

# Colour pattern as a single trait driving speciation in *Hypoplectrus* coral reef fishes?

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Theory shows that speciation in the presence of gene flow occurs only under narrow conditions. One of the most favourable scenarios for speciation with gene flow is established when a single trait is both under disruptive natural selection and used to cue assortative mating. Here, we demonstrate the potential for a single trait, colour pattern, to drive incipient speciation in the genus *Hypoplectrus* (Serranidae), coral reef fishes known for their striking colour polymorphism. We provide data demonstrating that sympatric *Hypoplectrus* colour morphs mate assortatively and are genetically distinct. Furthermore, we identify ecological conditions conducive to disruptive selection on colour pattern by presenting behavioural evidence of aggressive mimicry, whereby predatory *Hypoplectrus* colour morphs mimic the colour patterns of non-predatory reef fish species to increase their success approaching and attacking prey. We propose that colour-based assortative mating, combined with disruptive selection on colour pattern, is driving speciation in *Hypoplectrus* coral reef fishes.

**Keywords:** speciation; coral reef fishes; colour pattern; population genetics; assortative mating; aggressive mimicry

## 1. INTRODUCTION

The colours displayed by coral reef fishes are among the most visually stunning phenotypic traits in animals. Coral reef fish colour patterns have been claimed to serve such diverse functions as crypsis (Cott 1940), mimicry (Randall & Randall 1960) or poster coloration to conspicuously identify conspecifics (Lorenz 1966), but the role played by colour pattern in the process of coral reef fish speciation remains poorly understood (McMillan *et al.* 1999).

Caribbean coral reef fishes in the genus *Hypoplectrus* present an unparalleled opportunity to investigate colour polymorphism and its role in marine speciation. These fish, commonly called hamlets, include at least 11 distinct colour morphs (figure 1), with as many as seven morphs recorded from the same reef (Fischer 1980a). Colour pattern is genetically determined (Domeier 1994) and hamlets have been shown to pair assortatively by colour pattern (Fischer 1980a). Nonetheless, spawning events between different colour morphs as well as individuals with intermediate colour patterns have been observed in the wild (present study; Fischer 1980a; Domeier 1994), suggesting the potential for gene flow between colour morphs. There are no intrinsic post-zygotic barriers between colour morphs (Domeier 1994) and to date no ecological, behavioural or morphological trait other than colour pattern has been shown to clearly distinguish the different hamlet morphs

(Randall 1983; Böhlke & Chaplin 1993). Population genetic analyses have failed to reveal consistent differences between *Hypoplectrus* colour morphs (Graves & Rosenblatt 1980; McCartney *et al.* 2003; Ramon *et al.* 2003; Garcia-Machado *et al.* 2004), and although most colour morphs have been described as species (e.g. Poey 1852; Acero & Garzón-Ferreira 1994), a century of study and debate has not settled the question of whether hamlets are distinct species or a single species displaying ‘endless variations in colour’ (Jordan & Evermann 1896).

Randall & Randall (1960), followed by Thresher (1978), suggested that hamlet colour morphs might have evolved as aggressive mimics. According to this hypothesis, predatory hamlets that mimic the colour pattern of non-predatory coral reef fish models have a fitness advantage resulting from increased success approaching and attacking their prey. Seven putative mimic–model pairs have been identified (Randall & Randall 1960; Thresher 1978), but behavioural evidence of aggressive mimicry in hamlets is lacking.

Disruptive natural selection on the colour pattern of hamlets to match coral reef fish models provides one possible mechanism for the origin of colour differences among some *Hypoplectrus* colour morphs, and in association with the assortative pairing by colour observed by Fischer (1980a), may provide an explanation for both the origin and maintenance of colour variation in this group of reef fish. Maynard Smith (1966) pointed out that the combination of assortative mating and disruptive natural selection on a single trait, sometimes referred to as a ‘magic trait’ (Gavrilets 2004), establishes one of the most favourable scenarios for speciation in the presence of gene flow (see also Moore 1981;

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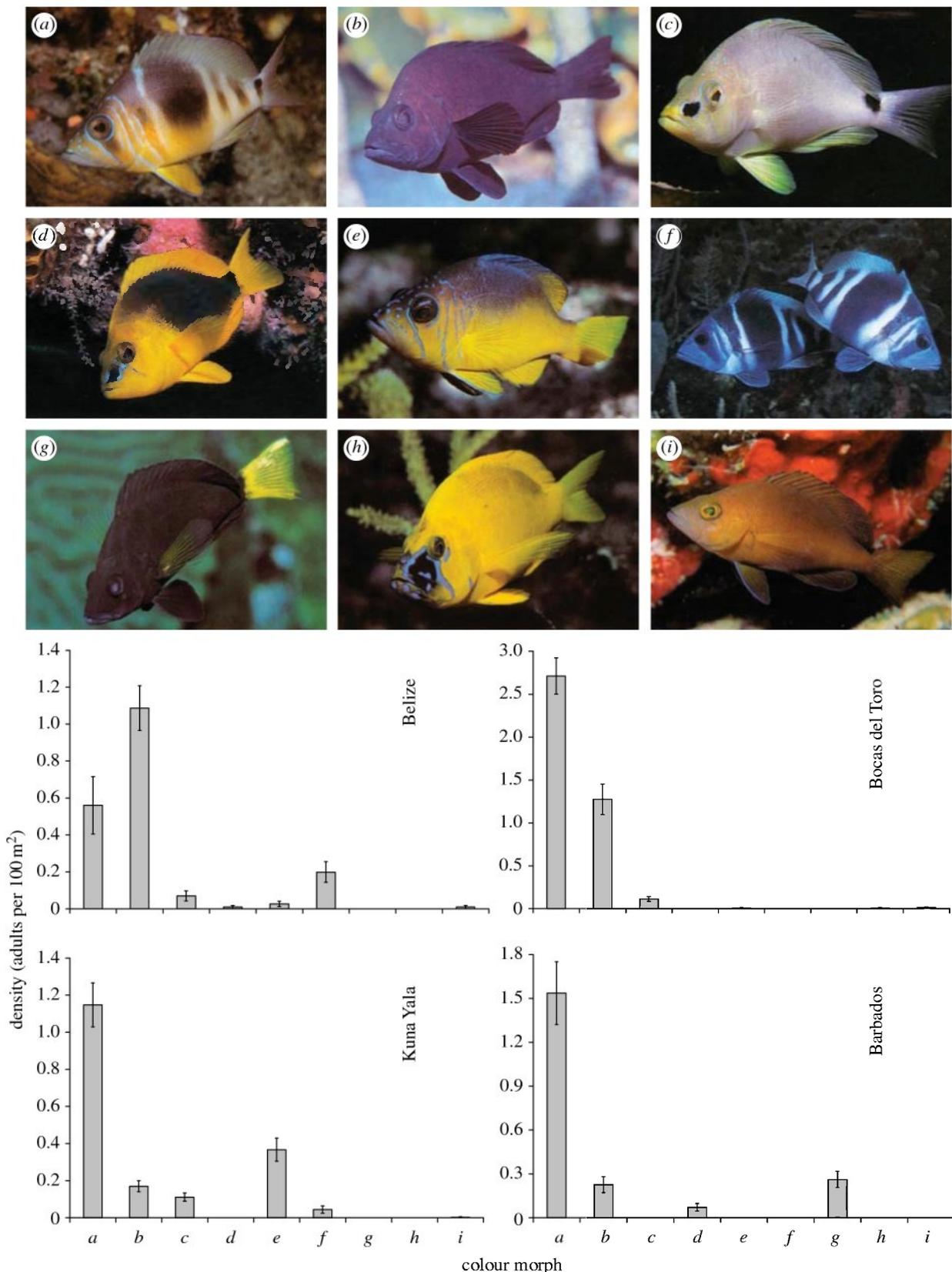


Figure 1. Nine *Hypoplectrus* colour morphs and densities (number of adults per 100 m<sup>2</sup>, mean  $\pm$  s.e.) in Belize, Bocas del Toro (Panama), Kuna Yala (Panama) and Barbados as assessed from 29, 40, 90 and 21 transects, respectively, covering a total of 72 000 m<sup>2</sup> of reef. (a), *H. puella*; (b), *H. nigricans*; (c), *H. unicolor*; (d), *H. guttavarius*; (e), *H. aberrans*; (f), *H. indigo*; (g), *H. chlorurus*; (h), *H. gummigutta*; (i), tan hamlet. Photographs with permission from Reef Fish Identification, New World Publications, © 2002, Paul Humann.

Slatkin 1982; Dieckmann & Doebeli 1999; Kirkpatrick 2000; Gavrilets 2004; Schneider & Bürger 2006). We provide the following three lines of evidence demonstrating the potential for colour pattern to drive speciation in the

genus *Hypoplectrus*: (i) significant genetic differences between sympatric barred (*Hypoplectrus puella*), black (*Hypoplectrus nigricans*) and butter (*Hypoplectrus unicolor*) hamlets in Belize, Panama and Barbados, (ii) behavioural

observations of colour-associated assortative mating in Belize, Panama and Barbados, and (iii) behavioural evidence of aggressive mimicry of *H. unicolor* in association with its putative model, the four-eye butterflyfish (*Chaetodon capistratus*) in Panama.

## 2. MATERIAL AND METHODS

### (a) Sampling

In order to investigate the relationship between assortative pairing and genetic differentiation between hamlet colour morphs, we collected large population samples of barred (*H. puella*), black (*H. nigricans*) and butter (*H. unicolor*) hamlets from Belize, Panama and Barbados. These morphs are ubiquitous throughout the Caribbean, thus permitting comparative analysis at a regional scale (figure 1).

Collecting, export and import permits were obtained prior to fieldwork. Sampling was undertaken in the vicinity of Carrie Bow Cay (Belize) in 2004, in Bocas del Toro (Panama) in 2004 and 2005, and along the west coast of Barbados in 2005. Hamlets were collected with microspears, while SCUBA diving over coral reefs at depths between 8 and 100 feet. Behavioural observations and fish collected for genetic analyses were carried out sequentially on the same reefs. Fish were killed in order to use specimens for an array of analyses, including meristic counts and stomach content analysis. The microspear method was efficient and highly selective, causing no damage to the reef or other fish species. The majority of the fish sampled were photographed underwater before and/or immediately after collection and all were preserved on ice until identified, labelled and photographed in the laboratory within a few hours after collecting. Gill tissue samples for genetic analyses were preserved in salt-saturated DMSO buffer. Entire fish were preserved in 10% formalin until accessioned and stored in 70–75% ethanol as voucher specimens in the Neotropical Fish Collection (Bermingham et al. 1997) at the Smithsonian Tropical Research Institute in Panama. Specimens, photographs, and DNA samples of all fish considered in this study are available upon request.

### (b) Genetic analyses

An enriched *Hypoplectrus* microsatellite library was constructed and five microsatellite markers were developed (electronic supplementary material). We used these markers as well as five previously developed loci (McCartney et al. 2003) to analyse a total of 371 *Hypoplectrus* samples, with an average sample size of 46 individuals per colour morph per location (electronic supplementary material).  $F_{st}$  between sympatric colour morphs were estimated following Weir & Cockerham (1984) and genetic differentiation was tested with 10 000 allele permutations using the  $G$  log-likelihood statistic (Goudet et al. 1996).

### (c) Hamlet densities and spawning observations

Hamlet densities were assessed during the day on the same reefs where spawning observations were made later in the early evening. Two SCUBA divers surveyed non-overlapping 100 × 4 m transects between 8 and 80 feet, with each diver counting all hamlets observed 2 m on each side of a 100 m transect tape.

Spawning observations were performed using SCUBA in water 10–60 feet deep, during a period beginning roughly 1 h before sunset and ending roughly 15–30 min after sunset. Only hamlets observed in the ritualistic head-to-tail spawning

position (Fischer 1980b) were counted as a spawning event. In order to avoid recording the same spawning pair twice, each reef section was surveyed only once.

### (d) Behavioural observations to test for aggressive mimicry

Two SCUBA divers observed 12 butter hamlets (*H. unicolor*), the putative aggressive mimic, in the wild during observation intervals lasting 45–60 min in Bocas del Toro in February and March 2006. The same observation methods and times were also employed to independently track 12 barred hamlets (*H. puella*), which we used as the experimental control given that this colour morph does not resemble the four-eye butterflyfish (*C. capistratus*), the putative model. Observations were performed between 08.40 and 14.10 at depths ranging from 11 to 43 feet. Time spent by *H. unicolor* tracking *C. capistratus* was recorded and the predatory strikes performed by *H. unicolor* while tracking *C. capistratus* and while swimming alone were counted. ‘Tracking’ was defined as *H. unicolor* actively staying within 30 cm of *C. capistratus*, with sharp changes in speed and/or direction in order to stay close to the putative model (video sequence 1 in electronic supplementary material). ‘Predatory strikes’ were defined as sharp and long accelerations performed by *H. unicolor* (video sequence 2 in electronic supplementary material). Moreover, the time spent by *H. unicolor* in association with all other coral reef fishes and the number of predatory strikes performed during such associations was recorded.

A two-sided Mann–Whitney test was performed to test whether *H. unicolor* spent significantly more time tracking *C. capistratus* than did the control. A two-sided Wilcoxon signed ranks test was performed to test whether both *H. unicolor* and the control, *H. puella*, displayed significantly higher predatory activity while tracking *C. capistratus* versus when swimming alone. We tested whether the proportion of predatory strikes while tracking the putative model was significantly greater than the proportion of time spent tracking the putative model. Bonferroni corrections for multiple tests were applied to test the significance of  $p$ -values.

## 3. RESULTS AND DISCUSSION

Microsatellite analysis demonstrates highly significant genetic differences between all pairs of sympatric colour morphs (table 1). The absence of significant genetic differentiation between two samples of *H. puella* collected in Bocas del Toro in 2004 and 2005 empirically confirms that our methodology does not detect significant genetic differences in cases where none are anticipated. This constitutes the first evidence of consistent genetic differentiation between sympatric *Hypoplectrus* colour morphs.

Spawning observations carried out on the same populations analysed genetically show that the assortative pairing of hamlets observed by Fischer (1980a) translates to assortative mating, with 247 out of 251 (98.4%) independent spawning events occurring between two individuals of the same colour morph (table 2 and §2). Different colour morphs were recurrently seen in close proximity during spawning observations, thereby discounting habitat selection as a possible cause of assortative mating. A total of 180 transects covering 72 000 m<sup>2</sup> of reef revealed considerable variation between sites in relative densities of hamlet colour morphs (figure 1), and in conjunction with the spawning observations indicate that

Table 1. Pairwise  $F_{st}$  estimates and test of genetic differentiation between *Hypoplectrus puella*, *H. nigricans* and *H. unicolor* from Belize, Bocas del Toro (Panama) and Barbados genotyped at 10 microsatellite loci. (Highly significant genetic differences were observed between all pairs of sympatric colour morphs tested except the control, *H. puella* collected in consecutive years in Panama.  $n$ , sample size; CI, confidence interval.  $F_{st}$  estimated following Weir & Cockerham (1984). Genetic differentiation tested with 10 000 permutations using  $G$  log-likelihood statistic (Goudet *et al.* 1996). \*, \*\*, \*\*\* significant  $p$ -value at the 0.05, 0.01 and 0.001 levels, respectively.)

locus	<i>H. puella</i> / <i>H. nigricans</i>	<i>H. puella</i> / <i>H. nigricans</i>	<i>H. puella</i> / <i>H. nigricans</i>	<i>H. unicolor</i> / <i>H. puella</i>	<i>H. unicolor</i> / <i>H. nigricans</i>	<i>H. puella</i> / <i>H. puella</i>
	Barbados	Panama	Belize	Panama	Panama	Panama
	$n=50/n=46$	$n=50/n=50$	$n=50/n=50$	$n=40/n=50$	$n=40/n=50$	$n=50/n=35$
	2005/2005	2004/2004	2004/2004	2004/2004	2004/2004	2004/2005
E2	0.001**	0.009*	0.014	0.023*	0.023***	-0.005
Gag010	0.033***	0.016	0.028***	0.007	0.025***	0.005
Hyp001	0.055***	0.003	0.033**	0.011	0.012	0.003
Hyp015	0.027***	-0.011	-0.003	0.000	0.003	-0.009
G2	0.027***	0.026***	0.000	-0.002	0.028***	0.002
H24	0.035***	0.077***	0.022**	0.026**	0.078***	-0.002
Hyp008b	0.113***	0.029**	0.018*	0.001	0.066***	0.009
Hyp016	0.079***	0.042***	0.004	0.025***	0.029***	0.002*
Hyp018	0.000	-0.007	-0.003	0.007	0.024	-0.001
Pam013	0.017***	0.011***	0.006	0.003	0.016***	0.004
overall	0.042***	0.023***	0.013***	0.010***	0.032***	0.001
95% CI	0.022–0.064	0.008–0.039	0.005–0.020	0.004–0.017	0.018–0.048	-0.002–0.004
$p$ -value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0778

hamlets mate assortatively even when rare (table 2 and §2). For example, yellowbelly hamlets (*Hypoplectrus aberrans*) from Bocas del Toro were observed spawning assortatively, despite a density estimated to one individual per 16 000 m<sup>2</sup> of reef in the area.

Spawning observations show that colour pattern and mate choice are tightly associated. This suggests that colour pattern is the cue for assortative mating, especially in this system where no ecological, behavioural or morphological trait other than colour pattern has been shown to clearly distinguish the different hamlet morphs (Randall 1983; Böhlke & Chaplin 1993). Formal demonstration that colour pattern is the cue for assortative mating is beyond the scope of this study and is not expected to be an easy task for several reasons. Experiments under monochromatic light typically used to establish that colour alone cues a behavioural response (e.g. Seehausen & van Alphen 1998) would not be entirely satisfying, given that patterns such as bars, lines and spots (figure 1), in addition to colour, are likely to be an important phenotypic aspect of mate recognition. Moreover, hamlets engage in an elaborate courtship (Fischer 1980b) that renders the use of artificial models difficult. Finally, colour pattern manipulation would be challenging owing to the complexity of hamlet colour patterns (figure 1). In the absence of additional experiments, colour pattern represents the best hypothetical cue for the high colour fidelity of mating hamlets.

Notwithstanding the strong assortative mating observed among hamlets, table 2 establishes that 4 out of 256 (1.6%) spawning events occurred between different *Hypoplectrus* colour morphs. Interestingly, three out of these four spawning events occurred between colour morphs that were locally abundant (table 2 and figure 1), suggesting that there is no straightforward relation between spawning

behaviour and colour morph densities. The occurrence of spawning events between different colour morphs in the wild, as well as the occasional occurrence of individuals with intermediate colour patterns, suggests the potential for gene flow between morphs. The analysis of individual microsatellite loci reveals that H24 is the only locus out of 10 systematically demonstrating highly significant differences between colour morphs. This genetic pattern is consistent with a scenario of speciation with gene flow where only a fraction of the genome—presumably linked to an ecological trait under selection—is systematically differentiated (Campbell & Bernatchez 2004). Given our limited assessment of the *Hypoplectrus* genome, we do not mean to imply that a single gene linked to H24 is responsible for speciation in hamlets, and additional genetic data are required to investigate the genetic architecture in *Hypoplectrus*. Complexes of multiple genes rather than a single gene are actually expected to underlie speciation in hamlets as colour pattern could result from the combination of several characters, and is shown here to be associated with complex foraging and spawning behaviours.

Altogether, spawning observations and microsatellite data show that although extremely closely related, sympatric *Hypoplectrus* colour morphs are reproductively isolated and can therefore be considered incipient species. Furthermore, the data on assortative mating provide the first line of evidence that colour pattern might be a phenotypic trait of the sort envisioned by Maynard Smith (1966) when he considered conditions under which speciation might proceed in the face of gene flow. But is hamlet colour pattern also under disruptive selection?

Randall & Randall (1960), followed by Thresher (1978), suggested that hamlet colour morphs evolved as aggressive mimics. According to this hypothesis, predatory



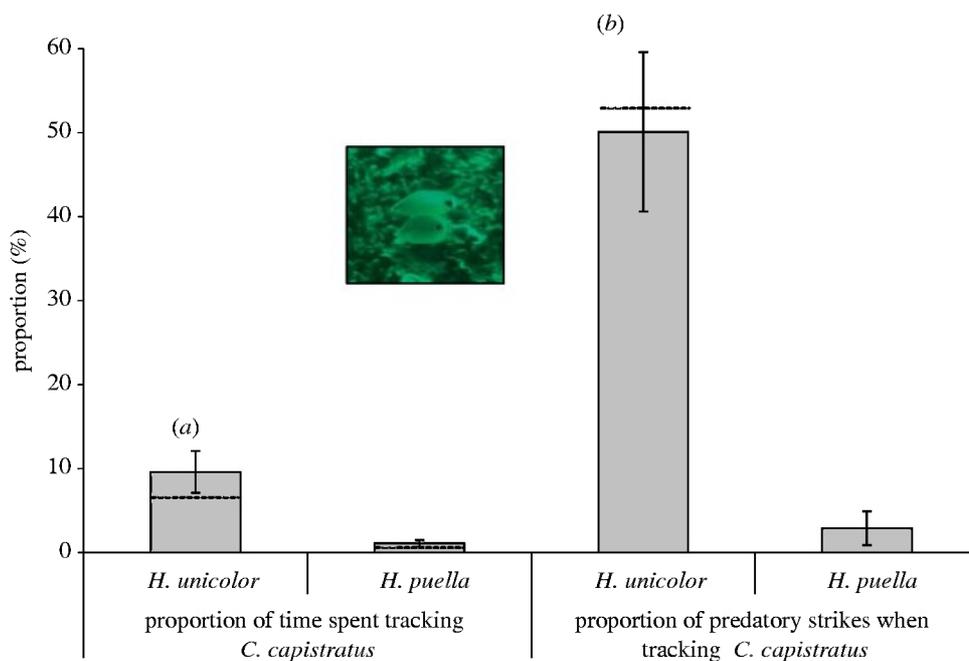


Figure 2. (a) Proportion of time spent by *H. unicolor* tracking its putative model, the four-eye butterflyfish (*C. capistratus*). (b) Proportion of predatory strikes performed by *H. unicolor* while tracking *C. capistratus* versus while swimming alone. *Hypoplectrus puella* served as the control for both analyses. Means  $\pm$  s.e., dashed lines: medians,  $n=12$  observations of 45–60 min for each species. Inset: *H. unicolor* (above) and *C. capistratus* (below).

(e.g. Nagel & Schluter 1998; Jiggins *et al.* 2001; Podos 2001), but both theoretical (Moore 1981; Slatkin 1982; Dieckmann & Doebeli 1999; Kirkpatrick 2000; Gavrillets 2004; Schneider & Bürger 2006) and experimental (Rice & Salt 1988, 1990) studies have shown that they offer one of the most plausible paths for speciation in the presence of gene flow. A question raised more recently by single trait models is whether speciation in the presence of gene flow is still possible when the costs of assortative mating are taken into account (Gavrillets 2004; Schneider & Bürger 2006). Our data show that incipient speciation is occurring, despite the fact that assortative mating is expected to be costly for rare colour morphs due to the difficulty of finding mates (e.g. *H. aberrans* in Bocas del Toro, figure 1). This study demonstrates that sympatric colour morphs of the genus *Hypoplectrus* are incipient species, and establishes a mechanism by which colour pattern can play an important role in marine speciation.

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## REFERENCES

- Acero, A. & Garzón-Ferreira, J. 1994 Descripción de una especie nueva de *Hypoplectrus* (Pisces: Serranidae) del Caribe occidental y comentarios sobre las especies colombianas del género. *An. Inst. Invest. Mar. Punt Betín* **23**, 5–14.
- Bates, H. W. 1862 Contributions to an insect fauna of the Amazon valley. Lepidoptera: Heliconidae. *Trans. Linn. Soc. Lond.* **23**, 495–566.

- Bermingham, E., Banford, H., Martin, A. P. & Aswani, V. 1997 Smithsonian tropical research institute neotropical fish collections. In *Neotropical fish collections* (ed. L. Malabarba), pp. 37–38. Puerto Alegre, Brazil: Museu de Ciências e Tecnologia, PUCRS.
- Böhlke, J. E. & Chaplin, C. C. G. 1993 *Fishes of the Bahamas and adjacent tropical waters*. Austin, TX: University of Texas Press.
- Campbell, D. & Bernatchez, L. 2004 Generic scan using AFLP markers as a means to assess the role of directional selection in the divergence of sympatric whitefish ecotypes. *Mol. Biol. Evol.* **21**, 945–956. (doi:10.1093/molbev/msh101)
- Cheney, K. L. & Côté, I. 2005 Frequency-dependent success of aggressive mimics in a cleaning symbiosis. *Proc. R. Soc. B* **272**, 2635–2639. (doi:10.1098/rspb.2005.3256)
- Côté, I. & Cheney, K. L. 2004 Distance-dependent costs and benefits of aggressive mimicry in a cleaning symbiosis. *Proc. R. Soc. B* **271**, 2627–2630. (doi:10.1098/rspb.2004.2904)
- Cott, H. B. 1940 *Adaptive coloration in animals*. London, UK: Methuen & Co. Ltd.
- Dieckmann, U. & Doebeli, M. 1999 On the origin of species by sympatric speciation. *Nature* **400**, 354–357. (doi:10.1038/22521)
- Domeier, M. L. 1994 Speciation in the serranid fish *Hypoplectrus*. *Bull. Mar. Sci.* **54**, 103–141.
- Fischer, E. A. 1980a Speciation in the hamlets (*Hypoplectrus*: Serranidae)—a continuing enigma. *Copeia* **1980**, 649–659. (doi:10.2307/1444441)
- Fischer, E. A. 1980b The relationship between mating system and simultaneous hermaphroditism in the coral reef fish, *Hypoplectrus nigricans* (Serranidae). *Anim. Behav.* **28**, 620–633. (doi:10.1016/S0003-3472(80)80070-4)
- García-Machado, E., Chevalier Montegudo, P. P. & Solignac, M. 2004 Lack of mtDNA differentiation among hamlets (*Hypoplectrus*, Serranidae). *Mar. Biol.* **144**, 147–152. (doi:10.1007/s00227-003-1170-0)
- Gavrillets, S. 2004 *Fitness landscapes and the origin of species*. Princeton, NJ: Princeton University Press.

- Goudet, J., Raymond, M., de Meeüs, T. & Rousset, F. 1996 Testing differentiation in diploid populations. *Genetics* **144**, 1933–1940.
- Graves, J. E. & Rosenblatt, R. H. 1980 Genetic relationships of the color morphs of the serranid fish *Hypoplectrus unicolor*. *Evolution* **34**, 240–245. (doi:10.2307/2407388)
- Jiggins, C. D., Naisbit, R. E., Coe, R. L. & Mallet, J. 2001 Reproductive isolation caused by colour pattern mimicry. *Nature* **411**, 302–305. (doi:10.1038/35077075)
- Jordan, D. S. & Evermann, B. W. 1896 The fishes of North and Middle America. *Bull. US Nat. Mus.* **47**, 1189–1194.
- Kirkpatrick, M. 2000 Reinforcement and divergence under assortative mating. *Proc. R. Soc. B* **267**, 1649–1655. (doi:10.1098/rspb.2000.1191)
- Lorenz, K. 1966 *On aggression*. London, UK: Methuen.
- Maynard Smith, J. 1966 Sympatric speciation. *Am. Nat.* **100**, 637–650. (doi:10.1086/282457)
- McCartney, M. A., Acevedo, J., Heredia, C., Rico, C., Quenoville, B., Bermingham, E. & Mcmillan, W. O. 2003 Genetic mosaic in a marine species flock. *Mol. Ecol.* **12**, 2963–2973. (doi:10.1046/j.1365-294X.2003.01946.x)
- McMillan, W. O., Weigt, L. A. & Palumbi, S. R. 1999 Color pattern evolution, assortative mating, and genetic differentiation in brightly colored butterflyfishes (Chaetodontidae). *Evolution* **53**, 247–267. (doi:10.2307/2640937)
- Moore, W. S. 1981 Assortative mating genes selected along a gradient. *Heredity* **46**, 191–195.
- Nagel, L. & Schluter, D. 1998 Body size, natural selection, and speciation in sticklebacks. *Evolution* **52**, 209–218. (doi:10.2307/2410936)
- Podos, J. 2001 Correlated evolution of morphology and vocal signal structure in Darwin's finches. *Nature* **409**, 185–188. (doi:10.1038/35051570)
- Poey, E. 1852 Memorias sobre la historia natural de la isla de Cuba. *Havana* **1**, 1–463.
- Ramon, M. L., Lobel, P. S. & Sorenson, M. D. 2003 Lack of mitochondrial genetic structure in hamlets (*Hypoplectrus* spp.): recent speciation or ongoing hybridization? *Mol. Ecol.* **12**, 2975–2980. (doi:10.1046/j.1365-294X.2003.01966.x)
- Randall, J. E. 1983 *Caribbean reef fishes*. Neptune City, NJ: T. F. H. Publications.
- Randall, J. E. & Randall, H. A. 1960 Examples of mimicry and protective resemblance in tropical marine fishes. *Bull. Mar. Sci.* **10**, 444–480.
- Rice, R. W. & Salt, G. W. 1988 Speciation via disruptive selection on habitat preference: experimental evidence. *Am. Nat.* **131**, 911–917. (doi:10.1086/284831)
- Rice, R. W. & Salt, G. W. 1990 The evolution of reproductive isolation as a correlated character under sympatric condition: experimental evidence. *Evolution* **44**, 1140–1152. (doi:10.2307/2409278)
- Schneider, K. A. & Bürger, R. 2006 Does competitive divergence occur if assortative mating is costly? *J. Evol. Biol.* **19**, 570–588. (doi:10.1111/j.1420-9101.2005.01001.x)
- Seehausen, O. & van Alphen, J. J. M. 1998 The effect of male coloration on female mate choice in closely related Lake Victoria cichlids (*Haplochromis nyererei* complex). *Behav. Ecol. Sociobiol.* **42**, 1–8. (doi:10.1007/s002650050405)
- Slatkin, M. 1982 Pleiotropy and parapatric speciation. *Evolution* **36**, 263–270. (doi:10.2307/2408044)
- Thresher, R. E. 1978 Polymorphism, mimicry, and the evolution of the hamlets (*Hypoplectrus*, Serranidae). *Bull. Mar. Sci.* **28**, 345–353.
- Weir, B. S. & Cockerham, C. C. 1984 Estimating *F*-statistics for the analysis of population structure. *Evolution* **38**, 1358–1370. (doi:10.2307/2408641)