radiations (only two species are represented for the sake of clarity). If the regional pool is panmictic (A), no spatial genetic structure within species is anticipated and the pattern of genetic divergence between species 1 and 2 is expected to be similar at different locations. If gene flow is restricted between populations (B, dashed arrows), spatial genetic structure within species is expected and the pattern of genetic divergence between species 1 and 2 might present spatial variation.

Our spatial analyses were limited to *H. puella* and *H. nigricans* as only these two *Hypoplectrus* species were present concurrently at our three study sites. Microsatellite data of other *Hypoplectrus* species such as *H. chlorurus* (yellowtail hamlet), *H. aberrans* (yellowbelly hamlet), *H. indigo* (indigo hamlet), and *H. unicolor* (butter hamlet) from McCartney *et al.* (2003) and Puebla *et al.* (2007) indicate that the levels of genetic differentiation between sympatric *H. puella* and *H. nigricans* reported in this study (Fig. 2) are representative of the genus. However, genetic and ecological data on additional species and locations are required for a comprehensive understanding of the process of radiation in *Hypoplectrus* and other marine groups.

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## Supplementary material

The following supplementary material is available for this article:

**Fig. S1** Graphical summary of clustering analysis of *H. nigricans* (black hamlet) and *H. puella* (barred hamlet) genotypes from Belize, Panama and Barbados pooled together.

**Table S1** Summary statistics per sample of the 10 loci considered in this study

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