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Genetic Evidence for Local Retention of Pelagic Larvae in a Caribbean Reef Fish

Michael S. Taylor* and Michael E. Hellberg

The pelagic larvae of many marine organisms can potentially disperse across hundreds of kilometers, but whether oceanographic or behavioral mechanisms can constrain dispersal over periods sufficient for the evolution of genetic differentiation remains unclear. Here, we concurrently examine larval duration and genetic population differentiation in a cleaner goby, *Elacatinus evelynae*, a member of the most species-rich genus of Caribbean reef fishes. Despite evidence for extended pelagic duration (21 days), populations of *E. evelynae* show strong genetic differentiation: among color forms (1.36 to 3.04% divergent at mitochondrial cytochrome b) and among island populations within color forms (Φ_{ST} up to 70%). These results suggest that marine populations can remain demographically closed for thousands of generations despite extended larval duration, and that recognition cues such as color may promote speciation when geographic barriers are transient or weak.

Many marine organisms have pelagic larvae that can potentially interconnect distant populations through dispersal on ocean currents. If these larvae disperse as passive propagules on advective current flow, they will be transported among both near and distant island populations (*1*). Species with such broadly dispersing larvae should be genetically homogeneous over large spatial scales, thus compromising their ability to adapt to local conditions (*2*). If, however, pelagic larvae are retained near their natal populations by behavioral (*3*) or physical oceanographic (*4*) mechanisms, then populations would have a greater opportunity for genetic differentiation and local adaptation. Should local retention persist over many generations, marine populations undivided by strong physical barriers might nonetheless form new species or at least differentiate to levels where different management or conservation strategies would be warranted for different populations.

Studies in which fluorescent tags and environmental trace elements were used as markers in otoliths—calcareous structures in the inner ear of fishes—from newly recruited juvenile fishes indicate that as many as 60% of a juvenile cohort may recruit to their natal populations, despite larval durations of 3 to 7 weeks (*5*, *6*). However, exchange rates averaging just a single larval individual per generation among populations can be sufficient to hinder genetic differentiation caused by drift or weak selection (*7*). In the absence of biogeographic barriers, genetic analyses to date have failed to reveal significant population differentiation for species with broad larval-dispersal potential (*8*–*10*), including one species (bluehead wrasse, *Thalassoma bifasciatum*) shown by trace-element studies to have high larval retention (*6*). Here, we test for genetic differentiation among island populations separated by hundreds of kilometers in a Caribbean reef fish with pelagic larvae.

Elacatinus (=*Gobiosoma*) *evelynae*, a reef-dwelling cleaner goby, is widely distributed throughout the Bahamas and Caribbean Sea (Fig. 1). It belongs to the most species-rich genus of fishes found on west Atlantic coral reefs, as well as to the largest family of marine fishes (Gobiidae) (*11*). Although long recognized as a single species on the basis of morphological criteria (*12*, *13*), *E. evelynae* has three brightly colored forms: yellow, blue, and white. However, individuals of the different color forms rarely co-occur, despite geographic separation by as little as 23 km.

The potential for larval dispersal between geographically proximal populations can be assessed with knowledge of currents and pelagic larval duration (PLD). Current patterns in the Caribbean have been well studied; typical current speeds average 1 to 2 km/hour (*4*). We determined PLD for *E. evelynae* by counting daily growth rings in the otoliths from the core (which forms as the planktonic stage begins after hatching) to the settlement check (which forms as the planktonic stage ends and individuals settle onto the reef) (*14*). Larvae of all color forms had a PLD of about 3 weeks (Table 1); the mean PLD of the yellow form (25 days) was slightly longer than that of blue or white forms (21 days; $P < 0.001$). Assuming passive dispersal and a conservatively estimated current speed of 1 km/hour (*8*), an individual with a 21-day PLD could potentially disperse more than 500 km per generation (corresponding to 1 year). Dispersal, however, may be influenced by factors other than PLD. Ecological requirements or behavioral attributes may cause larvae to develop near shore, rather than disperse (*15*). The pelagic larvae of gobies are typically found over or near reefs and not in open water (*16*), suggesting limited dispersal.

To assess the realized extent of genetic exchange among populations, we sampled 246 individuals from 17 Caribbean and Bahamian island populations representing all color forms (Fig. 1), and amplified and sequenced 400 base pairs of the mitochondrial cytochrome b gene by polymerase chain reaction (*14*). The different color forms of *E. evelynae* are genetically distinct and appear to be reproductively isolated. An analysis of molecular variation (*17*) indicates that 78.6% of the genetic variation is partitioned among color forms (Φ_{ST}, Table 1), and none of the 79 unique haplotypes (with the exception of 3) is shared among color forms (Fig. 2). Notably, haplotypes are not shared between blue and white forms from Puerto Rico, where they are separated by only 23 km.

Within color forms, few haplotypes are shared among populations of either the blue or the white forms, indicating that haplotypes unique to each population are not spreading (by larval dispersal) to other populations. Of the 32

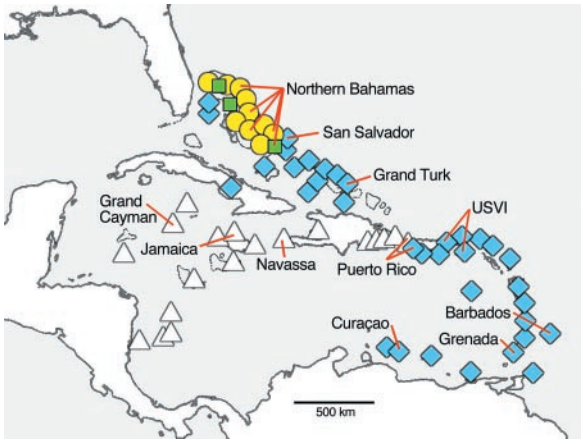


Fig. 1. Distribution of the yellow (circles), blue (diamonds), and white (triangles) color forms of *E. evelynae* across the Bahamas and Caribbean Sea. Green squares indicate localities where both blue and yellow forms have been reported. The 17 sampled populations are indicated with red lines. Northern Bahamas represents five sampled populations (north to south): Sweetings Cay, Eleuthera Island, Lee Stocking Island, Cat Island, and Long Island. Puerto Rico represents two sampled populations, Isla Desecheo (white form) and the main island (blue form). The U.S. Virgin Islands (USVI) represents two sampled populations: St. John and St. Croix.

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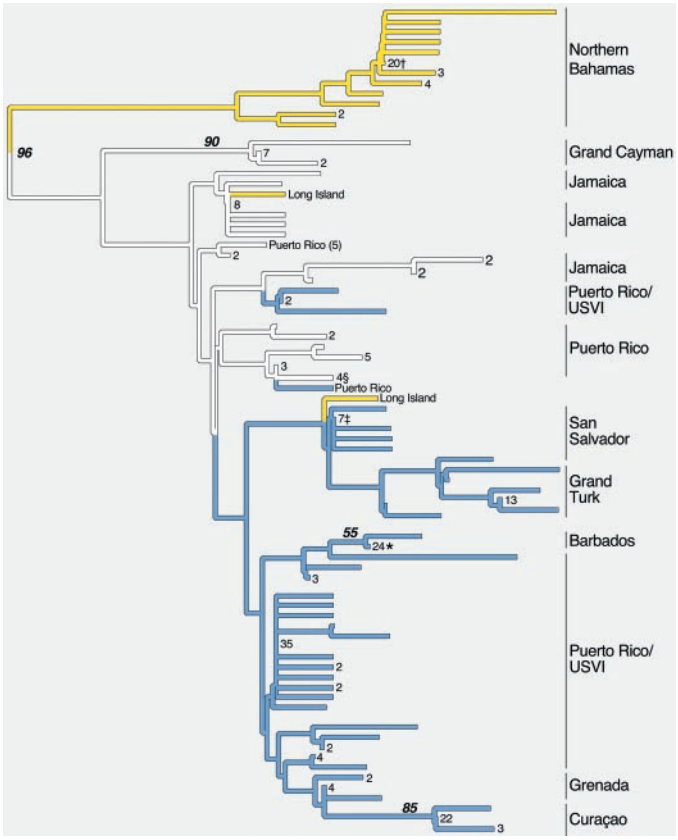


Fig. 2. Neighbor-joining tree of 79 mitochondrial cytochrome b haplotypes sampled from 246 *E. evelynae* individuals representing 17 populations across the Bahamas and Caribbean Sea. The three color forms are indicated on the branches. Average pairwise genetic distances (Kimura two-parameter) between color forms are white/yellow: 2.81%; blue/yellow: 3.04%; white/blue: 1.36%. Numbers at the branch tips indicate haplotypes shared by more than a single individual. Symbols: (†) includes one blue individual from San Salvador; (§) includes two white individuals from Jamaica, one blue individual from Grand Turk, and one yellow individual from Long Island; (‡) includes two yellow individuals from Long Island; (*) includes a single individual from Grenada. Bold, italicized numbers indicate bootstrap support (100,000 replicates) for monophyletic populations.

Table 1. Mean pelagic larval duration (PLD, in days), and genetic population subdivision (Φ_{ST}) within and among color forms.

Color form	Larval duration			Genetic subdivision			
	N	PLD	SE	Populations	*N*	Φ_{ST}	*P*
Blue	48	21.7	0.46	8	156	0.704	<0.0001
White	24	21.1	0.66	4	49	0.584	<0.0001
Yellow	20	25.2	0.72	5	41	0.038	>0.05
All	92	22.3	0.37	17	246	0.786	<0.0001

haplotypes found across blue-form populations (separated by 60 to 2000 km), only 6 occurred in more than 1 population (5 among Puerto Rico, St. John, and St. Croix, and 1 between Barbados and Grenada). Of the 19 white-form haplotypes (populations separated by 250 to 750 km), only 1 occurred in more than 1 population (Jamaica and Navassa). The blue-form populations are strongly subdivided (Φ_{ST} = 0.704; Table 1); the white form also shows considerable subdivision (Φ_{ST} = 0.584). Furthermore, several populations within the blue form and the white form are reciprocally monophyletic (or nearly so) (Fig. 2), indicating that gene flow among populations has been absent or restricted over many generations. Using a coalescent model (*14*), we estimate that populations at Barbados and Curaçao (separated by 1000 km) have been isolated from each other for between 75,000 and 103,000 years.

Some of the geographic subdivision we found could be due to a "sweepstakes effect," the genetic drift among larval cohorts that results from the random reproductive success of different small subsets of adults over time. If such sweepstakes effects are important, then different larval cohorts should be genetically differentiated (*18*). We sampled three populations repeatedly over as many as four generations, but found no evidence of temporal subdivision that would support a reproductive sweepstakes effect (Table 2). More detailed temporal sampling of marine species that are longer lived and have overlapping generations (attributes most favorable for sweepstakes effects) have also failed to detect sweepstakes effects (*19*).

Our results show that strong phylogeographic structure can develop in the Caribbean Sea between marine populations separated by as little as 23 km for species that have potential for long-distance larval dispersal. The amount of genetic subdivision between populations of all three color forms (Table 1) is similar to that found between populations of an Indo-West Pacific stomatopod separated by a strong biogeographic barrier (*20*), where lineages with long separate histories meet; however, no such barriers are currently recognized for the Caribbean. Instead, the reciprocal monophyly of populations within the blue form and the close proximity of genetically distinct color forms observed here suggest that local larval retention generates the strong phylogeographic structure observed in *E. evelynae*.

Table 2. Genetic differentiation (Φ_{ST}) among years for three populations. Analyses of molecular variation showed no evidence of temporal differentiation within populations ($P > 0.05$).

Population	Φ_{ST}	Years sampled (no. of individuals)
Barbados	0.016	2000 (11), 2002 (13)
Curaçao	−0.038	1999 (13), 2002 (13)
St. Croix	−0.004	2000 (13), 2001 (12), 2002 (15)

Thus, for at least some taxa, the simple assumption that extended PLD will result in broad dispersal is a faulty foundation for the management of fisheries resources (*4*) and for understanding the geographic context of speciation in the sea. Even in the absence of strong geographic barriers, persistent retention of larvae could allow rapidly evolving mate-recognition characters, such as the color differences seen here in *E. evelynae* and in other fishes (*21*, *22*) or the specificity of fertilization proteins in free-spawning animals (*23*), to follow independent, population-specific trajectories that could leave such populations reproductively isolated upon subsequent contact. If the apparent reproductive isolation of the gobies studied here is driven by coloration, then the genes underlying coloration (or color recognition) should be monophyletic among color forms, even when mitochondrial genes are not (*24*). Whatever the mode of speciation for *Elacatinus*, our data suggest that the diversity of reef fishes, even for well-studied species, remains underestimated and that the bright colors that attract popular interest in coral-reef fishes may in part be responsible for their remarkable biodiversity.

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Materials and Methods
References

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Long-Distance Signaling in Nodulation Directed by a CLAVATA1-Like Receptor Kinase

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Proliferation of legume nodule primordia is controlled by shoot-root signaling known as autoregulation of nodulation (AON). Mutants defective in AON show supernodulation and increased numbers of lateral roots. Here, we demonstrate that AON in soybean is controlled by the receptor-like protein kinase GmNARK (*Glycine max* nodule autoregulation receptor kinase), similar to *Arabidopsis* CLAVATA1 (CLV1). Whereas CLV1 functions in a protein complex controlling stem cell proliferation by short-distance signaling in shoot apices, GmNARK expression in the leaf has a major role in long-distance communication with nodule and lateral root primordia.

Multicellular organisms need to control the proliferation of pluripotent stem cells, also referred to as meristematic cells in the apices, cambium, and pericycle of flowering plants. Because organ differentiation of plants is predominantly postembryonic and does not involve cell migration, plant stem cells need to be controlled by short- and long-distance signals to achieve equilibrium between cell proliferation and differentiation. The role of short-distance signaling in plant development has been more extensively researched, and some of the key genes involved have been identified (*1*–*8*).

Legume nodulation is important in supplying nitrogen to ecological and agricultural systems. Nodule meristems form in response to mitogenic signals from symbiotic bacteria called rhizobia (*6*), but nodule proliferation is restricted by autoregulation of nodulation (AON) (*9*–*12*). Mutations affecting nodule meristems have been readily identified, including ones that confer supernodulation as a result of a defect in AON (Fig. 1A).

Allelic supernodulating (*nts*) mutants of soybean were first isolated by EMS (ethylmethane sulfonate) mutagenesis (*11*–*13*). These mutants altered at the *NTS-1* locus also develop more lateral roots, leading to a bushy root system in the absence of nodulation, and reduced root growth in the presence of prolific nodulation. Subsequently, additional mutations in the *NTS-1* locus were induced chemically or by fast neutrons (*14*–*16*). Isolation of mutants in other legumes confirmed the generality of AON (*17*–*19*), and reciprocal grafting of supernodulating and wild-type genotypes showed that long-distance signaling was involved and that the leaf genotype controlled proliferation of nodule primordia (*20*–*22*).

To elucidate the mechanisms of this long-distance signal exchange, we used map-based cloning to isolate the *NTS-1* locus. Mutant alleles were mapped to soybean linkage group H close to restriction fragment length polymorphism (RFLP) marker pA132. A subclone of pA132, pUTG132a, was placed 0.7 cM from *NTS-1* in a F_2 population of *nts382* (*G. max* Bragg) × *G. soja* (PI468.397) (*23*, *24*) and 1.3 cM from *NTS-1* in a *G. max nts246* × *G. soja* CPI 100070 population (*25*). *nts382* and *nts246* were identified in our original mutant screen (*11*). Amplified fragment length polymorphism (AFLP) marker UQC-IS1 also flanked *NTS-1* 1.9 cM away (Fig. 1B) (*25*). UQC-IS1 was the closest of 11 AFLP markers shown by bulk segregant analysis and genetic mapping to be linked to *NTS-1* (*25*).

Bacterial artificial chromosome (BAC) clones derived from a soybean PI437.654 library (*26*) were isolated by filter-hybridization to pUTG132a and UQC-IS1, and were verified to contain either pUTG132a or UQC-IS1 by sequencing each marker from the respective BAC clone. Both the pUTG132a and UQC-IS1 BAC contigs were oriented relative to *NTS-1* by mapping polymorphic BAC ends on F_2 recombinants (Fig. 1B).

Confirmation of mapping was aided by the fast neutron mutant *FN37* (*16*). Physical mapping of markers and complete BAC sequencing of BAC171O7 (135 kb) (Fig. 1B) showed that this mutant contains a chromosomal deletion in the *NTS-1* region. The southern and northern deletion breakpoints were localized within the BAC171O7 sequence and close to marker UQC-IS4, respectively (Fig. 1B). Arrangement of putative open reading frames from BAC171O7, sequenced BAC ends, and markers in the *NTS-1* region showed contiguous microsynteny to *Arabidopsis* chromosomes 2 and 4 (Fig. 1B). Seven genes (three from the northern contig and four from the southern contig) were syntenic between the *NTS-1* region and *Arabidopsis*. BAC92D22 contained three expressed sequence tags, highly syntenic

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