A SINGLE TRAIT DRIVES INCipient ECological SPECIATION IN SYmpatric COLOR MORPHS OF THE ARCEYE HAWKFISH

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DEDICATION

I dedicate this dissertation to Ed DeMartini, Alison Whitney and the hawkfish, without which this work would not have been possible.
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ABSTRACT

Coral reef fishes represent the most diverse assemblage of vertebrates on the planet, yet our understanding of the mechanisms driving this diversity remains limited. There is growing recognition that ecological adaptation shaped by natural selection may be a major driver of diversification on coral reefs. However, few examples of ecological speciation in nature currently exist. I integrate research on ecology, behavior and genetics to outline a novel case of incipient ecological speciation in sympatric color morphs of the arceye Hawkfish (*Paracirrhites arcatus*). First, I demonstrate that color morphs are exploiting different niches along a steep ecological gradient, likely driven by disruptive selection favoring color patterns that are better camouflaged in contrasting microhabitats. Second, mate preference experiments show that females prefer individuals of their own morph, indicating color morphs are mating assortatively. Third, I provide genetic evidence that these premating barriers have resulted in at least partial reproductive isolation between ecologically differentiated sympatric color morphs. Taken together, these results suggest that reproductive isolation between morphs may be arising as a by-product of divergent selection on ecological differences and enhanced by the isolating effects of assortative mating. I conclude that color alone is driving incipient divergence in this species, despite high gene flow and no geographic isolation. I argue that the characteristics of this system could be quite common and thus widely applicable to thousands of reef organisms. This dissertation emphasizes the role natural selection plays in initiating speciation and should help bring us one step closer to understanding the processes driving high biodiversity in tropical seas.
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CHAPTER I
INTRODUCTION
INTRODUCTION

Understanding speciation, the evolutionary process by which new species arise, remains a fundamental challenge in biology (Coyne & Orr 2004, Marie Curie Speciation Network et al. 2012, Shaw & Mullen 2014, Gavrilets 2014). The causes of most speciation events remain unidentified, thereby leaving huge gaps in our understanding of how biodiversity is generated. A particular enigma has been explaining the mechanisms responsible for the incredible radiation of coral reef fishes in tropical seas (Rocha & Bowen 2008). Coral reefs cover <0.1% of the ocean’s surface (Spalding & Grenfell 1997), yet contain an astounding one-third of all marine fishes, more than 5,000 species (Lieske & Myers 1999, Helfman et al. 2009). This paradox has inspired a constructive debate about the origins of tropical marine biodiversity (Rocha et al. 2005, Rocha & Bowen 2008, Bernardi 2013, Bowen et al. 2013).

For a variety of reasons, coral reef fishes are thought to challenge the classic allopatric model of speciation, which predicts that new species are created by divergences resulting from geographic isolation (reviewed in Rocha & Bowen 2008). First, the majority of coral reef fishes have a planktonic larval stage that is capable of dispersing long distances (Mora & Sale 2002), thereby connecting populations, at least over evolutionarily relevant time periods (Bowen et al. 2001, Lessios & Robertson 2006). Second, there appear to be only a handful of barriers in the sea, so geographic isolation may be difficult to achieve in such a continuous aquatic medium (Briggs 1995, Rocha et al. 2007, Briggs & Bowen 2013). These factors combined tend to result in high rates of gene flow between populations, which should greatly reduce opportunities for speciation via geographic isolation (Palumbi 1994). Nevertheless, the ocean houses an extremely rich biological diversity, particularly on shallow coral reefs, which rivals that of tropical rainforests. The existence of such a paradox highlights a huge gap in our understanding of how new species are generated in the oceans.

Ecological speciation may be an alternative mechanism capable of explaining the evolution of tropical reef fishes without geographic isolation. This model predicts that natural selection can act differently in alternative environments even in habitats directly adjacent to one another. If divergent natural selection drives the evolution of reproductive isolation, ecological speciation can occur (Mayr 1963, Schluter 2000). The idea that ecological partitions may be driving speciation in coral reef fishes has only recently begun to be explored and represents an
avenue of research with extraordinary potential to help explain the high diversity of reef fishes in tropical seas.

Speciation driven by natural selection may be especially prevalent on coral reefs – where the complex environment and rich ecological interactions create ample opportunities for niche divergence, especially for fishes (Price et al. 2011). Coral reefs harbor the greatest species richness of fishes on earth (Harmelin-Vivien 2002), with up to a thousand species coexisting within a single location (Bellwood et al. 2005) and are thought to be epicenters of speciation for fishes and other reef organisms (Alfaro et al. 2007, Kiessling et al. 2010). Briggs (2005) proposed that the large-scale patterns of high species diversity in Indo-Pacific fishes, particularly in the Coral Triangle, are best explained by ecological processes rather than physical isolation.

Evidence for the role of ecology in driving reef fish diversity has been provided by a number of phylogenetic studies that show breaks in sister clades separated by ecological factors in several speciose families including Scarids (Streelman et al. 2002), Tetraodontids (Alfaro et al. 2007), Labrids (Rocha et al. 2005) and Gobiids (Rüber et al. 2003, Taylor & Hellberg 2005). Rocha et al. (2005) provided a notable example of parapatric ecological speciation in Atlantic wrasses (*Halichoeres* spp.) by showing strong genetic partitions between adjacent yet ecologically distinct habitats despite high genetic connectivity observed between similar habitats separated by thousands of kilometers.

A few comprehensive studies combining research on ecology, genetics, and behavior have described the few confirmed cases of ecological speciation in reef fish. Perhaps one of the most compelling examples involves a well-studied case of host shift in coral-dwelling gobies (Munday et al. 2004). Coral gobies of the genus *Gobiodon* spend their entire lives on branches of *Acropora* corals. Intense competition for coral hosts drove a habitat shift where a previously unoccupied coral species was invaded (Munday 2001, Munday et al. 2004). Disruptive selection favors specialists on their preferred corals where they experience greater fitness and strong site fidelity of settling juveniles reinforces reproductive isolation (Munday et al. 1997, 2004).

Hamlets represent another noteworthy case study of ecological speciation-with-gene-flow in reef fish. In the Caribbean, more than a dozen *Hypoplectrus* color morphs, most of which are argued to be good biological species, have radiated in sympatry (Domeier 1994, Puebla et al. 2007, 2014). Predatory hamlets appear to mimic other non-predatory fishes in order to increase success in the approach and attack of prey. Occurrence of various model species in the same
environment provides a source of divergent selection on hamlet color pattern. Color is also a cue for assortative mating (Fischer 1980, Puebla et al. 2007), thereby providing a direct link between divergent selection and reproductive isolation, which has been confirmed by significant genetic differentiation between sympatric species (Puebla et al. 2007, 2014).

These cases help to highlight the three major ingredients needed for ecological speciation to proceed: (i) a source of natural disruptive selection, (ii) a mechanism of reproductive isolation, and (iii) a link between the two. The requisite link between natural selection and reproductive isolation appears to be more effective for ecological specialists. As exemplified by the coral gobies, when individuals prefer the habitat to which they are best adapted, and mating takes place in that habitat, reproductive isolation can result as a pleiotropic effect of divergent habitat preference. Scenarios where the ecological trait under selection is linked directly with mate choice are considered the most plausible paths to speciation (Maynard-Smith 1966, Gavrilets 2004). As it is in the hamlets, color is a potentially important trait as it can be both under disruptive selection and used as a cue in assortative mating.

With so few studies to date, it’s difficult to discern general patterns about the mechanisms and prevalence of ecological speciation in marine fishes. The lack of examples from nature may not be due to their rarity, but rather the difficulty in diagnosing a mode of speciation that has already occurred. Identifying modes of speciation in wild populations is inherently challenging, as the processes that led to the formation of most species are often obscured by time. The approach here is to examine the earliest stages of divergence, where the factors causing the split are still evident and testable. I aim to capture a glimpse of the process of divergence by analyzing what mechanisms drive the maintenance of a color polymorphism within the arceye hawkfish, Paracirrhites arcatus.

GOALS & OBJECTIVES
The central goal of this dissertation is to contribute to the understanding of large-scale patterns of marine biodiversity by investigating how ecological specialization can lead to population divergence even without physical barriers to gene flow. This dissertation integrates ecological, behavioral, and genetic research to test the hypothesis that ecologically-based disruptive natural selection on color pattern in combination with color-based assortative mating is driving the evolution of reproduction isolation between sympatric morphotypes of the arceye hawkfish,
Paracirrhites arcatus. Testing this hypothesis requires us to address three specific aims and their corresponding null hypotheses:

\[ H_0 \text{ I: Color morphotypes show no significant differences in niche space} \]
\[ H_0 \text{ II: Mating is at random with respect to color} \]
\[ H_0 \text{ III: Allele frequencies at microsatellite loci do not differ significantly between morphs} \]

**Chapter II: Assess degree of ecological divergence between morphotypes**
The ecological speciation model predicts that natural selection can drive the evolution of reproductive isolation between subsets of a single population when they become adapted to different environments or to exploit divergent resources (Schluter 2009). Thus, an in-depth understanding of the natural history and ecology of *P. arcatus* is required to determine if color pattern variation is correlated with ecological divergence. Chapter II focuses on characterizing the habitat preferences of each color morph, identifying important phenotype-environment correlations, and quantifying the degree of niche divergence between morphotypes.

**Chapter III: Determine if color morphs mate assortatively**
It is often assumed that vivid coloration is used as a signal for species recognition and mate choice, but few direct experimental tests are available (McMillan et al. 1999). If color acts as a mate recognition signal, this can lead to the evolution of assortative mating (like-with-like), which can create a strong barrier to gene flow and subsequently amplify reproductive isolation (Gavrilets 2004). Chapter III test the existence of pre-mating isolation between morphs by determining if color pattern acts as a mate recognition signal and can lead to assortative mating. I combine field observations of pairing behavior with lab-based experiments to assess the role of sexual selection in generating reproductive isolation.

**Chapter IV: Test for genetic evidence of reproductive isolation**
Under ecological speciation, it is predicted that reproductive isolation will evolve as a consequence of divergent selection on traits adapted to different environments. Chapter IV examines the degree of genetic divergence between morphotypes to assess levels of gene flow and ultimately evaluate the degree of reproductive isolation.
The overarching goal of this dissertation is to determine if ecological processes can drive divergence in morphotypes of a single species, even in the face of gene flow. In doing so, I aim to provide evidence for the role of natural selection in driving reproductive isolation in marine species, thereby helping to advance our understanding of the connection between adaptation and speciation in marine environments.

REFERENCES


CHAPTER II
ECOLOGICAL DIVERGENCE IN SYMPATRIC COLOR MORPHS
OF THE ARCEYE HAWKFISH
INTRODUCTION

Coral reef fishes represent the most diverse assemblage of vertebrates on the planet, yet our understanding of the mechanisms driving this diversity remains limited. There is growing evidence and recognition that ecological adaptations shaped by natural selection may be a major driver of diversification in reef fishes (Streelman et al. 2002, Munday et al. 2003, Rocha et al. 2005, Rocha & Bowen 2008, Puebla 2009, Bowen et al. 2013). The structural, spatial, and ecological complexities of reefs create environmental heterogeneity at small scales, which should allow substantial opportunity for niche diversification and ecological speciation (Bellwood & Wainwright 2002, Streelman et al. 2002, Alfaro et al. 2007). This environmental heterogeneity can create opportunities for fishes to exploit a wide range of resources, thus allowing disruptive selection to favor certain adaptive traits and potentially lead to reproductive isolation. Specifically identifying these environmental drivers and the phenotypic traits they shape is essential to our understanding of how marine biodiversity is generated.

What are the adaptations that foster niche divergence in coral reef fish? Trophic specializations are arguably the most significant trait facilitating adaptive niche divergence. The coevolution of habitat association and feeding mode is prevalent in reef fishes (Streelman et al. 2002) and many major groups are split along eco-morphological axes (Wainwright et al. 2012, Betancur-R et al. 2013, Near et al. 2013).

Coloration in coral reef fishes is also well known for its exceptional diversity. Yet the evolutionary mechanisms that maintain color pattern variation and the role color plays in speciation remain enigmatic (McMillan et al. 1999, Bernardi et al. 2002, Barreto & McCartney 2007, Lin et al. 2009, DiBattista et al. 2012). Color polymorphisms in reef fishes offer special opportunities for testing what ecological mechanisms drive diversification. Intraspecific color polymorphisms are common and widespread in reef fishes (Randall & Randall 1960, Thresher 1978, Larson 1980, Thresher 1984, Marliave 1985, DeMartini & Donaldson 1996, Munday et al. 2003, Drew et al. 2008). Color is likely to be a significant evolutionary trait in some reef fish families, as it is often the only morphological trait separating closely related species (Lieske & Myers 1999, Allen et al. 2005). Despite the potential evolutionary significance of color, the ecological conditions that promote divergence in color and ultimately reproductive isolation are largely unknown.
In this chapter, I investigate the mechanisms maintaining color variation using the Arc-eye Hawkfish, *Paracirrhites arcatus* (family Cirrhitidae), a small, coral-dwelling reef fish common on shallow reefs throughout the Indo-Pacific. *P. arcatus* exhibits a permanent color polymorphism that is behaviorally unmodifiable and as yet unrelated to size, sex, maturation, or ontogeny (Randall 1963, Donaldson 1990, Sadovy & Donaldson 1995, Myers 1999). There are two distinct, predominant color morphs (Fig. 2.1): a pink-white-stripe (PWS) morph with a pink to reddish body and a bright white-stripe running along the flank, and a melanistic (MEL) morph with a more uniformly olive to dark-brown body and lacking a stripe; as well as an intermediate morph (INT) that is reddish-olive with a white stripe (DeMartini & Donaldson 1996). With the exception of color differences, morphs appear to be morphologically and meristically identical (Randall 1963).

The two predominant color morphs are sympatric throughout the entire species range, from East Africa to the Hawaiian Islands, and were found to co-occur at 96% of sites (159 of 165) surveyed throughout the West, Central, and South Pacific (DeMartini & Donaldson 1996), although at varying relative abundances. The PWS morph is by far the most common throughout most of the Indo-Pacific (~80% of individuals), followed by the MEL morph (~15%), and the less common intermediate morph (<5%; DeMartini & Donaldson 1996). In the Hawaiian Islands, however, the two distinct morphs (MEL and PWS) are more equally abundant, and the melanistic morph is more frequent at several sites along the island of Hawaii (Randall 1963, DeMartini & Donaldson 1996). The reason for this higher prevalence of the melanistic morph is unknown.

*Paracirrhites arcatus* is a semi-sedentary diurnal ambush predator that frequently shelters in branching corals, particularly *Pocillopora* spp. (DeMartini & Donaldson 1996, Kane et al. 2009, Coker et al. 2015). Although co-occurring, color morphs do appear to have different habitat preferences. A Pacific-wide survey determined that morph frequencies are strongly correlated with bottom depth with the MEL morphs were more abundant shallow (<5m) and PWS morphs more abundant deep (>8m) (DeMartini & Donaldson 1996). To a lesser degree, frequencies were also correlated with the density of their preferred coral host, *Pocillopora meandrina* (DeMartini & Donaldson 1996). The authors concluded that morphs are likely partitioning resources based on either coral community or depth, but that more work would be needed to resolve habitat preferences and determine the adaptive function of color variation.
In this study, I evaluate the hypothesis that niche divergence among *P. arcatus* color morphs explains the maintenance of multiple color phenotypes. I hypothesize that alternative color patterns are being favored by disruptive natural selection in heterogeneous microhabitats, and that partitioning of microhabitats is driving the coexistence of multiple sympatric color morphs despite no geographic isolation. To test if the color polymorphism could be maintained by fitness differences among habitats I further examined the spatial distribution and patterns of microhabitat use of the three color morphs. I used fine-scale habitat surveys to (1) identify correlations between phenotypes and environmental gradients, (2) assess the relative contributions of ecological factors in explaining variation in morph frequencies, and (3) infer the adaptive function of color phenotypes in respective microhabitats.

**METHODS**

**Fieldwork**

To characterize the habitat preferences of each morph, I conducted a series of ecological surveys in 2010 at nine sites spanning 80 km of coastline on the leeward side of the Island of Hawaii (Fig. 2.2). Sites were required to have at least one morphotype present and a contiguous reef habitat extending over a depth gradient. Sites were chosen to include a representative range of habitats used by *P. arcatus* morphotypes, a variety of wave exposures, and topographies. At each site, I conducted quantitative reef surveys on 25 x 4m belt transects oriented parallel to the shoreline and along an isocline in the same overall habitat type. The most shoreward transect at each site was located in the surge zone and subsequent transects were laid in parallel at intervals of 5-10m further from shore to a depth where *P. arcatus* become scarce. Due to differences in reef profile between sites, the number of transects per site was not constant (mean = 7; range: 4-12). Collectively, transects covered a depth gradient of <1m to 18m and a distance from shore of 8 - 115m.

On each transect, a pair of divers counted all hawkfish and recorded the phenotype, microhabitat, and depth. Total body length (TL) was visually estimated to the nearest cm. I validated the accuracy of visual estimations by comparing to lengths measured in a subset of collected individuals. Visual estimations were consistently within 1 cm of measured lengths. Depth of each individual was measured using calibrated Oceanic Geo2 dive computers. Microhabitat was defined as the substratum upon which an individual was perched or sheltering.
in when first sighted, and included live corals (identified to species), dead coral, lava rock, and sand. The topographic feature to which the coral was attached, its relative position on that feature, and height above the seafloor were also recorded. Topographic features were categorized as boulders, walls, outcappings, ledges, rubble, or reef flat; and the position of the coral shelter was categorized as either on the top, side, or base of the feature.

I characterized the habitat on each transect by measuring physical factors associated with reef topography (rugosity, slope, depth, and distance to shore) and wave action, as well as biological factors describing the benthic community. I estimated rugosity using the chain-and-tape index method (McCormick 1994) by measuring the length of a light brass chain (2cm links) draped over the reef surface (along the transect center line) that is needed to cover the 25m transect. The amount of chain lying on the bottom that is necessary to span the 25m transect is then divided by the straight-line tape measurement to generate an index of rugosity for that transect. Vertical surfaces could not be measured for rugosity using this method. Slope was estimated by first dividing each 25m x 4m belt transect into a grid of five 5m x 4m blocks, in which I measured the vertical change (rise) by taking the difference between the shallowest and deepest bottom depth in each block, and the linear distance between those points (hypotenuse) using a meter tape and averaged across all five blocks. The slope of each transect was calculated as the arcsine of the ratio of mean rise to mean hypotenuse and ranges from 0° (completely flat) to 90° (vertical wall face). Wave action along each transect was qualitatively categorized into three levels (high, moderate, and low) based on in-situ observations at each transect over a period of two days to account for temporal variability.

To characterize the benthic community along each transect, I measured the species composition and percent cover of all sessile benthic organisms and substrates using 50cm x 70cm (0.35 m²) PVC photoquadrats (after Preskitt 2004) placed at 15 non-overlapping random locations laid alternately on opposite sides of transect center lines. Each quadrat was photographed using a mounted digital camera (Canon Powershot G9 with a Canon WP-DC21 underwater housing and digital slave strobe) and later interpreted using the software CoralPointCount v4.0 (Kohler & Gill 2006). Using random point counting (100 random points overlain onto each image), I calculated percentage cover of each taxon/substratum per transect by summing point counts over all 15 quadrats and dividing by 1500 (100 random points per quadrat x 15 quadrats = 1500 points). All taxa were identified to the lowest taxonomic level...
possible and abiotic substrates were categorized as rock, rubble or sand. A summary of the environmental variables recorded and their ranges are provided in supplemental Table S2.1.

**Statistical analysis**

I first aimed to describe patterns of co-occurrence among morphs and quantify the degree of spatial overlap at site and transect scales. To account for differences in abundance (as opposed to just presence or absence) I calculated Levins’ (1968) spatial overlap index ($B$), which factors in differences in abundance among transects and ranges from 0.0 (no overlap) to 1.0 (complete overlap). I also measured standardized Levins’ Niche Breadth for each morphotype, which ranges from 0.0 (narrow niche) to 1.0 (broad niche) and is a function of the uniformity of the distribution of abundance among the sites surveyed (Levins 1968, Pandit et al. 2009). All indices were calculated using the spaa package (Zhang et al. 2013) in R v3.1.2 (R Core Development Team).

To characterize the relationship between total abundance and environmental variables, I analyzed the spatial variation in total *P. arcatus* abundance between transects using generalized linear models (GLMs). Total abundance (sum of all three morphs) per transect (n=64) was modeled with a gamma distribution and a log-link function using eight environmental parameters as fixed effects. Explanatory variables were all standardized and percentage cover measures were logit transformed. I compared GLMs with different main effects, interactions, and quadratic effects using the Akaike Information Criterion (Akaike 1974). The model with the lowest AIC was selected and subsequently simplified by removing non-significant ($P > 0.05$) fixed-effect parameters in a backwards-stepwise process until all effects were significant (Zuur et al. 2009). Estimation of all GLMs was done using the glmmADMB package v0.7.7 in R (Fournier et al. 2012).

**Phenotype-environment relationships**

To examine the relationship between color morphotypes and ecological gradients, I first performed a redundancy analysis (RDA), which regresses a community response matrix on a matrix of environmental variables observed on the same transects. The response matrix consisted of abundance counts of the three *P. arcatus* color morphotypes per transect (1,866 individuals; 64 transects) transformed into relative abundance (Legendre & Gallagher 2001). The explanatory
matrix included three physical parameters (depth, slope and wave action) and percent live coral cover of *Pocillopora meandrina* and *Porites* spp. (*Por. lobata* and *Por. compressa*). Given that these three coral species account for 98% of the total coral cover, I am confident that they collectively provide a faithful representation of the coral community.

The coordinates of the RDA were constructed using linear combinations of constraining variables (linear constraint, LC, scores), which creates a multivariate habitat gradient that is calculated independently of the response matrix (Palmer 1993, Oksanen et al. 2015). Because RDA computes a linear model of explanatory variables and the morph-environment relationships are not necessarily linear, I also tested the effect of polynomials and transformations of each variable (Legendre & Legendre 2012), however, neither explained more variance than raw variables, thus I proceeded with non-transformed single-order variables. Total coral cover and distance from shore were excluded because of high correlations with *Por. lobata* cover ($r = 0.81$) and depth ($r = 0.93$) and rugosity was excluded because vertical wall transects resulted in empty cells, which are not tolerated in the RDA. All included independent parameters had relatively low multicollinearity (variance-inflation factors $< 2$ and $r < 0.5$; see supplemental Table S2.2 for correlation matrix). The RDA and variation partitioning (Borcard et al. 1992) were conducted using the Vegan package in R (Oksanen et al. 2015). Significance was tested with permutation (100,000 steps) and results were visualized with triplots using LC scores for the first two RDA axes. Adjusted $R^2$ ($R^2_{adj}$) values were used as unbiased estimates of the proportion of explained variance (Peres-Neto et al. 2006).

**Niche overlap**

My next objective was to determine if color morphs occupy different niche space. To test this I would first need to identify the distribution in niche space occupied by each morph, and then calculate the amount of niche overlap. To compare the multidimensional niche space used by each color morph, I used an approach introduced by Mouillot (2005) and modified by Geange et al. (2010) that combines probability distributions and density estimation techniques to produce comparable measures of niche overlap across several niche dimensions in a way that is independent of underlying distributions.

My analysis included habitat data from 1,714 *P. arcatus* of three morphotypes: MEL (n=723), INT (n=393), and PWS (n=598). I estimated niche overlap between each color morph
using 10 habitat measures, which included four categorical variables (coral perch type, microhabitat, wave action and *Por. compressa* cover), four percentages (*Poc. meandrina* cover, *Por. lobata* cover, total coral cover and rock cover) and two continuous measurements (depth and slope). Due to issues with zero-inflation, the percentage cover of *Por. compressa* had to be categorized into a factor with three levels (absent, 0.1-10.0%, >10.0%). These variables represent habitat resources measured on various spatial scales. Coral perch type, microhabitat and depth were recorded for each fish and represent habitat resources on the scale of centimeters to meters. Whereas slope, wave action and coral cover were estimated per transect and thus describe the habitat at the scale of meters to tens of meters. In addition, I also calculated niche overlap using just the primary axis of the RDA as a synthetic niche gradient as it encompasses just the most explanatory variables that can be used to establish the distribution of color morphs in multidimensional niche hyperspace (Laliberté & Legendre 2010).

I conducted a unified multivariate niche overlap analysis using the R scripts provided in Geange et al. (2010), which use mixture models to estimate probability distributions for each data type followed by kernel-density estimations to calculate the overlapping distributions of morphotypes. This gives rise to equivalent univariate measures of niche overlap ranging from 0.0 (no overlap) to 1.0 (complete overlap) that can be averaged over multiple axes to create a composite niche overlap. The question of interest is then whether niche overlap is sufficiently < 1.0 to provide evidence of niche differentiation. Permutation tests were used to produce a statistical null distribution by calculating pseudo values of niche overlap, which would arise if the null hypothesis (complete niche overlap) was true (Gotelli & Graves 1996, Gotelli 2000). To provide a null model of complete niche overlap, the color morph labels were permuted 100,000 times. Niche overlap (NO) statistics were calculated for each niche dimension and compared with null distributions to test significance of overlap for each niche axis separately and for the composite multidimensional niche space. *T*-tests were calculated to compare data-based NO with the null pseudo-values of NO for each morph-comparison (n=3) per axis (n=9). To protect against false positives (i.e., detecting niche differentiation that is not really there) sequential Bonferroni adjustments were used (Geange et al. 2010).

In order to visualize niche overlap in a manner consistent with how the statistic is calculated, I constructed kernel density plots for all quantitative axes, excluding the categorical variables perch and microhabitat. To visually represent the composite multidimensional niche
overlap, I also constructed a kernel density estimation plot of the first RDA axis (RDA1), as it represents the major six quantitative variables tested, accounts for the majority of the variation in those variables and has significant linear relationships with each.

**Microhabitat use and selectivity**
My next objective was to test if color morphs are partitioning ecological resources by using different microhabitats. *Paracirrhites arcatus* perch and shelter on hermatypic corals, which serve as platforms for foraging, courtship, spawning, and territorial displays. I compared microhabitat use between color morphs by assessing differences in use and selectivity of coral host species, the position of coral perches both among and within topographic features, as well as the vertical positioning (height above bottom) of coral shelters.

To determine if morphotypes show differential use and selectivity of different coral species as a perch or shelter I estimated the microhabitat preference using Manly’s selectivity index \( w_i \), which is the ratio of proportional use \( o_i \) relative to the proportional availability \( \pi_i \) of each habitat type (Manly et al. 2002). Microhabitat use was measured (per transect) as the number of individuals recorded on each coral species and availability was measured using percentage cover estimates from photoquadrats averaged per transect. I employed the adehabitatHS package in R (Calenge 2006) to calculate Manly’s index along with a log-likelihood \( \chi^2(X_L^2) \) for each transect, to test preference or avoidance of each habitat type and test for significant differences between selection indices. I used the Bonferroni method to adjust the significance of the \( w_i \) values for multiple comparisons. All coral species that were recorded being used less than 5 times were excluded from analysis as comparisons would be unreliable (Manly et al. 2002). For this reason, I excluded the corals *Pocillopora eydouxi* and *Porites evermanni*, which were relatively rare at the study sites.

I tested for differences in the use of topographic features as microhabitat among color morphs. To simplify analyses, structural features were categorized as having high complexity (i.e., vertical relief), which included boulders, walls, outcrops and ledges, or as having low complexity (i.e., flat or low structure), which included rubble patches, reef flats and bench pavement. Frequencies were tabulated for each feature type for the two common morphs, and compared using a \( \chi^2 \) test of independence. I also tested for differences in the vertical positioning of each color morph when perching on topographic features, which was categorized as either on
the top, side, or base of the feature. I performed a three-by-three $\chi^2$ test of independence using two color morphs and three vertical positions using position data from a total of 809 fish (MEL = 474, PWS = 335).

**RESULTS**

To test the hypothesis that color phenotype is independent of size or ontogeny, I compared body length-frequency distributions for each color type and found that distributions were similar among morphs (Fig. S2.1) and each phenotype was represented by individuals of all sizes, ranging from 4 to 15 cm TL. All three morphs had roughly equivalent average body lengths ~ 8 cm: MEL (7.6cm ± 1.7cm), INT (7.5cm ± 1.7cm), PWS (8.3cm ± 2.0cm). These results support the hypothesis that color phenotype is independent of size or ontogeny in *P. arcatus*, at least for the sizes tested here (>3cm). Furthermore, gross morphology of dissected gonads revealed that color variation was also independent of sex, as each color type had both male and females.

**Species-habitat patterns**

Before exploring the habitat patterns that differentiate color morphs, I first aimed to characterize the habitat relationships for the species (ignoring phenotype), by analyzing the variance in total abundance among and within sites. The density of *P. arcatus* varied significantly among sites (ANOVA: $F_{7,54}=17.61, P < 0.00001$), however, this was driven almost exclusively by site H (Wawahiwaa Point), which had an average density (85.4 fish/100m$^2$) 2-6 times greater than all other sites (Tukey HSD, $P < 0.00001$). The 608 individuals recorded at this site, represent more than a third (35.7%) of all fish, and more than half (57.5%) of all INT morphs. Densities did not vary significantly among the other seven sites (Tukey HSD, $P > 0.001$, Bonferroni adjusted $\alpha = 0.001$).

The GLM of total *P. arcatus* abundance revealed that *Poc. meandrina* cover and depth were the only habitat variables with a significant effect on abundance ($P < 0.00001$; Table S2.3). *Poc. meandrina* cover was the best predictor ($R^2 = 0.59$) and had a positive linear relationship with abundance (Fig. S2.2), whereas depth had a weak negative relationship ($R^2 = 0.023$). The exclusion of site H did not impact the overall result. In summary, these results indicate that the highest abundances were found on transects where *Poc. meandrina* has higher cover (> 20% cover), which occurs in relatively shallow depths (<10m). My results correspond closely with
those of Kane et al. (2009), who also found that the abundance of Pocillopora corals explained nearly two-thirds of the variation in abundance of P. arcatus at reefs in French Polynesia.

**Morph composition, abundance and co-occurrence**

I surveyed more than 6,400 m² of coral reef habitat and documented a total of 1,866 individual P. arcatus of three color morphotypes (Fig. 2.1): Melanistic (n = 741), Pink-White-Stripe (734), as well as the Intermediate phenotypes (391). Overall, the two predominant morphotypes, MEL and PWS, had equivalent relative abundances (39.6% and 39.3%, respectively), while the intermediate phenotype (INT) was roughly half as abundant (20.9%). All three morphs co-occurred at all sites, and thus were found to have no spatial isolation at the island and site scales. Relative abundances, however, did vary significantly among sites for both MEL (mean RA = 0.39; \( F_{7,52} = 4.07, P = 0.0013 \)), and PWS (mean RA = 0.42; \( F_{7,52} = 2.68, P = 0.019 \)), but not INT (mean RA = 0.19; \( F_{7,52} = 1.62, P = 0.144 \)). These differences were driven primarily by comparisons with site J, which had only 1% PWS. For comparison, I re-ran ANOVAs excluding site J and neither MEL (\( F_{6,47} = 1.718, P = 0.138 \)) nor PWS (\( F_{6,47} = 1.171, P = 0.338 \)) had significant differences in relative abundance among the remaining 8 sites.

Within sites, there were differences in morph composition. MEL and PWS co-occurred on 62% of transects, and all three morphs co-occurred on 50% of transects. Levin’s spatial overlap index, which accounts for differences in abundance among transects, indicated that the spatial overlap between MEL and PWS (\( B = 0.36; CI_{95\%} = 0.22-0.51; P < 0.001 \)) was less than expected at random. The intermediate phenotype (INT) was always found co-occurring with one or both dominant morphs (i.e., was never the only morph present on a transect) and had nearly twice as much spatial overlap with MEL (\( B = 0.67; CI_{95\%} = 0.49-0.87 \)) than with PWS (\( B = 0.38; CI_{95\%} = 0.24-0.55 \)). Levins niche breadth (LNB) indicated that the niches (measured by use of transects) of the two main morphotypes have similar breadths (MEL: LNB = 0.37; PWS: LNB = 0.40), while the niche of the intermediate MS phenotype was distinctly narrower (LNB = 0.23).

Differences in both occurrence and relative abundance among transects indicate that color morphs are not equally distributed among habitats. Transects were categorized into dominance groupings based on which morph had relative abundance greater than 0.5. Of 64 transects, 42% were dominated by PWS, 33% by MEL, 12.5% by INT, and the remaining 12.5% were considered mixed transects where no morph was numerically dominant. Each of the two
main color morphs were highly clustered onto transects in which they are numerically dominant. Less than 10% of all melanistic morphs are found on PWS-dominated transects, and less than 5% of PWS are found on MEL-dominated transects. Therefore, these groupings provide a useful means of classifying transects.

Despite co-occurring at all sites, MEL and PWS show significant partitioning of habitats within sites. The spatial segregation is evident when relative abundance is viewed along a distance to shore gradient, as MEL morphs have higher relative abundance closer to shore, and PWS morphs further from shore (Fig. S2.3). At four of eight sites, morph composition transitions from >90% MEL to >90% PWS along a depth or distance to shore gradient. All transects with 100% MEL are the most shoreward per site and all transects with 100% PWS are the deepest (most seaward) – with one exception that occurs in a protected, flat backreef. The spatial scale of the transition between MEL dominated and PWS dominated habitats varies from dramatic shifts in only a few meters to gradual transitions over 10’s of meters. For example, at Mailu Point and Kukio Bay relative frequencies shift from mostly MEL (80-100%) on vertical walls to mostly PWS (78-100%) on the adjacent flat shelf reef less than 5m away. In contrast, at sites with more gradual reef profiles, such as Noio Point, morph composition shifts from all MEL to all PWS gradually over 40+ meters in horizontal distance; thereby creating a longer transition zone. Interestingly, such transition zones are precisely where the majority of the intermediate phenotypes occur: 72.5% of all INT were observed in transition habitats where both MEL and PWS had similar relative abundances (i.e., both between 0.3-0.7 relative abundance). Therefore, INT morphs appear to occur almost exclusively in areas where MEL and PWS overlap.

**Phenotype-environment relationships**

Shifts in abundance of *P. arcatus* color morphs correlate strongly to habitat gradients (Table 2.1; Fig. 2.3; Table S2.4). Nine of ten environmental parameters varied significantly among the groups of transects categorized by numerical dominance (ANOVA; $P < 0.005$). Rugosity was the only variable tested that was not significantly different ($P = 0.261$) among groups. The two variables that failed to meet ANOVA assumptions were also tested using the Kruskal-Wallis rank test, which detected significant differences in both *Por. compressa* cover ($X^2 = 21.03$, df = 2, $P < 0.0001$) and wave action ($X^2 = 41.16$, df = 2, $P < 0.0001$). Tukey’s post-hoc comparisons between MEL and PWS dominated transects revealed significant differences in means of all
eight variables tested \((P < 0.001)\), which indicates that shifts in the relative abundance of the two dominant color morphotypes are associated with significant changes in multiple factors (Fig. 2.3, Fig. S2.4).

The RDA revealed that color morph community structure was significantly correlated with environmental gradients on these reefs. Seven environmental variables representing gradients in reef topography, coral community, and wave action collectively explained 66.5\% of the variance in relative abundance of three \(P. arcatus\) color morphs among transects \((F_{8,55} = 14.4, P < 0.00001;\) Table 2.2). The first axis (RDA1) explained 63.2\% of the total variability in morph relative abundance, while the second axis (RDA2) explained only 3.2\% in additional variance (Table 2.3). RDA1 represents a gradient of both physical and biological factors that strongly separates morphs in ecospace. All seven variables had high loadings with RDA1 axis. Wave action, slope, \(Poc. meandrina\) cover, and rock cover had negative loadings, while depth, distance to shore and percent \(Porites\) cover had positive loadings (Table 2.2; Fig. 2.4).

Linear constraint site scores clearly segregate into two discrete sides of the RDA1 gradient, which strongly correlates to morph relative abundance (Table 2.3; Fig. 2.4). Morph scores indicate that MEL and PWS have inverse loadings on RDA1 (-0.99 and +1.25, respectively), while INT is intermediate (-0.26), yet more similar to MEL (Table 2.3). The distribution of each color phenotype is thus strongly correlated to the ecological gradient represented by RDA1. Color morph community structure is a continuum ranging from transects with 100\% MEL \((n = 10)\) at one extreme to 100\% PWS \((n = 14)\) at the other, with the majority \((n = 40)\) in-between. The proportion of PWS to MEL has a strong relationship with RDA1 \((R^2_{\text{adj}} = 0.81, F_{1,62} = 316.5, P < 0.00001),\) and all transects where only one morph is present correspond to opposite ends of the ecological gradient (Fig. 2.5).

All transects dominated by MEL morphs have negative RDA values, which represent shallow, steep, topographically complex habitats with relatively higher water motion and a coral community dominated by \(Poc. meandrina\). In contrast, all PWS dominated transects (positive RDA values) are deeper, flatter habitats with less wave action and a coral community dominated by \(Por. lobata\) and \(Por. compressa\). The intermediate phenotype was more abundant in intermediate habitats characterized by moderate slope, moderate wave action, and a coral community transitioning between \(Poc. meandrina\) and \(Porites spp.\) INT morphs have higher relative abundance on transects that more closely overlap with MEL. Of the eight transects
where INT was the numerically dominant morph, seven fell within the ellipse representing the 95% confidence interval of MEL dominated transects (Fig. 2.4), whereas only one transect fell within the PWS ellipse.

Although morph relative abundance is clearly correlated with multiple covarying factors, gradients in topography and water motion appear to be the major driving force in segregating color phenotypes. When tested individually, topography and wave action (together) explain the highest individual fraction of variance (63%), followed by reef profile (47%), and coral community (38%). When combined, the largest partition of variation in morph relative abundance (30%) is jointly explained by all variables, which suggests high covariance among parameters (Fig. 2.6). The majority of the remaining variation is explained by wave action, either alone (12%) or jointly with reef profile (15%) and coral community (7%), which together explain only 3% of variation that was not shared with other factors (Fig. 2.6). The residual (unconstrained) axes represent the remaining 33.3% of variance left unexplained by the RDA model. I attempted to fit the random effect of site onto the residual axes to assess if it is driving the unexplained variance. Site had significant goodness of fit with residual axes ($R^2 = 0.29$), indicating that random effects varying among sites account for a significant proportion of the unexplained variance in the model.

Niche overlap
To determine if two color morphs occupy different niche space, I estimated niche overlap (NO) between each morph pairs for all niche axes separately then averaged niche overlap over all axes to create a composite output of multidimensional niche overlap. I also used the primary axis of RDA as a synthetic niche gradient that represents a combination of the most explanatory variables. I found significant evidence of niche divergence between MEL and PWS morphs across all nine niche dimensions ($P < 0.0001$), with niche overlap ranging from 0.41 (wave action) to 0.79 (depth; Table 2.4). The multidimensional niche overlap between MEL and PWS (NO = 0.64) was significantly less than would be expected under random assortment ($P < 0.0001$). This result demonstrates niche differentiation between color morphotypes, which can be summarized as having a 36% divergence (1-NO) in local realized niche space.

There was evidence of niche divergence among all three color morphs. The local realized niche of the intermediate INT morph had significant niche divergence with both MEL (NO =
0.78, \( P < 0.0001 \)) and PWS (NO = 0.65; \( P < 0.0001 \)). With respect to individual axes, INT had greater overlap with MEL (than with PWS) on all axes but depth, and nearly complete overlap on the coral host axis (NO = 0.98; Table 2.4). *Por. lobata* cover had the lowest niche overlap of any single axis for both MEL-PWS (0.54) and INT-PWS (0.44) comparisons. When averaging NO values over all three comparisons, *Por. lobata* cover and wave action had the lowest average niche overlaps (0.55 and 0.59, respectively), and therefore represent the most dissimilar niche dimensions of those tested.

Of all 27 single axis niche overlap comparisons (3 morph pairs x 9 axes), all but one (Perch, MEL-INT: NO = 0.98, \( P = 0.775 \)) were significantly less than with random permutation (\( P < 0.0001 \); Bonferroni adjusted \( \alpha = 0.0016 \)), thereby indicating differentiation across eight individual niche dimensions (Table 2.4). To provide a visual representation of niche overlap and divergence between morphs I constructed kernel-density estimation plots of each individual niche axis separately as well as the composite multidimensional niche space represented by RDA1 (Fig. 2.7A). The niche overlap analysis using just RDA1 produced very similar niche overlap estimates as when using nine individual axes. There was evidence of significant niche divergence in all three morph comparisons (\( P < 0.0001 \)) where MEL and PWS had the least amount of niche overlap (NO = 0.41), followed by INT and PWS (NO = 0.46); and INT and MEL had the highest overlap (NO = 0.71; Table 2.4; Fig. 2.7).

In summary, I found significant evidence of niche divergence between all three morphotypes. The lowest overlap occurred between PWS and the MEL morphs, indicating that the local realized niches of the two predominant morphs were most dissimilar. The intermediate INT morphs also had significant niche divergence from both MEL and PWS, but in niche space that is intermediate to the two, yet with greater overlap with MEL compared to PWS. Of single axes, they all differed the most with respect to *Por. lobata* cover and wave action and the least on the axes of perch and depth.

**Microhabitat use and selectivity**

I recorded microhabitat use for 1,866 individual *P. arcatus* (741 MEL, 734 PWS, and 391 INT). First, I compared the usage of host corals as sheltering microhabitat (ignoring differences in availability) using sums from all individuals on all transects. All three morphs were found predominately using *Poc. meandrina*, and to a lesser extent on *Por. lobata*, and rarely on *Poc.*
eydouxi and Por. evermanni. Whereas both MEL and INT used Poc. meandrina almost exclusively (>90%), PWS used Poc. meandrina (69.6%) predominantly, but would also perch on Por. lobata (22.6%) and to a lesser extent (6.9%) on Por. compressa (Fig. 2.8A). Percentage use of Poc. meandrina was 21.5% less in PWS (69.6%) compared to MEL (91.1%) and this difference was significant (Student’s t-test; $P < 0.05$). Although PWS used Por. lobata ~15% more than MEL and INT, this difference was not significant (ANOVA; $F_{1,94} = 3.21; P = 0.076$). Percentage use of Por. compressa however, was significantly different among morphs ($F_{1,94} = 11.34; P = 0.0011$); PWS used Por. compressa 6.9% of the time, while both MEL and INT were never recorded using it. In summary, PWS was found using Por. lobata and Por. compressa more than MEL and INT. Thus, PWS appears to be less dependent on Poc. meandrina and will perch on Por. lobata and Por. compressa proportionately more than the darker morphs (Fig. 2.8A).

To account for differences in availability of each coral type, I calculated the microhabitat selectivity using Manly’s index ($w_i$), which ranges from 0.0 (complete avoidance) to infinite (maximum positive selection), with values ~ 1.0 indicating that use is proportionate to availability (no selectivity; Manly et al. 2002). All three P. arcatus color morphs showed highly significant selectivity between coral microhabitats ($P < 0.001$): MEL ($X^2 = 4066; df = 21$), PWS ($X^2 = 3934; df = 41$), INT ($X^2 = 3154; df = 18$). Tests of selectivity of MEL and INT morphs were highly significant ($P < 0.001$) for each of the three coral habitats (Table 2.5; Fig. 2.8B): Poc. meandrina was highly preferred ($w_M = 2.4$), whereas Por. lobata was avoided ($w_L < 0.27$) and Por. compressa was completely avoided ($w_C = 0$). Selectivity tests of PWS morphs indicated that while Poc. meandrina was positively selected ($w_M = 2.53; P < 0.001$) and Por. lobata was avoided ($w_L = 0.40; P < 0.001$). The selectivity measure of Por. compressa by PWS ($w_C = 0.87; P = 0.231$) was the only $w_i$ that was not significantly different from null expectation ($w_i = 1.0$), indicating that it was used in proportion to availability (Table 2.5; Fig. 2.8B). Although I had to exclude Poc. eydouxi from the analyses due to small sample size, these corals appear to be highly preferred by all three morphs ($w_i > 5.0$). Despite their rarity in my study sites (~0.1% cover), nearly every Poc. eydouxi colony was inhabited by P. arcatus.

Comparisons of the selectivity of each coral species among color morphs shows that Poc. meandrina was the preferred coral species in all three color morphs, and the strength of selectivity is relatively equal among morphs ($w_m = 2.44-2.53$; Fig. 2.8B). Por. lobata is
negatively selected (avoided) by all three morphs and, although the strength of avoidance varied slightly among morphs, the differences were not significant (based on overlapping 95% confidence intervals). In contrast, the selectivity of *Por. compressa* differed significantly among morphs, as it was completely avoided by the melanistic and intermediate morphs (neither were ever recorded using it as a perch), but was used by PWS morphs in proportion to its availability. *Por. compressa*, however, is so rare in shallow habitats (<1% of cover) that the selectivity of MEL and INT morphs is probably closer to neutral than avoidance. In summary, these results indicate that all three morphs show similarly strong preference for *Pocillopora meandrina*, avoidance of *Por. lobata*, and differ mainly in their selectivity of *Por. compressa*, which is neutral to PWS and avoided by MEL and INT.

Use of topographic features was not independent of color type ($\chi^2 = 169.6; P < 0.00001$; Table 2.6): 90.4% of melanistic morphs were found perching on corals that were growing on topographically complex structures such as boulders, ledges, and outcrops, compared to 41% of PWS. In contrast, the majority of PWS (59%) were found perching on less complex features such as reef flats and rubble beds, compared to 9.6% of MEL. Therefore, color morphs are segregating into microhabitats with the majority of MEL morphs using features with high vertical relief, the majority of PWS morphs using less complex features, and intermediate morphs occupying transition zones. Furthermore, vertical positioning on structural features was not independent of color morph ($\chi^2 = 125.8; df = 4; P < 0.00001$; Table 2.7, Fig. 2.9). MEL morphs were positioned on either the sides or tops of structures nearly twice as frequently as PWS morphs (78% versus 40%), where the latter were more frequently perch on corals attached to the base of structures.

**DISCUSSION**

**Niche partitioning**

The data described in this chapter reveal strong ecological divergence between the two common color morphs of *Paracirrhites arcatus*. I found that morph frequencies correlate strongly with ecological gradients associated with wave action, reef profile, and coral community structure. Melanistic morphs were more frequent in shallow surge zones characterized by steeper slopes, higher wave action and higher relative cover of the branching coral *Poc. meandrina*. In contrast,
pink-white-stripe morphs were more frequent in deeper sub-surge zones characterized by proportionately more gradual slopes, less wave action and higher coral cover particularly of Porites corals (Por. lobata and Por. compressa). The redundancy analysis revealed that the combination of these gradients explains the majority (~65%) of the variability in morph relative abundance, and that the two common color morphs are utilizing distinctly different habitats (Fig. 2.4). The 24 transects with only 1 morph present are at opposite extremes of the gradients represented by RDA1 (Fig. 2.5). These opposite ends could be interpreted as the marginal edges of the habitat gradient, where each morph is experiencing the least inter-morph competition. Transects with steep slopes where Poc. meandrina is dominant contain only melanistic morphs, whereas flat areas with Porites corals contain only PWS morphs. These results provide robust evidence of a strong correlation between color phenotype and environment.

My results confirm trends observed previously and help to resolve the patterns and drivers of hawkfish habitat patterns. DeMartini & Donaldson (1996) concluded that morph frequencies were correlated most strongly to depth and less so to the density of Poc. meandrina. My results confirm that depth and Poc. meandrina density were both correlated with morph frequencies. I found, however, that depth was a poor predictor of morph relative abundance and did not account for any unique variation, suggesting that the correlation with depth is the result of covariance with other parameters, most likely wave action. Generally, changes in habitat from one reef zone to another coincide with depth and corresponding gradients in wave energy (Depczynski & Bellwood 2005). Although morphs are segregated along a depth gradient, my results indicate that differences in coral substrate and topography may be the driving forces. Therefore, I support DeMartini & Donaldson’s (1996) hypothesis that morphs may be partitioning resources based on coral substrate. My results indicate that variation in coral community and substrate type are the proximate mechanisms influencing color morph distributions.

The habitat choices correspond to discrete ecological zones typical of Hawaiian coral reefs (Dollar 1982). Transects dominated by MEL morphs are in the Poc. meandrina boulder zone, which is closer to shore and has less than 40% coral cover. Whereas transects dominated by PWS morphs are in the Por. compressa reef slope zone, which is furthest from shore and has greater than 60% coral cover. Mixed transects, where both morphs are present and neither are dominant, are generally found in the intermediate Por. lobata reef bench zone. Wave action and
topography are the primary mechanisms controlling coral reef community structure in Hawaii (Dollar 1982, Grigg 1983). The decrease in *Poc. meandrina* density with depth is driven primarily by increased competition from *Por. compressa*, which is competitively superior, but less tolerant of wave stress (Dollar 1982, Grigg 1983). Therefore, it seems likely that hawkfish are selecting habitat based on the substrate type and coral community, which in turn is controlled by wave action and topography.

Color morphs were found to differ in their use of coral hosts as a microhabitat, yet showed similar habitat selectivity. Usage of coral hosts differed slightly among phenotypes as the melanistic and intermediate morphs perched almost exclusively on *Poc. meandrina*, whereas the white-striped morph had a broader usage that included more *Porites* spp. corals. When comparing use relative to proportional availability, all three morphs had similarly strong preference for *Poc. meandrina*, avoidance of *Por. lobata* and used *Por. compressa* more or less in proportion to its availability. The preference for perching on live Pocilloporid corals is documented in Hawaii and throughout the Pacific (DeMartini 1996, DeMartini & Donaldson 1996, Kane et al. 2009, Coker et al. 2015). Several factors likely contribute to this preference. *Poc. meandrina* is the only closely branched coral common in the main Hawaiian Islands (Maragos 1972), its branching pattern creates refuge spaces (DeMartini 1996), and the surfaces are covered with verrucae (skeletal projections), which create frictional drag and offer grip to sheltering fish (Jokiel 1978).

The selectivity analysis indicates that differences in coral host use among phenotypes is driven more by availability than preference. Although all morphs showed similar preferences for *Pocillopora*, PWS is less dependent on these corals and will use *Porites* corals three times more frequently than the darker morphs. It is likely that PWS uses *Por. compressa* and *Por. lobata* as a perch more often because they are the dominant hermatypic corals in deeper reef zones preferred by the PWS morph. The PWS morphs slightly more generalized use of coral perches may help explain why it is able to exploit deeper habitats where melanistic morphs are rarely encountered.

Color morphs show strong segregation by topographic structures. Melanistic phenotypes occur primarily on substrates with more vertical relief such as walls, boulders, ledges, and outcrops, whereas the majority of PWS phenotypes occur in habitats with less vertical relief like reef flats and rubble beds (Fig. 2.9). Because these complex topographies tend to be shallower and more shoreward this habitat preference segregates morphs along a horizontal gradient.
Furthermore, morphs are vertically segregated as well. Melanistic morphs typically perch on the sides or on top of structures, while PWS morphs tend to perch at the base of structures or adjacent to them. On average, MEL morphs occurred 0.5m shallower than PWS morphs on the same transects. Therefore, color morphs are partitioning the reefscape on fine spatial scales on both horizontal and vertical axes.

**Functional aspects of contrasting microhabitats**

The two morphs are cryptic in their respective microhabitats. The PWS-associated sub-surge zone has a lighter substrate with less exposed basalt due to the higher coral cover, particularly of the lighter-colored *Porites* (Fig. 2.10A-C). The MEL-associated surge zone has a darker substrate due to more exposed basalt rock, and lower coral cover dominated by darkly colored *Poc. meandrina* (Fig. 2.10D-F). Furthermore, the intensity and behavior of light varies strongly by depth and topography, both of which correlate to microhabitats. The MEL habitat has more complex topographies that creates a more complex light regime with more shadows and backlighting when compared to the flatter benches and reef slopes associated with PWS habitat. Therefore, the patterns of habitat use exhibited by the two morphs could be explained as a response to visual backgrounds.

The correlation between variation of color pattern and visual environment suggests that divergent selection in light and dark habitats could be favoring alternate morphs, if they experience differential fitness. MEL morphs appear more cryptic against more darkly colored basalt rock and in the shadows and backlighting created on walls and outcrops (Fig. 2.11D-F). When backlit, melanistic morphs appear as just another coral lobe and are a good match to horizontal background when viewed off the edge of a topographic structure, such as a boulder or wall ledge (Fig. 2.11F). Also, the *Poc. meandrina* growth form on vertical surfaces creates perching positions underneath the coral that are usually shadowed, which may afford better crypsis for the darker coloration (Fig. 2.11E). Moreover, the increased wave action in the surge zone strongly impacts turbidity and light absorption, and adds to the darker visual environment where melanistic morphs are more frequent.

In contrast, PWS morphs are more cryptic in areas of higher coral cover, particularly with high cover of the lighter-colored *Porites* corals (Fig. 2.11A-C). The PWS morph has a reddish-green anterior that matches adjacent *Porites*, and brown shading on the posterior dorsal that
matches well to *Pocillopora* corals (Fig. 2.11A). Due to the rapid attenuation of red wavelengths with depth (Marshall et al. 2003a), the reddish hue of PWS morphs appear green in ambient light at depths where they are most frequent (>6m), and camouflages well to the greenish color of *Por. lobata* (Fig. 2.11A-C). In addition, most reef organisms are short-wavelength sensitive and can not easily detect differences in reflection beyond the green peak at 550nm (Lythgoe 1966, 1980, Losey et al. 2003). Therefore, the reddish color of PWS will match the average yellow-green background color on shallow reefs in Hawaii (Marshall et al. 2003a).

Increased crypsis could reduce the probability of detection in their respective habitats. Phenotypes that better match the visual background of a microhabitat should have a fitness advantage resulting from catching prey or avoiding predators. If the fitness of different morphs varies among habitat types, which seems likely, this will impose strong selection for genotype-specific habitat selection. These patterns suggest that varying habitat background and light conditions may be an important selective mechanism maintaining color polymorphism in *P. arcatus*, and that crypsis (matching habitat background) may represent the most likely ecological function of body color. Although I provide circumstantial support for this hypothesis, it will need to tested experimentally to determine the strength of selection and the function of color in these habitats.

The adaptive function of the white stripe remains unresolved, but I address a few hypotheses that could direct future work. First, the stripe could be a form of disruptive coloration that obscures the body outline (Stevens & Merilaite 2009), which may be easier to detect in the more directly lit microhabitats where white-striped morphs are more frequent. Second, the stripe could improve background matching on coral heads with bleached or dead tissue. Third, the stripe may serve as a communication signal for conspecific neighbors (DeMartini & Donaldson 1996). White-striped morph frequencies are inversely correlated with the density of *Poc. meandrina* (DeMartini & Donaldson 1996 and this study). Hence, it is possible that the stripe provides a visible signal that could be more easily detected by conspecifics across greater distances between sparser *Poc. meandrina* colonies in the sub-surge *Porites*-dominated zone (DeMartini & Donaldson 1996). DeMartini (unpublished data) tested this by examining the body positioning of striped morphs in various habitats and found that exposure of the stripe was inversely correlated with *Poc. meandrina* density, such that fish in areas of sparse *Poc. meandrina* perched higher atop the corals, presumably to make themselves more visible.
(DeMartini pers.comm). Finally, if the white stripe has a significant UV component, its utility may be less obvious to human observers as we lack the spectral sensitivity to see it. The white stripe of a related hawkfish (*Cirrhitops fasciatus*) is UV reflected (Marshall et al 2003b), therefore, it is plausible the function of the stripe in *P. arcatus* is dependent on UV sensitivity. Although the function of the stripe needs to be tested experimentally, these observations provide interesting insight that should inform future work.

The two discrete morphs are segregated with respect to vertical positioning, with corresponding differences in water flow conditions that are likely to influence the composition and density of zooplankton prey, and ultimately shape the diet and foraging strategies of each morph. Although, I do not address dietary differences in this study, others have shown that differing flow conditions among microhabitats on similarly small-scales have influenced feeding strategies of planktivorous reef fish (Finelli et al. 2009). For example, two co-occurring sister species of Caribbean blennies exploit shelters that vary in their height above the substrate (Smith-Vaniz & Palacio 1973, Clarke 1989, 1994). *Acanthemblemaria spinosa* lives in shelters in corals ~1m above the seafloor and feeds primarily on planktonic copepods, while *A. aspera* lives in shelters in corals closer to the seafloor and feeds mainly on benthic copepods (Clarke 1999). Hence hydrodynamic differences among *P. arcatus* microhabitats could be exposing color morphs to different prey species and densities in their respective microhabitats. Dietary analyses coupled with zooplankton collections will be useful for evaluating foraging ecology among color morphs.

**Habitat isolation and incipient divergence**

Niche divergence requires that (1) the environment is sufficiently heterogeneous, (2) that individuals occupy different niches (3) for which their phenotype has a selective advantage. Here I support the first two requirements, and offer speculation as to the third. First, the benthic environment on these coral reefs is sufficiently heterogeneous along a short depth gradient, which are generally attributable to differing flow conditions creating a gradient in benthic coral community (Dollar 1982, Grigg 1983). Second, color morphs occupy distinctly different microhabitats that have contrasting coral communities and substrate composition. Third, color morph crypsis in their respective habitats indicates a selective advantage. Overall, these results provide strong evidence for divergence in local realized niches among color morphs. Morph
frequencies and microhabitat varied substantially within sites and correlated strongly with one another, suggesting that morphs are experiencing heterogeneous visual environments with contrasting selection pressures on a small spatial scale.

Color morphs are experiencing incomplete microspatial habitat isolation, which could be a byproduct of disruptive natural selection favoring individuals with alternative color patterns that can better exploit opposing ends of a continuous ecological resource. Even though preferred habitats exist in close proximity and individuals and their gametes can disperse more widely, their reproductive encounters may be reduced by habitat preferences (see Chapter III). As a result, gene flow can be restricted by this microspatial habitat isolation (Coyne & Orr 2004).

We can infer that some degree of reproductive isolation is probable given that the spatial segregation resulting from ecological differences likely reduces mating rates among morphs. Selection on habitat choice can generate premating isolation, when mating takes place in the habitat (Bush 1969, Rice 1984, Rice & Salt 1990, Gavrilets 2004). The relatively sessile behavior of *P. arcatus* likely restricts mating opportunities to small spatial scales (within a few meters). Spawning occurs within a male’s territory, which both males and females aggressively defend (Donaldson 1990). The combination of small home ranges, territoriality and harem mating creates conditions that decrease the probability of mating with individuals in different microhabitats. Thus, divergent selection on color pattern could automatically lead to assortative mating via spatial isolation. This is the route by which sympatric speciation is thought to be most likely (Rice 1984, Rice & Salt 1990, Gavrilets 2004).

If color pattern also contributes to non-random mating, then speciation in the presence of gene flow is possible (Maynard-Smith 1966). Color has been identified as a potential automatic isolating trait for speciation with gene flow, because it is relevant to both niche divergence and communication (Gavrilets 2004, Servedio et al. 2011). In some cases, divergence of these so-called magic traits (Gavrilets 2004) is sufficient to induce reproductive isolation. For example, in *Heliconious* butterflies mimetic color pattern is both under divergent selection and generates sexual isolation via assortative mating (Jiggins et al. 2001). Additional cases of color pattern acting as a magic trait have been found in Caribbean Hamlets (Puebla et al. 2007), Poison-dart frogs (Summers et al. 1999, Reynolds & Fitzpatrick 2007), and *Lycaeides* butterflies (Fordyce et al. 2002). Experimental studies testing for assortative mating among color morphs are described in Chapter III.
**Spatial overlap and the intermediate phenotype**

The area of spatial overlap between morphs at each site varies with reef profile and the coral community structure. Sites with strongly contrasting topographies, such as flat reefs at the base of vertical walls have more distinct partitioning and less overlap between color morphs. Whereas, sites with more gradual transitions in slope and coral community have greater spatial overlap among morphs. The spatial scale of the transition between MEL dominated and PWS dominated habitats varies from dramatic shifts in only a few meters to gradual transitions over tens of meters.

Such transition zones are precisely where the intermediate phenotypes predominate. Intermediate morphs occurred almost exclusively in transition habitats where MEL and PWS co-occur. Their relative abundance has a unimodal curve along the synthetic niche gradient represented by the primary axis of the RDA (Fig. 2.7A), whereas the other two are more linear. In turn, the niche breadth of the INT morph is significantly narrower than the other two, likely because it is experiencing inter-morph competition from both directions. Although significant niche divergence exists between both MEL and PWS morphs, the INT morph has greater niche overlap with MEL morphs. Intermediate phenotypes may have an adaptive advantage in transition habitats, which could explain the relatively high frequency of these putative hybrids. Alternatively, intermediate phenotypes could be at a competitive disadvantage in MEL and PWS habitat and are being forced into these transition habitats. Future experimental work that tests for competition among morphs will be critical to advancing our understanding the selective mechanisms operating in this system.

**Caveats, considerations, and critiques**

A number of other factors warrant consideration when interpreting my results. First, there is not yet evidence that confirms whether phenotypes are genetically fixed, phenotypically plastic, or a combination of both. Even if coloration is partially plastic, it does not contradict a specialization in habitat use that can precede speciation (Pfennig et al. 2010). Plasticity can promote diversification because developmental pathways and variation in gene expression, which underlie environmentally induced phenotypes, consist of genetic components that can potentially respond to selection (Nijhout 2003, Windig et al. 2004, Pfennig et al. 2010). If coloration is plastic, the presence of both morphs in the same microhabitat and even on the same coral head
indicates that not all individuals respond equally to environmental cues. This variation in
sensitivity and/or response to cues could be based on genetic differences and may create
conditions for disruptive selection. Regardless, in the face of strong divergent selection between
habitats, this phenotypic variation might eventually lead to divergence and the emergence of
reproductively isolated ecotypes (Schluter 2000).

It also is uncertain whether or not the two color morphs will ultimately diverge to the
level of well-defined species. I stress that I am not arguing that hawkfish color morphs are or will
one day become distinct species, as it is impossible to predict future conditions. Instead, I
propose that disruptive selection via crypsis is favoring different morphs in heterogeneous
microhabitats and that this is likely the primary mechanism driving the evolution of color
polymorphism in \textit{P. arcatus}. Although this could certainly be the precursor for an evolutionary
branching point, reproductive isolation between phenotypes is required for evolutionary
divergence to proceed (Dieckmann & Doebeli 1999).

Although this study was restricted to a single island, my observations of this same pattern
of niche divergence throughout the main Hawaiian Islands confirm that these findings are not
unique to the Island of Hawaii. Similar patterns have been observed in multiple locations
throughout the Pacific (DeMartini & Donaldson 1996) and at Christmas Island in the Indian
Ocean (JP Hobbs pers.comm). This indicates relatively consistent and range-wide niche
partitioning between the two sympatric \textit{P. arcatus} color morphs.

CONCLUSION
This study has demonstrated that fine-scale partitioning of microhabitats allows sympatric color
morphs to coexist on the same shallow coral reefs. Niche partitioning is facilitated by
heterogeneity in substrate composition and topographic complexity on small spatial scales.
Differences in habitat use may represent adaptive strategies that allow each morph to increase
fitness in respective microhabitats. I propose that disruptive selection may have arisen because
different color patterns are cryptic in adjacent microhabitats. My results indicate that disruptive
natural selection on color pattern to increase crypsis provides a plausible mechanism for the
origin and maintenance of multiple color phenotypes in \textit{P. arcatus}. If divergence in these
functional traits also affects reproductive isolation, ecological speciation is likely to be promoted.
Further work will be required to determine whether morphs are differentiating further or are being maintained as a stable polymorphism. Therefore, I limit my inference to the suggestion that color morphs are subject to divergent selection on cryptic coloration and likely represent an early stage of evolutionary divergence driven by natural selection. Further, this system shows much promise for learning about the interface of polymorphism maintenance and speciation in coral reef fish.
REFERENCES


Clarke RD, Buskey EJ, Marsden KC (2005) Effects of water motion and prey behavior on
zooplankton capture by two coral reef fishes. Mar Biol 146:1145–1155


driving speciation in *Hypoplectrus* coral reef fishes? Proceedings of the Royal Society B: Biological Sciences 274:1265–1271


### TABLES

**Table 2.1.** Summary of habitat variables averaged over transects categorized into five dominance categories. Numerical dominance determined by relative abundance (RA) > 0.7, which corresponds to the dominant morph having at least twice as many individuals. MEL only (RAMEL=1.0), MEL dom (RAMEL=0.7-0.99), Mixed (both RA < 0.7), PWS dom (RAPWS=0.7-0.99), PWS only (RAPWS=1.0).

<table>
<thead>
<tr>
<th>Variable</th>
<th>MEL only</th>
<th>MEL dom</th>
<th>Mixed</th>
<th>PWS dom</th>
<th>PWS only</th>
</tr>
</thead>
<tbody>
<tr>
<td># Transects</td>
<td>10</td>
<td>13</td>
<td>13</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Depth (m)</td>
<td>2</td>
<td>3.3</td>
<td>5</td>
<td>6</td>
<td>8.7</td>
</tr>
<tr>
<td>Distance to shore (m)</td>
<td>7.1</td>
<td>15.6</td>
<td>19.1</td>
<td>26.3</td>
<td>31.1</td>
</tr>
<tr>
<td>Slope (°)</td>
<td>67</td>
<td>38</td>
<td>36</td>
<td>27</td>
<td>15</td>
</tr>
<tr>
<td>Rock cover (%)</td>
<td>32</td>
<td>39</td>
<td>34</td>
<td>28</td>
<td>13</td>
</tr>
<tr>
<td>Total coral cover (%)</td>
<td>27</td>
<td>37</td>
<td>41</td>
<td>50</td>
<td>55</td>
</tr>
<tr>
<td><em>Poc. meandrina</em> cover (%)</td>
<td>12</td>
<td>16</td>
<td>16</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td><em>Por. lobata</em> cover (%)</td>
<td>15</td>
<td>23</td>
<td>24</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td><em>Por. compressa</em> cover (%)</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>14</td>
</tr>
</tbody>
</table>

**Table 2.2.** Summary of redundancy analysis testing the influence of seven environmental variables on the relative abundance of three *Paracirrhites arcatus* color morphotypes. Results shown are Variables, degrees of freedom (df), Variance (Var.), $F$-ratio ($F$), $P$-value ($P$), Total % variance explained (Tot. var. expl.), Cumulative % variance explained (Cum. Var. expl.) and Loadings (correlations of constrained environmental variables with the two significant RDA axes). Result of overall model was highly significant ($F_{8,55} = 14.4, R^2_{ADJ} = 0.665; P < 0.00001$) based on 100,000 permutations. All variables are continuous except wave action (three-leveled factor: low, moderate, and high).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Df</th>
<th>Var.</th>
<th>$F$</th>
<th>$P$</th>
<th>Tot. var. expl.</th>
<th>Cum. var. expl.</th>
<th>Loadings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope (°)</td>
<td>1</td>
<td>0.082</td>
<td>43.2</td>
<td>&lt;0.0001</td>
<td>28.4</td>
<td>28.4</td>
<td>-0.67</td>
</tr>
<tr>
<td><em>Porites</em> cover (%)</td>
<td>1</td>
<td>0.028</td>
<td>14.8</td>
<td>&lt;0.0001</td>
<td>9.7</td>
<td>38.1</td>
<td>0.75</td>
</tr>
<tr>
<td>Wave action</td>
<td>2</td>
<td>0.023</td>
<td>6.1</td>
<td>&lt;0.0001</td>
<td>8.0</td>
<td>46.1</td>
<td>0.92</td>
</tr>
<tr>
<td><em>Poc. meandrina</em> cover (%)</td>
<td>1</td>
<td>0.023</td>
<td>12.0</td>
<td>&lt;0.0001</td>
<td>7.9</td>
<td>54.0</td>
<td>-0.53</td>
</tr>
<tr>
<td>Distance to shore (m)</td>
<td>1</td>
<td>0.022</td>
<td>11.7</td>
<td>&lt;0.0001</td>
<td>7.7</td>
<td>61.7</td>
<td>0.83</td>
</tr>
<tr>
<td>Depth (m)</td>
<td>1</td>
<td>0.011</td>
<td>5.5</td>
<td>0.007</td>
<td>3.6</td>
<td>65.3</td>
<td>0.67</td>
</tr>
<tr>
<td>Rock cover (%)</td>
<td>1</td>
<td>0.003</td>
<td>1.7</td>
<td>0.163</td>
<td>1.1</td>
<td>66.5</td>
<td>-0.54</td>
</tr>
<tr>
<td>Residuals</td>
<td>55</td>
<td>0.097</td>
<td>33.5</td>
<td>100.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2.3. Redundancy analysis variance partitioning by RDA axes. Results shown are the two significant RDA axes, variance (Var.), $F$-ratio ($F$), $P$-value ($P$), total % variance explained (Tot. var. expl.), cumulative % variance explained (Cum. Var. expl.) and morphotype scores, which are scaled proportional to eigenvalues on each canonical axis.

<table>
<thead>
<tr>
<th>Axes</th>
<th>Variance</th>
<th>$F$</th>
<th>$P$</th>
<th>Tot. var. expl.</th>
<th>Cum. var. expl.</th>
<th>Morphotype Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>RDA1</td>
<td>0.19</td>
<td>107.4</td>
<td>&lt;0.0001</td>
<td>63.2</td>
<td>63.2</td>
<td>-0.99, -0.26, 1.25</td>
</tr>
<tr>
<td>RDA2</td>
<td>0.01</td>
<td>5.5</td>
<td>0.005</td>
<td>3.2</td>
<td>66.5</td>
<td>0.20, -0.29, 0.10</td>
</tr>
<tr>
<td>Residual</td>
<td>0.10</td>
<td></td>
<td></td>
<td></td>
<td>33.5</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 2.4. Niche overlap estimates for nine individual niche axes and composite multidimensional niche. All bolded values are significant ($P < 0.0001$) after Bonferroni adjustment for multiple comparisons. Composite niche overlap values represent the mean ± sd over all nine axes.

<table>
<thead>
<tr>
<th>Niche axis</th>
<th>MEL-PWS</th>
<th>MEL-INT</th>
<th>PWS-INT</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Porites lobata</em> cover (%)</td>
<td>0.54</td>
<td>0.66</td>
<td>0.44</td>
<td>0.55</td>
</tr>
<tr>
<td>Wave action (3 levels: low/mod/high)</td>
<td>0.41</td>
<td>0.81</td>
<td>0.55</td>
<td>0.59</td>
</tr>
<tr>
<td>Total coral cover (%)</td>
<td>0.59</td>
<td>0.75</td>
<td>0.48</td>
<td>0.61</td>
</tr>
<tr>
<td>Slope (°)</td>
<td>0.61</td>
<td>0.70</td>
<td>0.70</td>
<td>0.67</td>
</tr>
<tr>
<td>Rock cover (%)</td>
<td>0.61</td>
<td>0.77</td>
<td>0.70</td>
<td>0.70</td>
</tr>
<tr>
<td><em>Pocillopora meandrina</em> cover (%)</td>
<td>0.75</td>
<td>0.83</td>
<td>0.63</td>
<td>0.74</td>
</tr>
<tr>
<td><em>Porites compressa</em> cover (%)</td>
<td>0.72</td>
<td>0.74</td>
<td>0.76</td>
<td>0.74</td>
</tr>
<tr>
<td>Depth (m)</td>
<td>0.79</td>
<td>0.76</td>
<td>0.83</td>
<td>0.79</td>
</tr>
<tr>
<td>Coral perch/shelter</td>
<td>0.77</td>
<td>0.98</td>
<td>0.76</td>
<td>0.84</td>
</tr>
<tr>
<td>Composite Niche Overlap (mean ± sd)</td>
<td>0.64 ± 0.12</td>
<td>0.78 ± 0.09</td>
<td>0.65 ± 0.13</td>
<td>0.69 ± 0.08</td>
</tr>
<tr>
<td>RDA1 Niche Overlap</td>
<td>0.41</td>
<td>0.71</td>
<td>0.46</td>
<td>0.53</td>
</tr>
</tbody>
</table>
Table 2.5. Estimation of selection indices for the occurrence of *Paracirrhites arcatus* perching on three hard coral species throughout all study sites. Sample counts, used proportion, and available proportion are calculated from all transects where each color morph was recorded. Whereas the mean selectivity index (Manly’s \( w \)) is averaged over \( w \), calculated for each transect where that morph was present (MEL, \( n = 48 \); PWS, \( n = 51 \); INT, \( n = 36 \)). Confidence intervals (95%) represent significance levels adjusted for multiple comparisons (Bonferroni adjusted \( \alpha = 0.016 \)). All negative lower confidence limits were replaced with 0.0.

<table>
<thead>
<tr>
<th>Habitat</th>
<th>Sample count</th>
<th>Used prop.</th>
<th>Available prop.</th>
<th>Manly’s</th>
<th>SE</th>
<th>95% CI</th>
<th>( P )</th>
<th>Manly’s ( \beta )</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Poc. meandrina</em></td>
<td>675</td>
<td>0.92</td>
<td>0.45</td>
<td>2.44</td>
<td>0.20</td>
<td>(1.96, 2.92)</td>
<td>&lt; 0.001</td>
<td>0.90</td>
</tr>
<tr>
<td><em>Por. lobata</em></td>
<td>60</td>
<td>0.08</td>
<td>0.55</td>
<td>0.26</td>
<td>0.06</td>
<td>(0.12, 0.41)</td>
<td>&lt; 0.001</td>
<td>0.10</td>
</tr>
<tr>
<td><em>Por. compressa</em></td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>(0.00, 0.00)</td>
<td>&lt; 0.001</td>
<td>0.00</td>
</tr>
<tr>
<td>Total</td>
<td>735</td>
<td>1.00</td>
<td>1.00</td>
<td>2.70</td>
<td></td>
<td></td>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td>PWS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Poc. meandrina</em></td>
<td>532</td>
<td>0.73</td>
<td>0.26</td>
<td>2.53</td>
<td>0.25</td>
<td>(1.94, 3.11)</td>
<td>&lt; 0.001</td>
<td>0.68</td>
</tr>
<tr>
<td><em>Por. lobata</em></td>
<td>147</td>
<td>0.20</td>
<td>0.65</td>
<td>0.40</td>
<td>0.06</td>
<td>(0.26, 0.54)</td>
<td>&lt; 0.001</td>
<td>0.11</td>
</tr>
<tr>
<td><em>Por. compressa</em></td>
<td>48</td>
<td>0.07</td>
<td>0.09</td>
<td>0.82</td>
<td>0.23</td>
<td>(0.27, 1.36)</td>
<td>0.230</td>
<td>0.22</td>
</tr>
<tr>
<td>Total</td>
<td>727</td>
<td>1.00</td>
<td>1.00</td>
<td>3.74</td>
<td></td>
<td></td>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td>INT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Poc. meandrina</em></td>
<td>363</td>
<td>0.94</td>
<td>0.36</td>
<td>2.48</td>
<td>0.23</td>
<td>(1.94, 3.02)</td>
<td>&lt; 0.001</td>
<td>0.93</td>
</tr>
<tr>
<td><em>Por. lobata</em></td>
<td>23</td>
<td>0.06</td>
<td>0.63</td>
<td>0.18</td>
<td>0.05</td>
<td>(0.05, 0.30)</td>
<td>&lt; 0.001</td>
<td>0.07</td>
</tr>
<tr>
<td><em>Por. compressa</em></td>
<td>0</td>
<td>0.00</td>
<td>0.01</td>
<td>0.00</td>
<td>0.00</td>
<td>(0.00, 0.00)</td>
<td>&lt; 0.001</td>
<td>0.00</td>
</tr>
<tr>
<td>Total</td>
<td>386</td>
<td>1.00</td>
<td>1.00</td>
<td>2.65</td>
<td></td>
<td></td>
<td></td>
<td>1.00</td>
</tr>
</tbody>
</table>
Table 2.6. Comparison of the use of topographic features as microhabitat among three color morphs (n = 754). Habitat categories included structural features with high complexity (boulder fields, ledges, and outcrops) and low complexity (reef flats and rubble patches). Data are shown as counts of individual fish recorded in each habitat type and the proportion of the total for each morph is in parentheses.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Melanistic</th>
<th>Intermediate</th>
<th>Pink-white-stripe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boulder</td>
<td>122 (0.37)</td>
<td>68 (0.46)</td>
<td>70 (0.27)</td>
</tr>
<tr>
<td>Ledge</td>
<td>40 (0.12)</td>
<td>22 (0.15)</td>
<td>19 (0.07)</td>
</tr>
<tr>
<td>Outcrop</td>
<td>139 (0.42)</td>
<td>18 (0.12)</td>
<td>18 (0.07)</td>
</tr>
<tr>
<td>Reef flat</td>
<td>15 (0.04)</td>
<td>28 (0.19)</td>
<td>120 (0.46)</td>
</tr>
<tr>
<td>Rubble</td>
<td>17 (0.05)</td>
<td>12 (0.08)</td>
<td>34 (0.13)</td>
</tr>
<tr>
<td>Total</td>
<td>333</td>
<td>148</td>
<td>261</td>
</tr>
</tbody>
</table>

Table 2.7. Comparison of the vertical positioning of each color morph when perching on topographic features (n = 1016 fish). Positions were categorized as either on top, side, or base of the feature. Data shown are counts with the corresponding proportion of the total observations for each morph in parenthesis.

<table>
<thead>
<tr>
<th>Morph</th>
<th>Position on Feature</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Base</td>
<td>Side</td>
<td>Top</td>
<td>Totals</td>
</tr>
<tr>
<td>Melanistic</td>
<td>103 (0.22)</td>
<td>200 (0.42)</td>
<td>171 (0.36)</td>
<td>474</td>
</tr>
<tr>
<td>Intermediate</td>
<td>78 (0.38)</td>
<td>59 (0.28)</td>
<td>70 (0.34)</td>
<td>207</td>
</tr>
<tr>
<td>Pink-white-stripe</td>
<td>200 (0.60)</td>
<td>82 (0.24)</td>
<td>53 (0.16)</td>
<td>335</td>
</tr>
</tbody>
</table>
Figure 2.1. Color morphs of *Paracirrhites arcatus*. A) Pink-White-Stripe (PWS), B) melanistic (MEL), and C) an intermediate phenotype (INT) with a faint white stripe.
Figure 2.2. Map of study site locations spanning the leeward coast of Hawai’i Island. Inset shows the position of the island within the Main Hawaiian Islands.
Figure 2.3. Correlation of habitat variables and abundances (log transformed) of the two common *Paracirrhites arcatus* color morphs (MEL=black, PWS=red) over all sites. Individual data points were omitted to highlight overall trends represented by loess smoothers (± 95% CI; span=1).
Figure 2.4. Redundancy analysis triplot showing the major influence of six environmental variables on the relative abundance of three *Paracirrhites arcatus* color morphotypes. Transects are plotted onto the two significant axes of the RDA using linear constraint (LC) site scores. Coordinates of sites (weighted and scaled) are expressed in the space of the constrained explanatory variables (i.e., calculated independent of relative abundance). The colored symbols indicate the numerically dominant color morph on that transect (relative abundance > 0.5): red triangles (▲) = PWS dominated, black circles (●) = MEL dominated, open blue squares (☐) = INT dominated. 95% CI ellipses (MEL=black, PWS=red) encompass the transects where each morph was dominant. The distances among ellipse centroids do not approximate Euclidean distance. The projection of each transect approximates its value along each of the variable vectors (grey arrows), the angles of which reflect correlations among constrained variables as well as between constrained variables and the response (Borcard et al. 2011). Continuous variables are slope, distance to shore, depth and percentage cover of *Pocillopora* spp. and *Porites* spp. corals. The three levels of the factor wave action (low, mild, and high surge) are shown underlined and without vectors. The morph scores (represented by photos) are shown scaled to site scores and the distances among them are approximations of their Euclidean distance in the multidimensional space.
Figure 2.5. Scatterplot of morph relative abundance (proportion of PWS to MEL morphs) along the synthetic niche gradient RDA1. The dashed line indicates the point on y-axis where both morphs are equally abundant, above which PWS is dominant and below which MEL is dominant. The blue smoother line signifies a binomial general linear model (± 95% CI) that highlights the strong correlation ($R^2 = 0.81$) between color phenotype and this ecological gradient.

Figure 2.6. Variation partitioning of redundancy analysis illustrated with Venn diagram indicating the proportion of variance in color morph relative abundance explained by three groups of explanatory variables: Reef profile (depth and distance to shore), Coral community (% cover of three dominant corals), and Topography (wave action and slope).
Figure 2.7. Niche overlap estimates of three Paracirrhites arcatus color morphs represented by kernel density plots of (A) RDA1 as a synthetic multidimensional niche gradient, and (B) six individual niche axes. Smoothing done with Gaussian kernels. (MEL = black; PWS = red; INT = blue dashed line).
Figure 2.8. Coral microhabitat use and selectivity. A) Stacked barplot illustrating proportional usage of coral hosts as a perch/shelter. Data shown are proportion of each coral used by the three color morphs. B) Estimation of microhabitat selectivity of three hard coral species used as a perch/shelter by three *P. arcatus* color morphs. Manly’s selectivity index ($w_i$) represents the mean $w_i$ calculated for each transect where that morph was present (MEL: n=48; PWS: n=51; INT: n=36). Error bars indicate the 95% confidence intervals, which have been adjusted for multiple comparisons. The dashed line ($w_i = 1$) indicates neutral selectivity, $w_i > 1$ indicates positive selection (preference) and $w_i < 1$ indicates negative selection (avoidance). * indicates that $w_i$ values were significantly different from random ($P < 0.001$) after Bonferroni corrections for multiple comparisons. Each morph showed similar preference for, and avoidance of *P. lobata*, however, they differed in their selectivity of *P. compressa*. 
Figure 2.9. Vertical position of microhabitat (3 levels) for 927 fish. Chi-square contingency test ($\chi^2=125.77$, df=4, $P < 0.0001$). MEL more frequently shelter on topographic structures (boulders, outcrops, walls, ledges) relative to PWS, which is more likely to shelter at the base of structures.
Figure 2.10. Representative examples of contrasting microhabitats associated with the two common color morphs of *Paracirrhites arcatus*. A-C) Habitats where pink-white-stripe (PWS) morphs are dominant are in deeper sub-surge zones characterized by gradual slopes, and higher coral cover, particularly of *Porites* spp. corals (i.e., *Por. lobata* and *Por. compressa*). D-F) Habitats associated with melanistic morphs are in shallow steep surge zones dominated the branching coral *Pocillopora meandrina* and algae-covered basaltic lava rock. All photographs were taken with ambient light, no color filters, and with the intention to represent natural color and light intensities in these habitats.
Figure 2.11. Representative examples of *P. arcatus* color morphs appearing cryptic in respective microhabitats. A) Pink-white-stripe (PWS) morph perching on *Poc. meandrina* in *Porites*-dominated sub-surge zone at 6m and B) 10m depth. C) PWS morph perching on *Porites* spp. at 8m. D-F) Melanistic morphs (MEL) perching on *Poc. meandrina* in *Pocillopora*-dominated surge zone. D) MEL showing good color matching to algae covered basaltic lava rock on at depth of 2m. E) MEL perching on the underside of *Poc. meandrina* colony at 2m. All photographs were taken with ambient light, no color filter and with the intention to represent natural color and light intensities in these habitats.
CHAPTER III
ASSORTATIVE PAIRING BEHAVIOR AS A MECHANISM FOR PREMATING ISOLATION AMONG ARCEYE HAWKFISH COLOR MORPHS
INTRODUCTION
Speciation with gene flow continues to be one of the most controversial concepts in evolutionary biology. Fifty years ago, Maynard Smith (1966) proposed that sympatric speciation is most plausible when a single ecologically important trait is linked directly to mate choice. In some cases, divergence of these so-called magic traits (Gavrilets 2004) can alone trigger the evolution of reproductive isolation without geographic isolation. There is a small, but growing list of studies of adaptive traits linked with assortative mating (i.e., when individuals with similar phenotypes mate more frequently than expected under a random mating pattern) including body size (Nagel & Schluter 1998, McPeek & Wellborn 1998, Jones et al. 2003, Rolán-Alvarez 2007), color (Summers et al. 1999, Fordyce et al. 2002, Reynolds & Fitzpatrick 2007, Puebla et al. 2007, Jiggins 2008, Maan & Cummings 2008), body shape (Langerhans et al. 2007, Hosoi & Hori 2008), foraging performance (Benkman 2003, Snowberg & Bolnick 2008, Snowberg & Benkman 2009), beak morphology in birds (Podos 2001, Badyaev et al. 2008), echolocation in bats (Kingston & Rossiter 2004), and electrolocation in electric fish (Feulner et al. 2008). Of the 18 putative cases of magic traits involving mate choice identified by Servedio et al. (2011), color pattern is the most common, plausibly because it is relevant to both niche divergence and communication (Gavrilets 2004). Several recent studies have shown links between mate choice and mimetic color pattern in Heliconious butterflies (Jiggins 2008), Caribbean Hamlets (Puebla et al. 2007) and poison-dart frogs (Summers et al. 1999, Reynolds & Fitzpatrick 2007, Maan & Cummings 2008).

Coloration in coral reef fishes is well-known for its exceptional diversity, yet our understanding of the evolutionary significance of color and color pattern remains unclear (McMillan et al. 1999, Bernardi et al. 2002, Barreto & McCartney 2007, Lin et al. 2009, DiBattista et al. 2012). Color is likely to be a significant evolutionary trait, as it is often the only diagnostic trait differentiating closely related species in several speciose families (Lieske & Myers 1999, Allen et al. 2005). The role of color pattern in species recognition is well-established (McMillan et al. 1999), but experimentally tested in only three genera of marine fishes including Chaetodon butterflyfishes (McMillan et al. 1999), Hypoplectrus hamlets (Fischer 1980, Domeier 1994, Puebla et al. 2007) and Plectrochromis dottybacks (Munday et al. 2003, Messmer et al. 2005, Cortesi et al. 2015).
Here I investigate the role of color in species recognition using an ecologically relevant polymorphism in the arceye hawkfish (*Paracirrhites arcatus*; family Cirrhitidae), a polychromatic species distributed throughout the Indo-Pacific. *P. arcatus* exhibits two sympatric color morphs: a pink-white-striped (PWS) morph that is pink to reddish brown with a bright white-stripe running along the flank, and a melanistic (MEL) morph that is more uniformly olive to dark-brown and lacks a stripe. A third, intermediate morph (INT) also is known and it is reddish-olive with a white stripe (DeMartini & Donaldson 1996). Color is not sexually dimorphic, and with exception of color differences, morphs appear to be meristically identical (Randall 1963).

Most hawkfish, including *P. arcatus*, are thought to be protogynous hermaphrodites based on mating systems and histological structure of gonads (Donaldson 1987, 1990, Sadovy & Donaldson 1995, Sadovy & Liu 2008). *P. arcatus* are sexually dimorphic, characterized by larger males, however sizes ranges overlap (Donaldson 1990). The mating system of *P. arcatus* is classified as harem polygyny with a single dominant male maintaining a territory including one or more females (Donaldson 1990). Females live and on coral in the male's territory, usually separate from other females, but will on occasion inhabit the same coral as another female. Males move between female occupied corals in their territories and likely monopolize mating opportunities (Donaldson 1990). Courtship is paired, occurs on female corals around dusk, and is relatively simple: males approach females on her coral, the pair align in parallel, rest, and eventually ascend ~1 m into the water column where they release pelagic eggs and sperm (Donaldson 1990, Tanaka 1991).

Mating opportunities are likely restricted to individuals within a few meters. *P. arcatus* is relatively sessile, and typically maintains a small home range of a few square meters around a central home coral (DeMartini 1996). In addition, spawning occurs within a male’s territory, which both males and females aggressively defend (Donaldson 1990, DeMartini 1996). Hence, the combination of high site fidelity and harem spawning likely restricts mating opportunities to individuals within a few meters.

Patterns of ecological divergence among color morphs in the Hawaiian archipelago suggest color is under disruptive selection, as color morphs are partitioned into contrasting microhabitats where each phenotype appears to be better camouflaged (Chapter II). Despite microspatial habitat segregation, color morphs still overlap spatially, and therefore have
opportunity for intermorph mating. If color is also used as a species recognition signal, this could lead to reproductive isolation capable of initiating genetic divergence between color morphs. In this study, I investigated whether color influences the likelihood of forming an association with the other sex. I combine laboratory measures of female preference with field-based observations of pairing to assess whether coloration is used as a species recognition signal and hence a basis for assortative mating.

METHODS
Overview
My main objective was to assess if *P. arcatus* exhibit a mate preference among color morphs, using both field observations and captive preference experiments. The first captive laboratory experiment consisted of a traditional dichotomous no-contact mate preference test where females observed two males behind transparent barriers that prevented physical contact and female behavior and association times were recorded. The second experiment evaluated the consistency of preference of a group of females across replicate trials. The third experiment substituted live males with model replicas to control for male behavior and other traits that may influence the female and isolated color as the only variable in choice tests.

As in many fishes, observing mating in hawkfish is a relatively rare event and therefore difficult to quantify directly. Mating in *P. arcatus* has been rarely observed in nature, most likely due to observer interference (Donaldson 1990, Sadovy & Donaldson 1995), and is difficult to induce under experimental conditions as they do not readily mate in captivity (however, see Tanaka (1991) for a notable exception). Many studies have relied on using female association time as a measure of female preference in two-stimulus choice tests (Kodric-Brown 1985, Ryan & Wagner 1987, Milinski & Bakker 1990, Basolo 1990, Shackleton et al. 2005, Rutstein et al. 2007), and a few have shown that female preference is a reliable predictor of reproduction (Bischoff et al. 1985, Walling et al. 2010). To remove effects of male-male competition, males are typically prevented from interacting with one another via a barrier. *P. arcatus* males are haremic and aggressively defend territories and pilot studies that allowed contact among males usually resulted in violent competition that made female behavior difficult to document. I was unsuccessful at designing an experimental tank big enough to allow two males to establish territories. I therefore adopted an experimental setup using a transparent divider that restricted
contact (Fig. 3.1), but did not prevent males and females from initiating courtship. Pilot studies revealed that initiation of courtship does occur across the divider, provided a coral is present on either side. Hermatypic corals, particularly of the genus *Pocillopora* are the courtship sites in *P. arcatus*. Given that courtship is dependent on the presence of a host coral, I incorporated coral replicas into the design (Fig. 3.1).

**Field surveys of intermorph pairing frequency**

My field observations use pair formation as a measure of assortative mating. To assess the frequency of intermorph pairing associations in the field, I conducted fish surveys at nine sites along the leeward side of the Island of Hawai‘i in 2010 (for detailed description of methods see Whitney et al. in prep). On each transect (n = 64), a pair of divers counted all hawkfish and recorded the phenotype of each fish. When two individuals were found inhabiting the same *Pocillopora* coral head, I recorded the composition of the pair and measured the body size (total body length; TL) of each fish using a handheld ruler. The smaller individual of the pair was assumed to be female and the larger individual assumed to be male based on findings of Donaldson (1990) that males were always the largest individual within a group. I recorded 182 occurrences of more than one individual *P. arcatus* in the same *Pocillopora* coral head, including 170 pairs, and 12 groups of 3-4 individuals. For simplicity, I limited my analysis to pairs, and pairs in which both individuals were equal in size (n=8) were excluded, because I could not infer gender (total = 162 pairs). The eight equal-sized pairs that were excluded included seven pairs of like color and one pair of mixed morphs.

To determine if females and males showed a preference for associating/pairing with like color morphs, I first compared counts of each pair type in a chi-square test of independence. Then to account for differences in the availability of each color morph, I calculated pair preference for different color morphs on each transect where pairs were recorded and both color morphs were present (n=36 transects). I used Manly’s (1973) preference index to assess if pairing preference is dependent on morph frequencies. Although Manly’s model has been used primarily for analyzing diet and habitat choice, it can be appropriately applied to mating behavior (Bots et al. 2015). Manly’s standardized preference index (Manly $\beta$) is calculated according to equation (9) in Manly (1973) as:
\[ \beta = \frac{(e_1/A_1)}{(e_1/A_1) + (e_2/A_2)}, \]

where \( e_1 \) and \( e_2 \) are the numbers of each morph pair and \( A_1 \) and \( A_2 \) are the total numbers of each morph available on each transect (like males were given subscript 1). Therefore, Manly \( \beta \) represents the strength of preference for like males and can range between 0.0 and 1.0, with 0.5 indicating no preference. Manly’s selectivity indices and significance was calculated using the *AdeHabitatHS* package in R (Calenge 2006). Significance of preference was determined using \( X^2 \) contingency tests comparing observed indices (\( \beta \)) with expected (0.5). When Manly \( \beta \) was significantly greater than 0.5, this was interpreted as evidence of preference for like males, whereas a Manly \( \beta \) less than 0.5 indicated avoidance. To allow comparisons of preference measured in the field and the laboratory, I also calculated Manly’s \( \beta \) for preference trials, where \( e_1 \) and \( e_2 \) were the number of minutes spent associating with each male morph, and \( A_1 \) and \( A_2 \) were equal relative proportions (0.5), which cancel out because of equal availability.

**Experiment 1: Female preference among two males**

Animals for laboratory experiments were captured using hand and screen nets from shallow coral reefs at three sites along the leeward coast of Hawai‘i Island (Honokohau Bay, Keanapukalua and Kapa’a) and a single site on Oahu Island (Kahe Point) and transported to the Hawai‘i Institute of Marine Biology (HIMB). Fish were housed separately and maintained at 25-31°C in flow through systems with continuously filtered and aerated 20gal (76 l) glass aquaria under a natural light cycle (12hr on/off) using 100W full-spectrum trichromatic fluorescent bulbs (Corallife, Franklin, Wisconsin). Fish were acclimated for at least one week prior to experiments and fed daily a mixed diet of krill, mysids, brine shrimp, and silversides. All fish were isolated from physical or visual contact with other fish for at least one week before the experiment.

I could not confirm the sex of individuals before trials, as there are no obvious diagnostic external differences among sexes. *P. arcatus* are likely protogynous hermaphrodites, and dissections of adults collected previously in Hawai‘i have revealed that most individuals with mature testes are > 9.0 cm (all measurements are total length TL) and all gravid females (n=14) ranged from 7.0 – 8.9 cm. Therefore, to increase the probability of using females in preference trials, I used only individuals < 8.5 cm as subjects in the preference trials. Following trials, presumed female fish were sexed using gross morphology of dissected gonads, and only those
individuals identified as female were included in analyses. Female subjects were experimentally naïve, sexually mature (>6 cm) fish ranging from 6 to 8.5 cm. All behavioral trials were conducted between 10:00 and 20:00 hours during 25 July to 30 September 2015.

Behavioral trials were conducted in a dichotomous choice setup where stimuli males of both color morphs (MEL and PWS) are placed behind transparent Plexiglas in the end compartments of an experimental aquarium (Fig. 3.1). The 120 x 30 x 48cm (length, width and height) glass aquarium was divided into three compartments including a large (76cm) middle section for the focal female, flanked by two identical smaller end regions (22cm) where males were confined behind transparent UV-transmittant acrylic Plexiglas (Arkema G-UVT Plexiglas), with 0.63cm holes drilled roughly every 5 cm to allow water and any chemical signals to flow through the dividers. Pilot studies revealed that initiation of courtship is dependent on the presence of a host coral. Thus, I incorporated two identical polyurethane models of their preferred coral *Pocillopora meandrina* (22 x 20 x 9m; Ocean Aquaria, Mandeville, Louisiana) into the design. Coral models were cut in half (perpendicular to the base) and siliconed to either side of the Plexiglas dividers, thereby allowing the female to interact with a male on the same coral head (as is required to initiate courtship), while still restricting contact (Fig. 3.1). To minimize reflections of fish, the interior bottom of the tank was lined with a 6.35mm thick white PVC floor and the exterior walls were covered with white non-glare vinyl sheeting, which also eliminated possible interference by external stimuli. In an effort to match a natural light regime, the tank was illuminated using four flood lamps (with 22cm reflectors) mounted obliquely 30cm above and to the outside of the tank: two lamps were fitted with 75-watt full spectrum bulbs (BR30 Agro light, Philips Lighting, San Marcos, Texas), and two were fitted with 26-Watt UVB emitting compact fluorescent bulbs (ExoTerra Repti-Glo10, Rolf C. Hagen Corp, Mansfield, Massachusetts).

The experimental focal female was placed in the middle compartment which was delineated into three observational zones: two 14cm association zones near each end that encompass the female’s half of the coral model, and a 48cm neutral zone in the center. Pairs of MEL and PWS males were matched for standard length and body mass, placed on opposite ends of the preference tank, and allowed to acclimate one hour before the introduction of the female. This acclimation period would allow males to acclimate and perch atop the coral models where they are visible to the focal female. Trials would not be initiated until both males were perched
and visible to the female. Each pair of males was tested with five to 10 females. To account for any positional biases, male presentation (left versus right side) was alternated every five trials.

At the beginning of a trial, a female was placed in the middle of the center compartment and her behavior and association times with each male recorded for 60 min. To exclude observer interference, behavioral trials were recorded using a wirelessly controlled high-definition digital video camera (GoPro Hero3, GoPro, Inc., San Mateo, California) mounted above the tank, and saved for subsequent analysis. Using Fiji (Schindelin et al. 2012), video from each trial was converted into a 8-bit gray scale JPEG image sequence at a rate of 1 frame per second and scale was calibrated to dimensions of the trial arena. The female’s position in the preference tank was tracked manually every 5 sec over the 60 min trial (n=721 points per trial) using the MTrackJ plugin (Meijering et al. 2012) for ImageJ v1.50a (Rasband 2012, Schindelin et al. 2012). From my pilot study, a duration of 60 min was chosen for the test trial because it allowed sufficient time for females to interact with both males.

I measured female association time with males as follows. A focal female associated with a male when she was occupying the coral model or positioned within the association zone (<14 cm from divider). Association preference was measured as the difference in association time between stimuli males of the subject’s own color morph minus that of the alternate morph and was evaluated with a paired t-test. Variation in the degree of preference between the two female morphs was tested with a one-way ANOVA. The difference in association time between like and unlike males was the dependent variable and the female’s morph was the fixed effect. The difference between association times had homogenous variance among female morphs (Bartlett’s test $K^2 = 0.095, P = 0.76$), but failed tests of normality (Shapiro-Wilk test $W = 0.90, P < 0.001$). Therefore, nonparametric statistical tests are also reported (i.e., Wilcoxon rank sum tests and Kruskal-Wallis tests). Alternatively, I also used the preference index (Manly’s $\beta$: proportion of association time with like male) as the dependent variable, which was arcsine transformed to better adhere to assumptions of normality. To determine if there was a relationship between female size and strength of preference, I used an ANOVA to compare preference indices among two female size classes (<8 cm and >8 cm), which had similar sample sizes ($n=25$ and $n=19$, respectively). In addition, I tested for differences in the outcomes of trials, which were nominally classified as the subject females either choosing MEL males, PWS males or no choice. Choice was defined as the subject spending $\geq 60\%$ of the trial time associating with one stimulus male.
Trials with no difference in association time between males were considered no choice. A Pearson’s chi-square test of independence was used to determine whether females spent more time with males of like color than expected by random chance. All statistical analyses were performed with the statistical package R (R Core Development Team).

**Experiment 2: Consistency of female preference**

To determine whether individuals show consistent behavior in repeated trials, I retested a group of 16 females (n=8 of each color morph) with a second replicate trial. Each female was tested with the same pair of males, switched to opposite sides with respect to her first trial, using the same experimental procedure as described above. I assessed female consistency using three procedures. First, the proportion of association time spent with like males (preference index) was compared among paired replicates for each female using a paired *t*-test. Second, I tested for consistency in trial outcomes by comparing proportions of trials (choosing like versus unlike males) across successive trials using a Cochran-Mantel-Haenszel chi-square test for repeated tests of independence (McDonald 2014). Third, I calculated a coefficient of variation (CV = sd/mean) for the proportion of association time spent with like stimuli males (arcsine transformed) for each female and used a linear regression to test for a relationship between CV and preference strength as in Cummings and Mollaghan (2006).

**Experiment 3: Preference trials using model replica males**

I could not control for variability in behavior among subject males. To determine if male behavior influenced female choice I conducted an additional series of trials using model replicas of males (n=14 females, 11 PWS and 3 MEL). Uneven sample sizes were due to time constraints and practical challenges in collection and housing the live fish. This technique avoids any confounding effects resulting from differences in behavioral or chemical cues from live stimulus males and provides a more restrictive trial that isolates male color as the only variable upon which choice could be based. Two replica male fish casts (Fig. 3.2) were made from the same mold of a frozen adult male *P. arcatus* (11cm TL). Identical castings were made using EpoxAcast 690 resin set in a mold of Dragon Skin 10 rubber (Smooth-On, Inc., Macungie Pennsylvania). Replica pectoral fins were cut from clear plastic sheet and epoxied onto the cast along with a 10-gauge wire inserted into the belly of the cast to be used for handling. Eyes were
constructed using 6mm glass fish eyes (Van Dyke’s Taxidermy, Woonsocket, South Dakota) and the ocular orbits and surrounding detail was sculpted using Aves Apoxie Sculpt (Aves Studio, Hudson, Wisconsin). Each fish cast was then painted to match reference photos of morph coloration patterns using an airbrush (Paasche Airbrush, Chicago, Illinois) with Life Tone Airbrush paints (McKenzie Taxidermy, Granite Quarry, North Carolina) and then sealed with a final thin layer of translucent aquarium-safe epoxy (Smooth-On). The replicas appeared to match the color pattern of real males reasonably well and provided a faithful representation of color and hue, but do not account for differences in UV reflectance/absorbance. Before trials, replica males were placed in natural looking perching positions on top of coral replicas in preference tanks (described above). Subject females were presented with two replica stimuli males and association time was measured over a 60min trial as in experiment 1.

RESULTS

Field surveys of intermorph pairing frequency

Results from field pairs indicated females have a highly significant preference for individuals of like color (Pearson’s $x^2 = 73.61$, df = 1, $P < 0.00001$; Table 3.1). Together, MEL and PWS females paired with like males in 90 of 100 pairs and only 10 pairs were mixed. This preference for pairing with like males was also significant for each female color morph when tested separately (Table 3.1). The majority of the PWS-MEL mixed pairs (6 of 10) were observed at a single site (Wawahiwaa Point), which had the highest abundance over all sites (more than 600 individuals) and the highest spatial overlap between MEL and PWS morphs (Chapter II). Of all mixed pairs that included an INT morph, the majority (27 of 31) were paired with MEL individuals. When MEL paired with other morphs they were usually (65%) the smaller of the two individuals (23 of 37). Results were the same when testing for male preference, larger individuals paired with smaller individuals more frequently than alternate morphs, and this preference was significant for all three male color morphs (MEL: $x^2 = 53.82$, $P < 0.0001$; PWS: $x^2 = 58.36$, $P < 0.0001$; INT: $x^2 = 26.03$, $P < 0.0001$). In summary, $P. arcatus$ showed a strong preference for pairing with like individuals, consistent among genders and color morphs.

Even when accounting for differences in availability, females had a strong preference for males of like color (Manly’s $\beta = 0.75$), which was greater than expected by chance ($H_0: \beta = 0.5$, $P < 0.001$). Both female types exhibited strong preference for males of their own color morph.
Female preference for pairing with like males was independent of the frequency of like males (linear regression, $F_{1,32} = 1.47, R^2 = 0.067, P = 0.49$). Therefore, females preferred to pair with like colored males disproportionately greater than their availability and avoided pairing with males of alternate morphs even when abundant. The strength of preference did not differ among female color morphs ($t$-test, $t = 0.44, df = 28, P = 0.66$; Wilcoxon $W = 175, P = 0.53$). Field-based Manly’s $\beta$ were slightly higher ($\beta = 0.75$) when compared to preference trials ($\beta = 0.64$), however these differences were not significant ($t = -0.706, df = 68, P = 0.48$; Wilcoxon $W = 610, P = 0.0712$). Therefore, both field pairing data and preference tests provided complimentary evidence that females preferred to associate with males of their own color morph.

**Experiment 1: Female preference among two males**

Although 52 females participated in experiment 1, I was only able to evaluate 44 female trials as I excluded six trials in which the female did not approach either male and two trials in which one of the two stimuli males left it’s perch and was no longer visible to the subject female. Overall, females spent 91.9% of time in experiments on coral models in association zones, and only 8.1% in neutral zones. The response of females to live stimuli males was high, as 81.8% of trials (36 of 44) resulted in a detectable preference (i.e., ≥ 60% of time spent associating with one stimulus male). In most trials, the females moved back-and-forth between the two males, but spent more time in one male’s association zone. Comparing just the 36 trials that resulted in a choice, the preference of females was found to be dependent on male color (Pearson’s $\chi^2 = 7.37, df = 1, P = 0.0073$; Table 3.2). Overall, females showed a preference for like males in 26 of 36 trials (72.2%), more than twice as often as alternative males (Fig. 3.4). The results hold when each female is tested separately (Table 3.2).

Over all 44 trials, female *P. arcatus* spent approximately twice as much time on average associating with males of their own color morph than the alternative males (means ± s.e.: 35.5 ± 3.33min *versus* 18.7 ± 2.95min; paired $t$-test: $t = 2.76, df = 43, P = 0.004$; Wilcoxon rank sum test $W = 719.5, P = 0.004$). Results hold when each morph is analyzed separately (Table 3.3). MEL females spent twice as much time associating with like males (35.2 ± 3.96min *versus* 17.9 ± 3.47min; paired $t$-test: $t = 1.99, df = 21, P = 0.0297$); and PWS females spent twice as much time associating with like males (35.9 ± 3.53min *versus* 19.6 ± 3.37min; paired $t$-test: $t = 1.87, df
I did not detect any statistical difference in the strength of preference among female color morphs ($t$-test, $t = -0.822, \text{df} = 42, P = 0.935$; Wilcoxon test $W = 238.5, P = 0.944$), and therefore both females appear to have equally strong preferences for like males.

There was no significant tendency for females to prefer the fish placed on a particular side of the tank (unpaired $t$-test, $t = 1.28, \text{df} = 85, P = 0.204$). Also, the strength of preference for like males (difference in association time between like and unlike males) was not significantly affected by female collection site ($t$-test, $t = 0.59, \text{df} = 41, P = 0.5585$; Wilcoxon $= 265.5, P = 0.589$). There was, however, a significant difference in preference strength among female size classes (ANOVA, $F_{1,42} = 7.505, P = 0.009$; Kruskal-Wallis = 8.454, $P = 0.015$). Smaller females (<8cm, n=25) had a significantly stronger preference for like males (difference = +30.32min; Manly’s $\beta = 0.75$) when compared to larger females (>8cm, n=19), which showed no preference for male color (difference = -1.0min; Manly’s $\beta = 0.49$). The effect of size did not vary significantly among female morphs, based on the lack of significant interaction between size class and female morph ($F_{1,42} = 0.866, P = 0.36$).

**Experiment 2: Consistency of female preference**

I evaluated the consistency of female preference by comparing preference behavior across successive repeat trials. There was no significant difference in preference strength among replicate trials (paired $t$-test, $t = -0.123, \text{df} = 16, P = 0.90$), which indicates preference behavior of the group was consistent and there was no detectable bias related to location in the experimental tank. This result was consistent when female morphs were tested separately (MEL: $t = -1.055, P = 0.326$; PWS: $t = 1.593, P = 0.150$). I also compared trial outcomes (like, unlike or no choice) between replicate trials in a 2x3 test of independence and found the same result. Odds ratios did not differ among replicates (Cochran-Mantel-Haenszel $\chi^2_{\text{MH}} = 0.665, P = 0.4148$) indicating that the group-level preference for like males did not vary significantly among replicate trials. There was, however, variation in consistency among females. The relationship between association time for like males among replicate trials was not significant (linear regression: $R^2 = 0.22, Y = 0.465X + 16.69; F_{1,14} = 3.955, P = 0.067$), indicating that preference changed among replicates for some females. In general, females showing the greatest strength of preference (>60% time with like males in first trial) also showed the greatest consistency in their behavioral preference (mean CV = 0.39), whereas females showing weaker preference (<60%...
preference with like male) showed greater variation (mean CV = 0.75). The relationship between consistency and strength of female preference for like males, however, was not significant (linear regression: $R^2 = 0.15$, $Y = -1.22X + 1.36$, $F_{1,15} = 2.78$, $P = 0.116$).

**Experiment 3: Preference trials using model replica males**

Results from experiments using replica stimuli males were similar to those using live males. On average, female *P. arcatus* spent roughly twice as much time associating with replica males matching their own color morph (37.6 ± 6.58min) than the dissimilar color morph (19.3 ± 6.36min). This difference, however, was not statistically significant (paired $t$-test: $t = 1.43$, $df = 13$, $P = 0.089$). The lack of significance is likely attributable to small sample sizes ($n = 14$ trials). Nevertheless, when comparing the relative preference for like colored stimuli males (i.e., the association time of like males relative to total association time of both stimuli males), there was no significant difference in preference among trials using dummy males compared to trials using live males (ANOVA, $F_{1,58} = 0.073$, $P = 0.789$; Kruskal-Wallis = 0.455, $P = 0.50$; Fig. 3.6), and no interaction among female color and trial type ($P = 0.57$). These results are consistent with results from the live male presentation experiments, and that observed female associations were not due to male behavior. Moreover, because similar results were observed using both live and model stimuli, this also confirms that preference was based upon color pattern and not some other, unmeasured variable.

**DISCUSSION**

This chapter presents empirical evidence for assortative pairing among color morphs of the hawkfish *Paracirrhites arcatus*. I used a combination of field surveys and experimental preference trials to test for the presence of behavioral mechanisms promoting reproductive isolation among color morphs. Results indicate a strong association bias for females to pair with males of like color morph. These findings were corroborated by field surveys. Altogether, results from field surveys and trials using both live and replica males indicated that individuals with similar phenotypes pair with one another more frequently than would be expected under random pairing pattern (assortative pairing).
I provide evidence of a direct link between assortative mating and ecologically important coloration within a single species. Results from Chapter II indicate that color may be an ecologically adaptive trait and is likely under divergent selection in *P. arcatus*. Color morphs occupy distinct microhabitats, to which phenotypes appear to be adaptive via increased crypsis. Melanistic color morphs occur primarily on steep and exposed microhabitats located in *Pocillopora*-dominated surge zones, whereas pink-white-stripe morphs occur mostly in relatively flatter and less exposed microhabitats located in *Porites*-dominated sub-surge zones and reef slopes. Thus, color morphs are experiencing microspatial habitat isolation that is most likely a byproduct of disruptive natural selection favoring alternative color patterns in respective microhabitats. This study shows that color pattern also contributes to assortative mating in *P. arcatus*. Therefore, color pattern is both under disruptive selection and generates sexual isolation via assortative mating.

Establishing that mate preference is based on an ecologically important trait has important consequences for reproductive isolation. Although evidence of assortative mating from pairing experiments alone is not sufficient to show that there is reproductive isolation, I argue that the low frequency of intermorph pairing on the reef indicates reduced intermorph mating rates, because it reflects the combined influence of behavioral and habitat preferences. The relatively sedentary behavior of *P. arcatus* likely restricts mating opportunities to individuals within a few meters. Spawning occurs within a male’s territory, which both males and females aggressively defend (Donaldson 1990, DeMartini 1996). The combination of small home ranges and territories, and harem mating creates conditions that decrease the probability of mating with individuals in different microhabitats. Therefore, habitat isolation among color morphs may first limit opportunities for intermorph mating, which is reinforced by behavioral preference for pairing with like individuals. Thus, the relatively low proportion of pairing between phenotypic extremes (< 10%) observed in zones of overlap provides strong evidence for reproductive isolation among sympatric color morphs, as like color morphs are nearly ten times more likely to pair with their own color morphs than the opposite morph.

In theory, assortative mating can initiate speciation even when geographic barriers are absent and gene flow is high (Dieckmann & Doebeli 1999). Speciation with gene flow is facilitated when traits subject to disruptive selection also contribute to non-random mating (Maynard-Smith 1966). In some cases, divergence of these so-called magic traits (Gavrilets
is sufficient to induce reproductive isolation. When mate choice is based on an ecologically important trait, speciation is more likely because divergence in mating behavior can occur as a byproduct of ecological divergence (Nosil 2012). Color has been identified as a potential magic trait for speciation with gene flow because it is relevant to both niche divergence and communication (Gavrilets 2004, Servedio et al. 2011). This has been documented, however, in a limited number of cases. Recent studies have shown mimetic color pattern is both under disruptive selection and generates sexual isolation via assortative mating in recently derived sister species of Heliconious butterflies (Jiggins 2008), Caribbean hamlets (Puebla et al. 2007), and Poison-dart frogs (Summers et al. 1999, Reynolds & Fitzpatrick 2007, Maan & Cummings 2008). This study provides evidence linking mate choice to an ecologically important color polymorphism within a single species. These results suggest that color evolution could be driving incipient speciation in P. arcatus in the Hawaiian archipelago.

To my knowledge, there are only two other studies that have experimentally evaluated the role of color pattern in mate recognition in coral reef fish. In Hypoplectrus hamlets there is strong assortative mating among 12 Caribbean morpho-species based on mimetic color pattern (Fischer 1980, Domeier 1994, Puebla et al. 2007). Among three sister species of butterflyfishes (genus Chaetodon), McMillan et al. (1999) found that one species (C. multicinctus) showed preference for pairing with their own species, but color pattern was not linked to pairing behavior in the other two sister species. Additional genetic studies have provided evidence of reproductive isolation among color morphs that indicates assortative mating. For example, color morphs of the spinytail damsel (Acanthochromis polyacanthus) on the Great Barrier Reef display genetic evidence of assortative mating in contact zones, and also forage in different habitats where they match the color patterns of other planktivorous fishes in mixed foraging flocks (Planes & Doherty 1997). This study makes a valuable contribution toward the goal of elucidating the role of color pattern in mate recognition in coral reef fish by providing experimental evidence confirming a direct link between mate choice and an ecologically important color polymorphism within a single species.
REFERENCES


TABLES

Table 3.1. Composition of 162 pairs observed during field surveys. The color morph of the male that each female was paired with was classified as melanistic (MEL), pink-white-striped (PWS) or intermediate (INT) color morphs.

<table>
<thead>
<tr>
<th>Females</th>
<th>No. Pairs with Males</th>
<th>Pearson’s Chi-Square</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MEL</td>
<td>PWS</td>
</tr>
<tr>
<td>Melanistic (MEL)</td>
<td>48</td>
<td>7</td>
</tr>
<tr>
<td>Pink-white-stripe (PWS)</td>
<td>3</td>
<td>42</td>
</tr>
<tr>
<td>Intermediate (INT)</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
<td>50</td>
</tr>
</tbody>
</table>

Table 3.2. Outcomes of 44 trials with naïve females (no replicates) indicating female preference for associating with males of two color morphs.

<table>
<thead>
<tr>
<th>Females</th>
<th>Trial Outcome (Preference)</th>
<th>Pearson’s Chi-Square</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Like</td>
<td>Unlike</td>
</tr>
<tr>
<td>Melanistic</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Pink-white-stripe</td>
<td>14</td>
<td>4</td>
</tr>
<tr>
<td>All females</td>
<td>26</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 3.3. Difference in association times of females with like versus unlike males in dichotomous no-contact trials for all females and broken down by female color morph. All tests are one-tailed (i.e., time associating with like male > unlike male).

<table>
<thead>
<tr>
<th>Females</th>
<th>Mean Difference (min)</th>
<th>Paired $t$-test</th>
<th>Wilcoxon signed-rank test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$t$</td>
<td>df</td>
</tr>
<tr>
<td>All females</td>
<td>+16.80</td>
<td>2.76</td>
<td>43</td>
</tr>
<tr>
<td>Melanistic</td>
<td>+17.30</td>
<td>1.99</td>
<td>21</td>
</tr>
<tr>
<td>Pink-white-striped</td>
<td>+16.29</td>
<td>1.87</td>
<td>21</td>
</tr>
</tbody>
</table>
FIGURES

Figure 3.1. Diagram of experimental tank used for female preference trials. Males (MEL or PWS) were placed behind transparent plexiglas in the end zones (22cm) of an experimental aquarium (120 x 30 x 48cm; length, width and height). Focal females were placed in the center compartment (76cm) with three zones: 14cm association zones near each end of the observational zone that encompass identical coral models and a 48cm neutral zone in the center.

Figure 3.2. Replica fish casts of male Paracirrhites arcatus color morphs. A) Melanistic color morph. B) pink-white-stripe morph. Identical castings were made of epoxy resin from a rubber mold of an 11cm (TL) male and then each replica was airbrushed to match male color patterns.
Figure 3.3. Manly’s selectivity ratios calculated from field pairs demonstrates female preference to pair with males of like color morph. The dashed line represents random association, above which indicates preference and below which indicates avoidance (less than expected based on availability). Both females exhibited strong preference for males of their own color morph and avoidance for males of the alternate color.

Figure 3.4. Outcomes of 44 no-contact female preference trials.
Figure 3.5. Time females spent associating with live stimuli males of two color morphs in 60 min trials (n = 44). Mean association time (± 95% confidence intervals).

Figure 3.6. Comparison of female preference using live versus model males.
CHAPTER IV
GENETIC DIFFERENTIATION AMONG ARCEYE HAWKFISH COLOR MORPHS
INTRODUCTION

Speciation, the evolutionary process by which new species arise, remains a fundamental challenge in biology (Coyne & Orr 2004, Marie Curie Speciation Network et al. 2012, Shaw & Mullen 2014, Gavrilets 2014). A particular enigma has been explaining the mechanisms responsible for the incredible radiation of coral reef fishes in tropical seas (Rocha & Bowen 2008). Until recently, allopatric speciation had been considered the predominant and almost exclusive driving force for marine biodiversity. Now there is a growing appreciation that ecological adaptation may be initiating speciation in the sea, particularly on coral reefs (Briggs 2005, Bernardi 2013, Bowen et al. 2013).

The role of ecology in driving reef fish speciation was initially supported by phylogenetic studies in several speciose families including Scarids (Streelman et al. 2002), Tetraodontids (Alfaro et al. 2007), Labrids (Rocha et al. 2005) and Gobiids (Rüber et al. 2003, Taylor & Hellberg 2005). Rocha et al. (2005) offered a noteworthy case of parapatric ecological speciation in Atlantic wrasses (*Halichoeres* spp.) by showing strong genetic partitions between adjacent but ecologically distinct habitats despite high genetic connectivity between similar habitats.

Recent studies combining ecology, morphology and genetics have shown that divergent selection on traits adapting to contrasting environments can create barriers to gene flow even in sympatry (Munday et al. 2004, Bongaerts et al. 2011, Bird et al. 2011, Puebla et al. 2014). The critical ingredients needed for ecological speciation-with-gene-flow are divergent selection, a mechanism of reproductive isolation and a link between the two. Comprehensive studies that have identified all three ingredients and the ecological trait under selection in reef fishes are extremely limited. The two most compelling examples involve host shift in coral-dwelling *Gobiodon* gobies (Munday et al. 2004) and aggressive mimicry in color polymorphic *Hypoplectrus* hamlets (Puebla et al. 2007).

These studies highlight the potential mechanisms by which reproductive isolation can result from divergent selection on ecologically important traits. As exemplified by the coral gobies, when individuals prefer the habitat to which they are best adapted, and mating takes place in that habitat, reproductive isolation can result (Munday et al. 2004). As seen in the hamlets, color is a potentially important pleiotropic trait as it can be both under disruptive selection and used as a cue in assortative mating (Puebla et al. 2007). Scenarios where the trait...
under selection is linked directly with mate choice are considered the most plausible paths to ecological speciation (Maynard-Smith 1966, Gavrilets 2004).

The lack of examples from nature may not be due to their rarity, but rather the difficulty in diagnosing a mode of speciation that has already occurred. Identifying modes of speciation in wild populations is inherently challenging, as the processes that led to the formation of most species are often obscured by time. Here, I use the natural color polymorphism in the arceye hawkfish (*Paracirrhites arcatus*) to investigate early reproductive isolation during a potential scenario of incipient ecological speciation-with-gene-flow. *Paracirrhites arcatus* is a polychromatic species with two distinct, described color morphs (Randall 1963) throughout their wide distribution (East Africa to the Central Pacific). The color morphs are sympatric, however, they segregate across an ecological cline on shallow coastal reefs (DeMartini & Donaldson 1996; Chapter II). Each color morph is likely experiencing higher fitness in the reef habitat that more closely match its color and provides better camouflage (Chapter II). Disruptive natural selection on color to match microhabitats provides a plausible mechanism for the differences among color morphs.

Adaptation to different microhabitats in association with assortative mating by color could provide the barriers to gene flow necessary to initiate reproductive isolation. The combination of small home ranges, territoriality and harem mating (Donaldson 1990, DeMartini 1996) decrease the probability of mating with individuals in different microhabitats (Chapter III). Therefore, habitat isolation among color morphs may limit opportunities for intermorph mating, which could be reinforced by behavioral preference for like individuals. Thus, the relatively low proportion of pairing between phenotypes on reefs (< 10 %) provides a mechanism of premating isolation among sympatric color morphs.

**Population genetics**

The goals of this chapter are to evaluate genetic evidence indicating that color morphs are reproductively isolated, and if so, identify the early barriers to gene flow. There are several patterns we might expect to find under a scenario of incipient ecological speciation (Via 2009, Thibert-Plante & Hendry 2010, Nosil 2012, Feder et al. 2012). First, when divergent selection causes reproductive isolation among adaptive ecotypes, it reduces the average effective migration rate globally across the entire genome and facilitates genetic divergence at neutral loci.
(Gavrilets 2004, Rundle & Nosil 2005, Hendry et al. 2007, Thibert-Plante & Hendry 2010, Feder et al. 2012). Second, if ecological barriers reduce gene flow, we would expect that genetic differentiation should be greater between contrasting environments than between similar environments (Rocha et al. 2005, Nosil 2007, Wang & Summers 2010). Third, divergence early in ecological speciation with gene flow is expected to be restricted to genomic regions containing traits under selection, whereas the rest of the genome is largely homogenized by ongoing gene flow (Via 2009). Therefore, in a scenario of divergence-with-gene flow, we should expect to find highly heterogeneous differentiation across the genome, with the majority of neutral loci showing little to no divergence, and few regions showing pronounced differentiation (Schluter & Conte 2009).

**Color candidate gene**

Color patterning in fish involves six types of pigment cells: melanophores (dark), xanthophores (yellow), erythrophores (red), leucophores (white), cyanophores (blue), and iridophores (silvery) (Fujii 2000). The combination of these cell types generates the complex range of body tones and color patterns in fish. The genetic pathways underlying color have been described in a few model fishes, such as zebrafish (*Danio rerio*) and medaka (*Oryzias latipes*; Parichy 2003). Some genetic variants of these pigmentation genes have even been associated with color differences in natural populations (Salzburger et al. 2007, Miller et al. 2007).

In the Arc-eye Hawkfish, the darker melanistic pigmentation is most likely the result of increased melanin (Ahmed & Setlow 1993). Melanistic coloration is widespread in fish and is reported to have several adaptive functions such as greater protection from damaging UV-light (Ahmed & Setlow 1993; Zellmer 1995), thermoregulation (Trullas et al 2007), disease and parasite resistance (Chakarov et al. 2008), cryptic coloration (Marjerus 1998), and even reduced vulnerability to stress (Overli 2008).
The melanocortin receptor 1 gene (Mc1r) encodes a G-protein coupled transmembrane receptor found primarily in melanocytes. It plays an essential role in the regulation of melanin synthesis in vertebrates (Barsh 1996), by controlling the type and density of melanin synthesized for deposition in tissues (Lin & Fisher 2007). Mutations in the Mc1r coding region have been linked to color polymorphisms in a wide range of vertebrates (reviewed in Hoekstra 2006a), including birds (Mundy 2005), mammals (Ritland et al. 2001, Eizirik et al. 2003, Mundy & Kelly 2003, Nachman et al. 2003, Hoekstra 2006b), reptiles (Rosenblum et al. 2004), and fish (Protas et al. 2006, Selz et al. 2007, Richardson et al. 2008, Henning et al. 2010). Given its widespread association in color evolution and role in melanin synthesis, I targeted Mc1r as a promising candidate gene to investigate the underlying genetic basis of color polymorphism in *P. arcatus*.

Here, I combine data from (presumably) neutral microsatellite markers and mc1r to investigate the roles of natural selection and nonrandom mating in driving genetic divergence between color morphs. To test the hypothesis that *P. arcatus* color morphs are reproductively isolated from one another, I first estimate neutral levels of divergence across phenotypes using 30 microsatellite loci. Next, I combine microsatellite and habitat data to test for the relationship between genetic and environmental distance as expected under divergence-with-gene-flow. Finally, I test for genetic signatures of selection in the Mc1r gene by comparing levels of differentiation with neutral markers.

**METHODS**

**Field Collections**

A collection of 292 adult *P. arcatus* individuals from the western (leeward) coast of the Island of Hawai‘i was made during July-August 2010 and September 2012. Fish were collected using micropole spears while on SCUBA along specific transects described in Chapter II. Upon collection, each fish was measured, photographed on a standardized background (using both ambient light and flash), and then dissected. During dissections, a chunk of white muscle tissue and caudal fin were removed and preserved in either 95% ethanol or saturated salt (NaCl) solution with 20% DMSO for genetic analysis. Gonads and stomachs were preserved separately in 95% ethanol for sexing and diet analysis, respectively. When possible carcasses were saved and frozen at -20° C.
Each individual’s color phenotype was scored using two qualitative traits that distinguish *P. arcatus* color morphs (1) body color: melanistic (uniform brown to black), intermediate (light brown to dark red), and pink (light pink to red with lighter ventral), and (2) the presence/size of the white-stripe running along the flank: no-stripe, faint-stripe, and full-stripe. Individuals were initially scored immediately after collection and then confirmed using voucher photographs. To simplify analyses, the nine possible phenotypic groupings were reduced into three discrete color morphs: melanistic (MEL; dark body, no-stripe), pink-white-stripe (PWS; light pink/red body, full white stripe), and intermediate (INT), which included all fish with either intermediate body color, faint-stripes, other combinations (i.e., dark body with full-stripe, or light body with no-stripe).

**Microsatellite amplification and genotyping quality control**

To estimate fine-scale population genetic structure and gene flow between color morphs, I genotyped 256 individuals at 30 microsatellite loci designed specifically for *P. arcatus* (Whitney & Karl 2012). Genomic DNA (gDNA) was extracted from muscle tissue following the HotSHOT protocol (Meeker et al. 2007). Loci were amplified using four PCR multiplexes in 5 µl reactions following the protocol of Whitney & Karl (2012). Fluorescently-labeled PCR products were resolved using an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA). Alleles were called manually using GENEMAPPER v4.0 with GS500LIZ size standards (Applied Biosystems).

Multiple steps were taken to ensure a robust microsatellite analysis. To minimize PCR amplification bias in microsatellites, samples that had poor amplification due to low DNA quantity or quality were re-amplified using less diluted or re-extracted gDNA. Questionable samples with hard-to-call genotypes (e.g., extensive stutter, faint alleles, low fluorescence peak heights, and >2 obvious alleles) were re-amplified or removed. Allele calls for individuals that were re-run were manually compared over all loci to create a consensus genotype. To minimize allele scoring error, once the entire collection of samples was genotyped, I manually re-analyzed all allele calls locus-by-locus to ensure consistency in calling and minimize the chances of misinterpreting artifact peaks and stutter patterns. To ensure the accuracy of scored genotypes, I estimated allele scoring error rate by repeating marker amplification and allele scoring in 20 randomly selected individuals (Selkoe and Toonen 2006). Genotypes that passed quality control
were exported from Genemapper as raw estimated fragment sizes and transformed into binned integer allele calls using TanDEM (Matschiner & Salzburger 2009). I then screened the microsatellite data set for abnormalities such as null alleles, large allele drop-out and stutter using Microchecker v2.2.3 (van Oosterhout et al. 2004) using 99% confidence intervals. Individuals missing more than 15% of data (i.e., no genotype for >4 loci) were excluded from analysis. Two loci (Paa12 and Paa45) had unreliable amplification and were removed from all analyses, thus reducing the dataset to 30 loci. The software program PDGSpider v2.0.0.0 (Lischer & Excoffier 2012) was used to convert genotype matrices into various formats for analysis.

Diversity & differentiation at microsatellite loci
Measures of genetic variation including allelic diversity, frequency, and heterozygosity were calculated using Genepop v4.2 (Raymond & Rousset 1995, Rousset 2008), Genalex v6.5 (Peakall & Smouse 2006, 2012) and Arlequin v3.5 (Excoffier & Lischer 2010). I tested for conformance to Hardy-Weinberg equilibrium (HWE) in Genepop using the Markov chain method (Guo & Thompson 1992) with 10,000 permutations (dememorization =10000, batches = 100, iterations per batch = 5000). All pairs of loci were tested for linkage disequilibrium using Genepop via LinkDos (http://genepop.curtin.edu.au/linkC.html; Garnier-Gere & Dillmann 1992). The false discovery approach of Benjamini & Hochberg (1995) was used to adjust critical P-values. I estimated the proportion of null alleles at each locus separately for each morphotype using FreeNA (Chapuis & Estoup 2007), according to the expectation maximization algorithm of Dempster et al. (1977).

Genetic differences between the two predominant color morphotypes were analyzed in several ways. First I tested for significant differences in allele frequencies using the genic differentiation option in Genepop, which provides an unbiased estimate of the P-value with a G{log-likelihood-based exact test (Goudet et al. 1996)}. I adjusted significance thresholds for multiple comparisons by controlling the false discovery rate (FDR < 0.05) using the q-value method (Storey et al. 2004) implemented in the R package qvalue v 2.2.2 (Dabney et al. 2015).

Second, I evaluated the partitioning of genetic variance both among color morphs and locations using a hierarchical allelic distance-based Analysis of Molecular Variance (AMOVA) (Excoffier et al. 1992) using Genodive v2.0b27 (Meirmans & van Tienderen 2004) with
significance tested by 10,000 random permutations and assuming an infinite allele model. I grouped individuals into two populations based on location of collection site: Kohala in the north (N=106; 54 MEL, 52 PWS) and Kona in the south (N=105; 53 MEL, 52 PWS). Collection sites were defined into these geographic regions because they were the most geographically separated groups that contained sufficient sample sizes (n>50 per morph per region).

Third, I calculated locus-specific and multilocus estimates of genetic divergence among phenotypes using $F$-statistics ($F_{ST}$) in GENALEX v6.5 as per Weir and Cockerham (1984) and Michalakis and Excoffier (1996), assuming an infinite allele model. Fixation indices (e.g., $F_{ST}$) may be poor estimators of population divergence when using markers with high allelic diversity because $F_{ST}$ is reduced proportionately to within-population levels of heterozygosity (Jost 2008, Meirmans & Hedrick 2010, Bird et al. 2011). I employed GENALEX, GenoDive, and SMOGD v1.2.5 (Crawford 2010) to calculate the standardized allele-frequency based estimators of population differentiation, $F''_{ST}$, $G''_{ST}$, and Jost’s D respectively (Hedrick 2005, Jost 2008) following the recommendations of Meirmans & Hedrick (2010). These estimators provide an index of genetic differentiation relative to their maximum achievable values and are scaled to a range of 0.0 to 1.0, with the upper limit of 1.0 achieved when populations have completely non-overlapping sets of alleles (Bird et al. 2011). Significance for all estimates of genetic differentiation were tested by comparing observed data with null distributions generated from 10,000 random permutations, and variances were estimated via jackknifing and bootstrapping over loci. Despite the limitations of $F_{ST}$, I report it along with standardized metrics to allow for a direct comparison with older literature (Weersing & Toonen 2009). Sample sizes for the intermediate morphotypes were prohibitively low (<50), and thus were excluded from all analyses with the exception of assignment tests (described below).

**Assignment tests**
Multilocus genotypes from 107 MEL, 104 PWS and 45 intermediate morphotypes (13 no-stripe, INS; 16 faint-stripe, IFS; and 24 white-stripe, IWS) were used to assess population structuring using both STRUCTURE 2.3 (Pritchard et al. 2000) and discriminant analysis of principal components (DAPC) in Adegenet 1.4-2 for R 3.1.0 (Jombart 2008, R Core Development Team). For STRUCTURE I used a model assuming admixture and correlated allele frequencies, as I expect current gene flow and many shared alleles among morphotypes due to common ancestry (Falush
et al. 2003). Phenotype was used as prior information to assist the structuring (the locprior model) as recommended for weak signals of structuring (Hubisz et al. 2009). The use of location priors can assist STRUCTURE in clustering without biasing analyses towards spurious structure (Hubisz et al. 2009). I ran the model using values of $K$ from 1 to 5, with 5 replicate runs for each $K$ and a burn-in of 500,000 steps, followed by 2,000,000 MCMC iterations. I used the delta $K$ method of Evanno et al. (2005) implemented in STRUCTURE HARVESTER v0.6.94 (Earl & vonHoldt 2012) to determine the most likely number of clusters. Membership scores from five replicate runs were aligned and averaged using CLUMPP v1.1.2 (Jakobsson & Rosenberg 2007), and visualized with DISTRUCT v1.1 (Rosenberg 2004). Membership probabilities in each of the two clusters were arcsine square root transformed and a $t$-test was used to compare values between the two putative clusters for each color morph.

DAPC was used to explore population structure among morphotypes. DAPC is a multivariate approach that attempts to improve discrimination of individuals into clusters by partitioning genetic variation in a way that maximizes the between group component, while minimizing the within-group component (Jombart et al. 2010). This method yields information similar to STRUCTURE, but does not rely on a particular population genetics model, and is thus free of assumptions about Hardy-Weinberg and linkage equilibrium (Jombart et al. 2010). I imposed the number of clusters ($K$) of two and ran two analyses. First, I clustered individuals without any priors and then using phenotype as a prior group assignment. The optimal number of PCs retained for the DAPC was chosen using an optimization procedure (Jombart 2008). The DAPC was performed using 30 PCs (representing ~50% of the total genetic variation) and one discriminant function, which is the maximum when $K = 2$. Membership probabilities in each of the two clusters were arcsine square root transformed, and a $t$-test was used to compare values between the two putative clusters for each color morph.

Although one might expect that more loci should be more informative, more loci can actually cause reduced performance in individual assignment in some cases (Guinand et al. 2004). Assignment accuracy is dependent on the number and variability of markers used, the levels of co-ancestry and degree of genetic differentiation among groups (Bjørnstad & Røed 2002, Guinand et al. 2006). Smouse & Chevillon (1998) recommend using a moderate number of informative loci when estimating mixture composition. In some cases, the selection of informative microsatellite markers is required for improving individual assignment efficiency (Li
Therefore, my goal was to identify a subset of microsatellite loci with high discriminatory power for informing individual assignments to phenotypic clusters.

I used backward elimination locus selection (BELS; Bromaghin 2008) to evaluate the power of each microsatellite locus to improve individual assignment estimation, by excluding loci (one by one) that cause the least reduction in performance. A test set (baseline) of 100 randomly individuals classified into two groups based on color phenotype (50 MEL, 50 PWS) was resampled 1000 times to produce 200 randomly generated genotypes from each groups. The average individual assignment accuracy across both groups (0.80) was used as the performance measure to evaluate locus utility. The set of loci required to meet the performance measure of 80% individual assignment accuracy was used in STRUCTURE (using parameters described above) to test if individuals could be assigned to groupings by phenotype and assess the degree of admixture of individuals of intermediate phenotypes. As a comparison, I executed the same BELS procedure using groupings defined by geographic location (North versus South regions of Hawai‘i Island) to see if individuals could be assigned to the geographic cluster.

**Distance-based redundancy analysis**

To test whether habitat-related variables explained some of the genetic variation observed among *P. arcatus* color morphs, I performed a partitioning of a genetic distance matrix using distance-based redundancy analysis (dbRDA; Legendre & Anderson 1999, McArdle & Anderson 2001). This procedure examines the extent to which each predictor variable explains significant genetic variability among individuals by regressing a response matrix of genetic distances on various explanatory matrices including geographic distance, phenotype, and habitat. This analysis was restricted to 100 individuals (50 MEL and 50 PWS) that were collected on transects linked with high-resolution habitat survey data (see Chapter II). Genetic distances among individuals were calculated using Euclidean distance in GENODIVE (Meirmans & van Tienderen 2004). Geographic distances between individuals were calculated from the approximate latitude and longitude. Phenotype was categorical with two levels (MEL and PWS), and habitat type had three levels corresponding to reef zones: (1) surge zone dominated by *Pocillopora* corals, (2) sub-surge zone co-dominated by *Pocillopora* and *Porites* corals, and reef slope dominated by *Porites* corals. The relationship between the response matrix (genetic distance) and each
predictor variable was analyzed separately using dbRDA with Vegan (Oksanen et al. 2015) in R. Statistical significance was determined using 10000 permutations.

**Diversity and differentiation of Mc1r**

An 850-bp protein-coding fragment of the Mc1r gene was amplified using conserved primers MC1RFor and MC1RRev of Eytan et al. (2012). PCRs were performed in 15 µl reactions of 7.5 µl BioMixRed (Bioline, Tanton, MA, USA), 0.3 µl of each primer (10 µM), 5.9 µl deionized water, and 1µl of genomic DNA with the following cycles: one cycle of 94 °C for 45 s, 50 °C for 45 s, 72 °C for 90 s followed by 35 cycles of 95 °C for 1 min, 63 °C for 1 min, and 72 °C for 90 s, and a final cycle of 94°C for 40s, 50°C for 45 s, and 72°C for 10min. I purified PCR amplicons with exonuclease I and thermosensitive alkaline phosphatase (FastAP) and sequenced purified product in both directions using ABI 3730xl Sequencers (Applied Biosystems). Individuals with poor sequence quality were re-sequenced. Sequences were edited and aligned using GENEIOUS v7.0.3 (Biomatters, Ltd, Auckland, New Zealand). Nucleotide and amino acid polymorphisms were viewed in MEGA v6 (Tamura et al. 2013). Double peaks of approximately equal height present in the same sequence from both directions were scored as heterozygous. Haplotype phases were inferred using PHASE 2.1.1 (Stephens et al. 2001).

I estimated diversity of the Mc1r gene as haplotype number and diversity \( h \) (Nei 1987) overall and for subpopulations defined by color phenotype. Nucleotide diversity was estimated with Waterson’s \( \Theta \) (Watterson 1975) and Nei’s \( \pi \) (Nei 1987). I calculated Tajima’s \( D \) (Tajima 1989) to test for departures from neutral expectations. Diversity metrics were all calculated using the software DNASP (Librado & Rozas 2009). To assess the proportion of genetic variation that is explained by color phenotype, I ran AMOVA tests in GENALEX and GENODIVE using both allele frequencies \( F_{ST} \) and individual-based distances \( \Phi_{ST} \). Statistical significance was estimated using randomization tests with 10000 random permutations. To estimate the degree of genetic differentiation between color morphs at this locus, I also calculated Jost’s \( D \) (Jost 2008) in GENODIVE (Meirmans & van Tienderen 2004).

**\( F_{ST} \) outliers**

The genetic divergence for some loci may exceed neutral expectations (Nosil et al. 2009), which may indicate evidence of selection. Microsatellite DNA is generally noncoding and often
assumed to act as a neutral marker in population genetics. However, divergent selection at a locus physically linked to a neutral microsatellite can cause inflation of measures of genetic differentiation, a process called genetic hitchhiking (Nielsen et al. 2006, Feder et al. 2012). To assess if any microsatellite loci or the Mc1r gene were more divergent than expected under neutral conditions, I used the FDIST2 outlier analysis described in Beaumont & Nichols (1996) and implemented in LOSITAN v1.0 (Antao et al. 2008). The Fdist method uses simulations to generate a null distribution of neutral $F_{ST}$, based on the assumption that all $F_{ST}$’s are equal among loci, and then uses the relationship between $F_{ST}$ and the expected heterozygosity to identify loci that have excessively high or low $F_{ST}$ compared to neutral expectations (Antao et al. 2008). I ran 50,000 simulations using the neutral $F_{ST}$ and force mean $F_{ST}$ options (both recommended), which increase the reliability of the mean $F_{ST}$ by running an initial simulation that removes potential outliers. I ran simulations under the infinite allele (IAM) model with a false discovery rate of 0.05 and 99% confidence intervals. For the Mc1r gene, I used the SNP with the highest $F_{ST}$ (Mc1r_023) to represent the gene.

RESULTS

Quality control of microsatellites

Microsatellite loci were successfully genotyped in 256 individuals of three color morphs (107 MEL, 104 PWS, 45 INT). All 30 loci were polymorphic with the number of alleles ranging from six to 34 (mean = 16.3). Repeat genotyping of 20 samples revealed 13 inconsistencies across 1122 alleles over 30 loci – an error rate of 1.2%. The discrepancies were not distributed evenly among loci: 18 loci had no discrepancies in allele calls, whereas the remaining 12 loci had error rates ranging from 2.0-5.9%.

The overall population (when pooling all loci) showed significant departures from HWE ($F_{IS} = 0.112, P < 0.001$), and the same was true when both subpopulations were tested separately (MEL: $F_{IS} = 0.113, P < 0.001$; PWS: $F_{IS} = 0.109, P < 0.001$; Table 4.1). Twenty loci had genotype frequencies that departed significantly from HWE expectations under $P < 0.05$ criterion due to heterozygote deficiency, however, only 12 remained significant after Bonferroni adjustments for multiple comparisons (Table S4.1). Lower than expected estimates of heterozygosity (and resultant deviation from HWE) at a locus was most probably due to the
presence of null alleles, which were detected in all loci failing HWE. The estimated frequencies of null alleles ranged from 0 to 0.26, and varied slightly between subpopulations (Table S4.2).

I estimated null allele frequencies for each locus and explored their impact by comparing locus-by-locus estimates of $F_{ST}$ obtained from the original dataset and with a second dataset adjusted for estimated null allele frequencies. Taking into account a null allele class, based on Chapuis & Estoup (2007), had no detectable impact on individual $F_{ST}$ estimates. The corrected and uncorrected $F_{ST}$ estimates are strongly correlated (Pearson’s correlation, $r = 0.99$, $P < 0.00001$) with a mean difference of -0.000087 (95% CI: -0.0005, 0.0003). Using the corrected dataset did not change the results of any statistical test. Thus, only values obtained from the original (uncorrected) dataset are provided.

MICROCHECKER detected significant stutter in three loci (Paa55, Paa65, and Paa76), and large allele dropout was not detected at any locus. Five of 440 pairwise tests for linkage disequilibrium (1.1%) were significant after applying Bonferroni adjustments (Table S4.3), however, none of these pairs were detected as being linked when each morphotype was tested separately. The lack of a repeatable pattern of linkage disequilibria across subpopulations suggests that any linkage disequilibria is more likely due to population structure than physical linkage of loci.

From these quality control screening procedures, five loci (Paa55, Paa65, Paa67, Paa71, Paa76) were flagged for possible removal due to (1) failing HWE in both morphotypes, (2) significant evidence of null alleles (ENAF > 0.15), and/or (3) significant evidence of stutter. To assess the impact of these loci on estimates of genetic differentiation I ran all analyses both with and without them and found their exclusion/inclusion had no significant impact on the inference made from any test result. Therefore all subsequent results will be reported using the full set of 30 microsatellite loci.

**Diversity & differentiation at microsatellite loci**

The analysis of 30 microsatellite loci of 211 individuals from the two predominant color morphs (107 MEL; 104 PWS) revealed high levels of genetic variability within populations: mean numbers of alleles per locus ($A_R$) were 14.7 (MEL) and 15 (PWS) and expected heterozygosities ($H_E$) were 0.83 for both MEL and PWS (Table 4.1). Estimates of unbiased heterozygosity ($H_S$) were consistently high (0.83) across morphotypes, and ranged from 0.50 – 0.95 across loci.
Levels of genetic diversity were not different between morphotypes, neither in terms of allelic richness ($P = 0.851$) nor heterozygosity ($P = 0.591$). Each morphotype had private alleles ($A_P$) with a total of 34 (mean $\bar{A}_P$ per locus = 1.1) in MEL to 45 ($\bar{A}_P$ per locus = 1.5) in PWS, however, no private allele had frequency $> 0.05$ (Table 4.1).

Microsatellite variation shows low levels of genetic differentiation among morphotypes. Multilocus exact $G$ log-likelihood tests revealed significant differences in allelic distributions ($P = 0.0003$), and genotypic distributions ($P = 0.0340$) among color morphs. $F'_ST$ estimates were highly heterogeneous across the genome, ranging from $-0.035$ to $0.072$ (mean $F'_ST = 0.014$) among loci. Locus-by-locus comparisons revealed significant allelic differentiation in six loci ($P < 0.05$; Table S4.4), four of which remained significant after controlling for a 5% false discovery rate (Storey et al. 2004). If we were to assume that all the null hypotheses are true ($F'_ST = 0$), given a criterion of $\alpha = 0.05$, we would expect that up to 5% of loci could show significant differences by chance alone. Using exact $G$ tests, 20% of loci (6/30) detected significant differentiation at $P < 0.05$. Therefore, after accounting for possible false positives, I could consider at least four loci demonstrating significant divergence among phenotypes. Together these four loci (Paa55, Paa60, Paa72, Paa88) had a mean $F'_ST = 0.044$, whereas the remaining 26 loci had a mean $F'_ST = 0.004$. Thus, estimates of differentiation between color morphs vary considerably among loci.

According to the two-level hierarchical AMOVA, nearly all genetic diversity (99.9%) was partitioned among individuals within morphotypes, indicating high genetic similarity overall (Table 4.2). Global $F'_ST$ estimates revealed very slight, but significant overall genetic structure among morphotypes ($F'_ST = 0.006$; $P = 0.025$), yet no detectable structure among locations (Kohala & Kona) neither when nested in morphotype ($F'_ST = -0.012$, $P = 0.977$) nor when pooling both morphotypes ($F'_ST = -0.003$, $P = 0.822$). Comparisons of sampling year also revealed no structure between samples collected in 2010 versus 2012 ($F'_ST = -0.001$, $P = 0.666$).

Pairwise multilocus comparisons of allelic distribution also revealed higher divergences among color morphs, even within a geographical cluster, than between geographical clusters (Table 4.3). There were no significant differences between Kohala and Kona regions when pooling all individuals ($P = 0.130$) or when each morph was tested separately (MEL: $P = 0.354$; PWS: $P = 0.187$; Table 4.3). Therefore, there was more differentiation among color morphs ($D_{EST} = 0.002$, $P = 0.025$) than among locations ($D_{EST} = -0.001$, $P = 0.130$).
Assignment tests

Results from the STRUCTURE analysis indicate that color morphs form discrete clusters when considered in the context of differentiation at a subset of microsatellite loci, whereas they do not group when all loci are taken together (Fig. 4.1). Simulations using all 30 microsatellite loci, indicated that $K = 2$ had the highest likelihood ($\text{lnL} = -21,747$) of the five models examined ($K = 1$ to 5). Because the Evanno et al. (2005) method cannot identify situations in which the most probable grouping is $K = 1$, the results were examined further following the recommendations of Pritchard et al. (2010). Specifically, if there is no population structure ($K = 1$), group assignment probabilities ($q$) should be roughly symmetric $\sim 1/K$ in each population. Therefore, if we assume $K = 2$, yet there really is no structure (true $K = 1$), most individuals in both clusters would be expected to have $q$ scores $\sim 0.5$, which is the case when using all 30 loci. Therefore, I conclude that the most likely number of genetic clusters identified in STRUCTURE was one, but I present the result of STRUCTURE clustering for $K = 2$ (Fig. 4.1A), in order to test whether color morphs form separate clusters when two genetic clusters are assumed. When based on genotypes at all 30 loci, an examination of the probability of assignment to either of the two clusters for $K = 2$, shows no distinguishable pattern (Fig. 4.1A) and there is no significant difference in membership of cluster 1 among phenotypes regardless of whether I use the mean posterior membership scores of five simulation runs ($F_{1,205} = 1.86, P = 0.174$) or the model with the highest likelihood ($F_{1,205} = 0.893, P = 0.346$).

Using the panel of seven loci selected by BELS based on their power to discriminate phenotypic groups (Table S4.5), STRUCTURE was able to correctly assign individuals to their phenotypic cluster (when phenotype is used as a priori grouping). Mean posterior membership scores for each cluster (averaged over five runs), differed significantly among the main color morphs ($F_{1,205} = 865.6, P < 0.00001$; Fig. 4.1B). The intermediate morphotypes clustered together, and cluster membership did not vary significantly among the three intermediate subtypes based on striping (absent, faint, full white stripe; $F_{2,42} = 2.857, P = 0.0917$; Fig. 4.1B). Though I only expected differentiation between MEL and PWS, the intermediates showed mixed ancestry between the two clusters, with mean membership in cluster 1 (0.596; associated with MEL morphs) significantly ($t = 9.26, P < 0.00001$) higher than in cluster 2 (0.404; associated with PWS morphs). For comparison, using the panel of seven loci selected by BELS based on
the power to discriminate locations (north versus south Hawai‘i Island; Table S4.5), mean posterior membership scores did not vary significantly among samples collected in northern versus southern sites ($F_{1,205} = 2.857, P = 0.0924$; Fig. 4.1D).

In the DAPC, thirty principal components and 1 discriminant function were retained, and the most likely number of clusters was $K = 2$, based on the lowest BIC score. When using phenotype as the prior group assignment, the mean posterior probabilities of assignment of PWS and MEL individuals to the cluster representing their own color morph was significantly higher (0.72 ± 0.22) than the alternate cluster ($F_{1,205} = 57.75, P < 0.00001; t = 10.5, P < 0.00001$). When individuals were clustered without $a priori$ groups defined by phenotype, however, clusters did not correspond well to color morphs, and there was no significant difference in membership probability in the two clusters ($t = 0.586, P = 0.56$).

**Distance-based redundancy analysis**
I detected significant isolation by habitat in the distance-based redundancy analysis. Habitat type defined as reef zone (i.e., surge, sub-surge, or reef slope) based on coral community and topography had a significant relationship with genetic distance ($F_{2,93} = 1.34, P = 0.025$), explaining 5.9% of the genetic variability among individuals. Concordant with the results of pairwise allelic differentiation, geographical distance did not significantly influence the patterns of genetic distance ($P = 0.327$). Color morph explained only 1.5% of genetic variability, but was highly significant ($P = 0.007$), and had significant interaction terms with both habitat type (% var = 3.2, $P = 0.001$) and geographic distance (% var = 1.6, $P = 0.019$; Table 4.4). When the spatial variation was taken into account by using distance as a covariable in a partial dbRDA, a significant relationship between genetic variation and habitat was still detected ($P = 0.001$). None of the other environmental variables tested (depth, slope, wave action, and coral percent cover estimates) showed a statistically significant relationship with genetic distance ($P > 0.05$).

**Diversity and differentiation of Mc1r**
I sequenced 741 basepairs of the coding region of the nuclear gene Mc1r for a total of 85 individuals (42 MEL, 43 PWS). Six of the 741 basepairs were segregating sites (all biallelic). Haplotype diversity was high ($h = 0.816 ± 0.035$) with 15 haplotypes estimated using those considered most likely by PHASE. Nucleotide diversity also was high ($\pi = 0.0026, \Theta = 0.008$),
and maximum sequence divergence between any two haplotypes was 1.26%. All segregating sites were synonymous (5 transitions, 1 transversion) and therefore do not directly affect protein coding (Tajima’s $D = 0.58$, $P > 0.10$). Nevertheless, Mc1r does show statistically significant differentiation among PWS and MEL morphotypes ($F_{ST} = 0.032$, $D_{EST} = 0.012$, $P = 0.022$), thereby indicating some evidence for genetic divergence between color morphs at this locus. An analysis of molecular variance of the whole gene indicated that 5.1% of the variation at Mc1r was partitioned between color phenotypes and that this was significantly greater than with random permutations of $\Phi_{ST}$ ($\Phi_{ST} = 0.051$, $P = 0.031$; Table S4.6). Individual SNP estimates revealed significant differences in allele frequencies between color morphs at a single variable site (Mc1r-023: $F'_{ST} = 0.216$, $F_{ST} = 0.175$; $P < 0.001$), whereas the remaining five SNPs were uninformative (mean $F'_{ST} = -0.004$; $P > 0.40$). Substitutions at the Mc1r-23 position are in the third codon position for glutamic acid (GAA/GAG), and do not change the amino acid coding. Therefore, Mc1r does show a correlation with color phenotype, however, the relationship is not mechanistic as none of the substitutions appear to generate any differences in protein coding that could change the function of the Mc1 receptor.

$F_{ST}$ outliers

The Fdist analysis conducted by LOSITAN identified the Mc1r gene as a significant outlier ($P < 0.00001$) and thus a candidate for positive selection. The most strongly segregating SNP in Mc1r had a conspicuously large $F_{ST}$ value ($F_{ST} = 0.175$) compared to the neutral microsatellite loci ($F_{ST} = 0.003$) and exceeded the 99.9% confidence interval (Fig. 4.2). Although there was variation in uncorrected estimates of $F_{ST}$ among loci (-0.004 to 0.007), all microsatellite loci were well within neutral expectations based on simulated null distributions.

DISCUSSION

Genetic variation at 30 putatively neutral microsatellite loci and a single candidate gene was used to evaluate the population structure of arceye hawkfish both within and between color morphs. Results indicate low, but significant genetic differentiation at microsatellite loci between ecologically differentiated sympatric color morphs. As discussed below, differentiation in neutral loci is only likely in the presence of various barriers to gene flow between divergent ecotypes,
and I conclude that sympatric color morphs are at least partially reproductively isolated. Outlier tests identified a single SNP in the Mc1r coding region as a significant outlier, as it was strongly differentiated between color morphs. Taken together, I argue that the genetic pattern emerging from these data match well with those expected during the initial phase of ecological speciation-with-gene-flow.

The most fundamental result in this study is the genetic divergence between ecologically differentiated sympatric color morphs. Estimates of genetic differentiation were highly heterogeneous across loci ($F'_ST$ ranges from -0.035 to 0.072, mean = 0.014). The majority of microsatellites showed little to no structure between color morphs, whereas 20% showed significant differences in allele frequencies between color morphs ($P < 0.05$). The comparison of STRUCTURE results when using all loci versus the subset selected by BELS well illustrates the variability in estimates of gene flow across the genome (Fig. 4.1). The pattern of a few loci showing significant divergence while the majority show high allelic exchange matches expectations of divergence-with-gene-flow (Feder et al. 2012, Andrew & Rieseberg 2013).

The observed pattern of neutral genetic differentiation among habitats is also in agreement with predications of isolation-by-adaptation (Nosil et al. 2009, Mendez et al. 2010, Nosil 2012). Results from the distance-based redundancy analysis (dbRDA) indicated that habitat and color phenotype, but not geographic distance, were significantly correlated with genetic distance among individuals, and together explained ~11% of genetic variation in microsatellite genotypes. Hence, I detected greater genetic differences between adjacent microhabitats and phenotypes than between the same habitats in different geographic locations. This pattern of reduced gene flow between ecologically diverged phenotypes provides compelling evidence for ecological barriers driving restricted gene flow on very fine spatial scales. Here I discuss the role of divergent natural selection in driving incipient divergence in hawkfish color morphs/ecotypes.

Nonrandom mating could sufficiently explain the overall genetic differentiation at unlinked neutral loci and corresponds well to patterns of ecological and behavioral divergence observed among color morphs. First, the strong phenotype-environment correlation suggests that phenotypic variation has evolved in response to divergent selection among habitats, as ecological divergence is often used as a proxy for the presence of selection (Nosil et al. 2009). Second, because mating in hawkfish occurs in their preferred habitat, habitat preferences are capable of
preventing random mating, irrespective of mate choice. This is supported by results from field surveys that show fish pair with like color nearly an order of magnitude more frequently than different colors (Chapter III). Moreover, the strong relationship between genetic differentiation and habitat type indicates that divergent habitat preferences are acting as an ecological barrier constraining gene flow. Third, behavioral experiments suggest that color is used as a mate recognition signal, and that color morphs are mating assortatively (Chapter III). Therefore, even when divergent phenotypes co-occur in the same habitat, behavioral divergence is reinforcing premating isolation. Overall, habitat choice and assortative mating are synergistically forming a potent premating barrier that is facilitating the evolution of reproductive isolation in the face of gene flow. The morphologically and genetically intermediate color forms, however, indicate that this assortative mating is not absolute. Interestingly, the intermediate forms also occupy an intermediate habitat.

Early ecological speciation-with-gene-flow is characterized by a genetic mosaic, where most of the genome is homogenized by ongoing gene flow, but divergent selection on regions harboring ecologically important traits will have reduced realized gene exchange (Via & West 2008). This pattern has been supported by genomic data in several empirical examples of ecological speciation in sympatry (Turner et al. 2005, Nosil et al. 2009, Andrew & Rieseberg 2013). These results reveal low \( F_{ST} \) across the genome in neutral microsatellite loci, but high differentiation at \( Mc1r \), which was detected as an \( F_{ST} \) outlier and thus potentially influenced by divergent selection. Outlying loci that show a peak in genetic differentiation (relative to background distribution) are often assumed to be the subject of natural selection. Using simulation-based methods for detecting selection, I confirmed that \( Mc1r \) was a potential candidate for positive selection (Fig. 4.2) or at least is likely physically linked to areas under selection. The most parsimonious explanation based on these data is that adaptive parts of the genome are differentiating, despite nearly unrestricted gene flow in neutral loci. As I have shown that color differences can cause premating isolation (Chapter III) any gene affecting color pattern is potentially important to hawkfish speciation.

As no polymorphism had a detectable change in the function of this melanocyte receptor, color differences between arceye hawkfish are \textit{not} attributable to variation detected in the \( Mc1r \) locus. Therefore a nonsynonymous substitution elsewhere in the gene or epistasis between \( Mc1r \) and another gene may explain results. Noncoding flanking regions of the \( Mc1r \) gene have been
found to regulate gene expression. Stahl and Gross (2015) recently reported that cave dwelling forms of *Astyanax* with no coding sequence variation in *Mc1r* still demonstrate reduced expression due to mutations in the 5’ regulatory region. Mutations in regulatory elements of two other pigment genes, *yellow* in *Drosophila* (Jeong et al. 2006) and *Agouti* in deer mice (Kingsley et al. 2009), can affect pigmentation via altered protein expression. Therefore, it is possible that the color polymorphism in hawkfish could be controlled by variation in *Mc1r* regulatory regions and/or other genes involved with melanin synthesis.

There are many steps along the biosynthetic pathway leading to tissue deposition of melanin that could be affected by genetic variation. The genes coding for melanocortins and the hormones that bind to and stimulate Mcr receptors, also are involved with melanistic pigmentation in teleosts and are thus promising candidates for future association studies. For example, melanocyte-stimulating hormones (MSHs) and adrenocorticotropic hormone (ACTH) are known to be involved in physiological color changes in teleosts (Fujii 2000, Richardson et al. 2008). Mutations in these genes could be responsible for unexplained variation in *Mc1r* expression. For example, the neotropical midas cichlid complex (*Amphilophus citrinellus*) have a melanistic and amelanic (gold) phenotype – with gold determined by the dominant allele at a single locus (Henning et al. 2010), however the gene has not yet been identified. *Mc1r* was found to be upregulated in the skin of gold fish, but there was no sequence polymorphism between morphs in coding or noncoding regions that could effect phenotype (Henning et al. 2010). Whole genome scans combined with targeted sequencing of potential candidate genes associated with color, particularly melanin metabolism, will no doubt help to further elucidate the genetic architecture of color polymorphism and speciation in this and other taxa.

**What is the biological significance of low levels of genetic differentiation?**

Inequality of gene flow estimates across loci is expected in ecological speciation, because differentiation at neutral loci is a product of stochastic genetic drift (Schluter & Conte 2009). However, adaptive divergence can cause genome-wide reductions in gene flow and resultant differentiation at unlinked neutral loci (Gavrilets 2004, Gavrilets & Vose 2007, Thibert-Plante & Hendry 2010). Although a barrier to gene flow should affect allele frequencies at all loci, variation in differentiation among microsatellites could be attributed to the stochastic influence of drift. Among-locus variation could also reflect variation in mutation rates, rather than
selection, however, mutation rate is not predicted to strongly affect patterns of genetic differentiation (Beaumont & Nichols 1996, Balloux & Moulin 2002, Hedrick 2005).

The relatively low and variable genetic differentiation observed among color morphs could be a consequence of either recent divergence and/or ongoing gene flow. First, it is certainly feasible that reproductive isolation has been so recent that not enough time has passed to accrue neutral genetic differences via drift. It can take time for neutral differentiation to accumulate to expected levels even when selection acts directly on linked loci. Second, ongoing gene flow is also likely given that color morphs are not completely spatially isolated and mixed morph pairs are observed in zones of overlap, albeit at low frequency. The occurrence of intermediate color morphs also suggests a strong potential for gene flow between morphs. Quantifying the current magnitude of gene flow is difficult at such low levels of differentiation. For reliable estimates of $Nm$ based on $F_{ST}$, the levels of differentiation need to be moderate to large ($F_{ST} > 0.05–0.10$; Allendorf et al. 2013). Thus estimates of gene flow and migration are unreliable at low levels of genetic divergence (Faubet et al. 2007).

Detecting any level of significant differentiation on such a fine-spatial scale is surprising. Most marine fishes only show significant genetic differences over much greater geographical distances, and a common denominator of most genetic studies of marine organisms is the low levels of genetic differentiation among putative populations (Waples 1998, Waples & Gaggiotti 2006, Eble et al. 2015). Therefore, finding structure on such a small spatial scale in marine fish is suggestive that selective forces are potentially driving allele frequency patterns. The level of genetic differentiation I have reported between hawkfish color morphs is comparable to that of well supported cases of incipient divergence-with-gene-flow (Powell et al. 2014). For example, the measured genetic structure between hawkfish color morphs is on par with differentiation (measured with microsatellites) between some pairs of sympatric color morphs in Midas cichlids, which are argued to be incipient species (Barluenga & Meyer 2004, Elmer et al. 2009). My observed levels of $F_{ST}$ are also comparable to that of *Hypoplectrus* reef fishes (Puebla et al. 2007). Estimates of $F_{ST}$ among various morpho-species pairs ranged from 0.01 to 0.04 and had between 30-90% of microsatellite loci showing significant differentiation among sympatric species pairs (Puebla et al. 2007); and a genome scan using 100,000 SNPs showed extremely slight genome-wide divergence ($F_{ST} = 0.0038$). In the context of these empirical examples, the
observed levels differentiation in hawkfish color morphs are in line with diverging ecotypes in sympaty.

**Could pattern be false detection?**
The relatively weak signal of genetic divergence that emerges from these data implies that hawkfish color morphs are partially isolated. An alternative hypothesis could be that these results reflect random divergence among populations that by chance have led to the expected signature. When true differentiation is low (or absent), it is hard to detect, and various errors such as nonrandom sampling (Allendorf & Phelps 1981), temporal fluctuations (Turner et al. 2002) and genotyping errors (Bonin et al. 2004) can have greater influence and thus a higher likelihood of leading to false conclusions (Waples 1998). Here I address these concerns and provide multiple lines of evidence supporting the unlikelihood of a false conclusion. First, the absence of genetic differentiation between samples collected in replicate years (2010, 2012) and locations (northern & southern sites), demonstrates that I did not detect significant genetic differences in comparisons where they were not expected. Second, by using temporal and geographic replicate sampling, I show that differences among phenotypes are consistent over time and location, and should therefore indicate that the genetic signal is real.

Third, genetic divergence among environments is difficult to detect with high gene flow. Using simulations, Thibert-Plante and Hendry (2010) demonstrated that when gene flow is high and ecological differences are strong, finding the expected signature of isolation-by-adaptation often indicates adaptive divergence. Detecting false positives in the absence of divergent selection is much more likely when gene flow is low, because all populations drift apart to a similar degree, and any differentiation between populations could be due to drift (Thibert-Plante & Hendry 2010). However when gene flow among ecotypes is high, as the evidence here suggests, detection is difficult (and type I error low) because divergent selection is often not powerful enough to reduce gene flow to the point where neutral genetic divergence can proceed (Thibert-Plante & Hendry 2010). That is unless environmental differences (strong divergent selection) or sexual isolation create a generalized barrier to gene flow. Therefore, detection of genetic divergence at high levels of gene flow and when strong environmental differences exist is more likely to reflect true differentiation.
CONCLUSION

The major ingredients of ecological speciation have been identified in *P. arcatus*, specifically a source of natural divergent selection (crypsis), a mechanism of reproductive isolation (habitat preference and color-based assortative mating) and a link between the two (pleiotropic effect of color). Here I provide genetic evidence that these mechanisms have resulted in partial reproductive isolation between color morphs in adjacent habitats. When physical barriers are absent, evidence of even partial reproductive isolation indicates that divergent selection is acting on phenotypes to reduce gene flow. Genetic and ecological patterns observed in hawkfish provide compelling evidence for partial reproductive isolation and strong assortative mating.

Disentangling the proportion of reproductive isolation that is due to premating (habitat and mate choice) versus postmating isolation (selection against migrants or hybrids) is beyond the scope of this study. Further experimental studies that directly measure fitness differences in each habitat will be necessary to evaluate the relative strength of isolating mechanisms. Nevertheless, the genetic pattern that emerges from these data is consistent with the signatures of natural selection. The peak in divergence in the Mc1r gene, amidst a background signal of low, but heterogeneous differentiation across the genome matches well to expectations under incipient ecological speciation with gene flow. Although unclear if this locus affects color directly, the potential relationship to the actual genes underlying the trait presumably under selection is intriguing. The application of genome-wide SNP scans together with targeted sequencing of additional candidate genes along this steep ecological gradient will surely provide insight into the architecture of these diverging genomes.

Speciation is a continuum of differentiation, and based on these data, hawkfish color morphs are at the early stages of evolutionary divergence. Despite showing evidence of ecological, behavioral, and genetic divergence between color morphs, the weak genetic differentiation leads us to caution against considering them incipient species. It’s possible that color morphs represent a balanced polymorphism, held in a dynamic equilibrium between selection and gene flow that could be stable indefinitely (e.g., Barton & Hewitt 1985, Butlin et al 2008). Whether complete reproductive isolation will develop between color morphs remains speculation. However, when mate choice is linked to an ecologically important trait under selection, rapid divergence may occur in sympatry (Rice 1984, Rice & Salt 1990, Schluter 2000). This is considered the most probable scenario for speciation-with-gene-flow (Maynard-Smith
1966, Gavrilets 2004), and therefore has a high likelihood of completing to speciation. Regardless of its fate, the genetic differentiation between ecologically specialized phenotypes provides empirical support that partial reproductive isolation can evolve (or at least persist) in the face of continuous gene flow. The addition of this case to the growing body of evidence supporting the role natural selection plays in initiating speciation should help bring us one step closer to understanding the processes driving high biodiversity on coral reefs.
REFERENCES


Butlin, RK, Galindo J, Grahame JW (2008) Sympatric, parapatric or allopatric: the most important way to classify speciation? Philosophical Transactions of the Royal Society B 363:2997–3007


DeMartini EE (1996) Sheltering and foraging substrate uses of the arc-eye hawkfish (Paracirrhites arcatus). BMS 58:826–837


divergence of neotropical cichlid fish. Evolution 63:2750–2757


Hoekstra HE (2006a) Genetics, development and evolution of adaptive pigmentation in vertebrates. Heredity 97:222–234


Watterson GA (1975) On the number of segregating sites in genetical models without recombination. Theoretical Population Biology 7:256–276


### TABLES

Table 4.1. Summary of diversity statistics for 30 microsatellite loci for each color morph. An asterisk indicates significant deviation from HWE after Bonferroni adjustments.

<table>
<thead>
<tr>
<th>Statistic</th>
<th>MEL</th>
<th>PWS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of genotypes</td>
<td>107</td>
<td>104</td>
</tr>
<tr>
<td>Total number of alleles</td>
<td>441</td>
<td>450</td>
</tr>
<tr>
<td>Mean number of alleles per locus ($N_A$)</td>
<td>14.70</td>
<td>15.00</td>
</tr>
<tr>
<td>Mean effective number of alleles per locus ($N_E$)</td>
<td>8.30</td>
<td>8.30</td>
</tr>
<tr>
<td>Total number of private alleles ($A_P$)</td>
<td>34</td>
<td>45</td>
</tr>
<tr>
<td>Number of loci w/ private alleles</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>Mean number of private alleles per locus</td>
<td>1.10</td>
<td>1.50</td>
</tr>
<tr>
<td>Number of private alleles with frequency &gt; .05</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Observed heterozygosity ($H_O$)</td>
<td>0.74</td>
<td>0.74</td>
</tr>
<tr>
<td>Expected heterozygosity ($H_E$)</td>
<td>0.83</td>
<td>0.83</td>
</tr>
<tr>
<td>Number of loci fail HWE $P &lt; 0.05$ (after Bonferroni)</td>
<td>15 (8)</td>
<td>13 (8)</td>
</tr>
<tr>
<td>Hardy-Weinberg equilibrium ($F_{IS}$)</td>
<td>0.11*</td>
<td>0.11*</td>
</tr>
</tbody>
</table>

Table 4.2. Analyses of molecular variance (AMOVA) in *Paracirrhites arcatus* color morphs (and collection site) for 30 microsatellite loci.

<table>
<thead>
<tr>
<th>Level</th>
<th>% var</th>
<th>$F_{ST}$</th>
<th>$F'_{ST}$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within color morph</td>
<td>99.9</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Among locations nested in color morph</td>
<td>0.0</td>
<td>-0.002</td>
<td>-0.012</td>
<td>0.977</td>
</tr>
<tr>
<td>Among locations</td>
<td>0.0</td>
<td>-0.001</td>
<td>-0.003</td>
<td>0.822</td>
</tr>
<tr>
<td>Among color morphs</td>
<td>0.1</td>
<td>0.001</td>
<td>0.006</td>
<td>0.025</td>
</tr>
</tbody>
</table>
Table 4.3. Pairwise comparisons of genic differentiation. Columns indicate groups being compared, overall $P$-value ($P$), the number of loci showing significant differentiation at $\alpha = 0.05$ and after Bonferroni correction (in parentheses), and Jost's $D_{EST}$. An asterisk indicates significance after Benjamini and Hochberg (1995) correction. Sample size was 211 overall (Kohala $n = 106$; 54 MEL, 52 PWS, Kona $n = 105$; 53 MEL, 52 PWS).

<table>
<thead>
<tr>
<th>Pairwise comparison</th>
<th>$P$</th>
<th>No. sig. loci</th>
<th>$D_{EST}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Between Regions</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>North v. South - ALL</td>
<td>0.130</td>
<td>1 (0)</td>
<td>-0.001</td>
</tr>
<tr>
<td>North v. South (within MEL)</td>
<td>0.354</td>
<td>1 (0)</td>
<td>-0.001</td>
</tr>
<tr>
<td>North v. South (within PWS)</td>
<td>0.187</td>
<td>2 (0)</td>
<td>-0.004</td>
</tr>
<tr>
<td><strong>Between Color morphs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEL v. PWS (overall)</td>
<td>$&lt; 0.001^*$</td>
<td>6 (1)</td>
<td>0.002</td>
</tr>
<tr>
<td>MEL v. PWS (in South only)</td>
<td>0.116</td>
<td>2 (0)</td>
<td>-0.004</td>
</tr>
<tr>
<td>MEL v. PWS (in North only)</td>
<td>0.087</td>
<td>1 (0)</td>
<td>0.005</td>
</tr>
<tr>
<td><strong>Color morph x Region</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEL - North v. PWS-South</td>
<td>0.027$^*$</td>
<td>4 (1)</td>
<td>-0.003</td>
</tr>
<tr>
<td>MEL - South v. PWS North</td>
<td>0.006$^{**}$</td>
<td>6 (0)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Table 4.4. Test for relationship between genetic distance among 100 *Paracirrhites arcatus* individuals and environmental variables, phenotype, and geographic distance using distance-based redundancy analysis (dB-RDA). The column %var indicates the percentage of the multivariate genetic variation explained by each predictor variable. Habitat types include surge zones, sub-surge zones, and reef slope.

<table>
<thead>
<tr>
<th>Variable</th>
<th>df</th>
<th>% var</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Habitat</td>
<td>2</td>
<td>5.9</td>
<td>1.13</td>
<td>0.025</td>
</tr>
<tr>
<td>Color morph</td>
<td>1</td>
<td>1.5</td>
<td>1.44</td>
<td>0.007</td>
</tr>
<tr>
<td>Geographic distance</td>
<td>1</td>
<td>1.1</td>
<td>0.95</td>
<td>0.327</td>
</tr>
<tr>
<td>Habitat x Color morph</td>
<td>3</td>
<td>3.2</td>
<td>1.38</td>
<td>0.001</td>
</tr>
<tr>
<td>Habitat x Geographic distance</td>
<td>3</td>
<td>1.3</td>
<td>1.10</td>
<td>0.218</td>
</tr>
<tr>
<td>Color morph x Geographic distance</td>
<td>1</td>
<td>1.6</td>
<td>1.35</td>
<td>0.019</td>
</tr>
<tr>
<td>Residual</td>
<td>85</td>
<td>85.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 4.1. Bayesian population assignment tests of 256 *Paracirrhites arcatus* individuals. Clustering results using color morph as *a priori* grouping, based on (A) all 30 microsatellite loci, and (B) seven loci selected by BELS (backward elimination locus selection) to improve the power of discrimination among color morphs. Each vertical line represents one individual and its assignment likelihood to the two clusters. Black vertical lines represent the limit between predefined groups based on color morph: melanistic (MEL; n = 107), pink-white-stripe (PWS; n = 104), and intermediate morphotypes (n = 45; 13 no-stripe, INS; 16 faint-stripe, IFS; and 24 white-stripe, IWS). (C-D) Clustering results using geographic location as *a priori* grouping, based on (C) all 30 microsatellites, and (D) seven loci selected by BELS to discriminate among sampling location: Kohala (North; n = 128) and Kona (South; n = 128). In each barplot, the cluster membership scores represent the average of five runs for each analysis.
Figure 4.2. Plot of Fdist outlier test showing pairwise $F_{ST}$ estimates of 30 polymorphic microsatellite loci (grey circles) and the Mc1r exon (black diamond) between 107 MEL and 104 PWS color morphs. Per-locus $F_{ST}$ is plotted against expected heterozygosity ($H_E$) to identify potential loci subject to selection. The dashed line corresponds to the median $F_{ST}$ value, and the solid lines indicate upper and lower limits of the 99.9% confidence interval estimated for the neutral model. Plot space above the upper confidence limit is shaded red, and below is shaded yellow. A single SNP in the Mc1r gene (Mc1r-023) was identified as a significant outlier ($P < 0.0001$) as the observed $F_{ST} = 0.175$ was higher than any value in all 50,000 simulations.
CHAPTER V
SUMMARY & SYNTHESIS
Coral reef fishes represent the most diverse assemblage of vertebrates on the planet, yet our understanding of the mechanisms driving this diversity remains limited. There is growing evidence and recognition that ecological adaptation shaped by natural selection may be a major driver of diversification on coral reefs. Nevertheless, speciation-with-gene-flow continues to be one of the most controversial concepts in evolutionary biology and the scarcity of empirical examples continues to hinder our ability to assess the role ecology plays in generating marine biodiversity.

The central goal of this dissertation was to contribute to the understanding of large-scale patterns of marine biodiversity by investigating how ecological specialization can lead to population divergence even without physical barriers to gene flow. Using *Paracirrhites arcatus* as a model, my approach was to test the hypothesis that ecologically-based disruptive natural selection on color pattern in combination with color-based assortative mating can drive the evolution of reproduction isolation between sympatric morphotypes. Throughout this dissertation I provide evidence suggesting that disruptive natural selection on color pattern in combination with color-based assortative mating is driving divergence in sympatric populations of the arceye hawkfish.

In Chapter II, I provide evidence for divergence in local realized niches among *P. arcatus* color morphs. The strong correlation between phenotype and environment suggests that each color type has a fitness advantage in their respective habitats, the function of which is hypothesized to be related to crypsis. Thus, I suggest that color morphs are experiencing microspatial habitat isolation that is most likely a byproduct of disruptive natural selection favoring alternative color patterns that can better exploit opposing ends of a steep ecological gradient.

In Chapter III, I used field surveys and preference experiments to demonstrate that color morphs pair assortatively. The relatively low proportion of pairing between color morphs (<10%) observed in zones of overlap provides strong evidence for premating isolation among sympatric color morphs, as like color morphs are nine times more likely to pair with their own color morphs. Therefore, color appears to act as a species recognition signal, which has led to the evolution of assortative mating (like-with-like) and can subsequently amplify reproductive isolation.
In Chapter IV, I provide genetic evidence that these premating barriers have resulted in at least partial reproductive isolation between ecologically-differentiated sympatric color morphs. The relationship between neutral genetic differentiation and both environmental and phenotypic divergence indicate that ecological barriers restrict gene flow between phenotypes inhabiting contrasting environments. This pattern of reduced gene flow between ecologically relevant phenotypes provides compelling evidence for ecological barriers driving restricted gene flow on very fine spatial scales. The genetic pattern that emerges from these data suggests that ecologically-based divergent selection has created a general barrier to gene flow that is responsible for driving at least partial reproductive isolation among color morphs.

Taken together, these results suggest that reproductive isolation between morphs is arising as a by-product of divergent selection on ecological differences and enhanced by the isolating effects of assortative mating. The strong association between genetic variants, color phenotypes, and ecological gradients all suggests that the species has evolved genetically-based alternative phenotypes that are adapting to spatially variable environments, despite living on the same reefs. Results from ecological surveys and behavioral experiments indicate that habitat choice and assortative mating are synergistically forming a potent premating barrier that is maintaining genetic and phenotypic divergence even in the face of gene flow.

The major ingredients of ecological speciation have been identified in *P. arcatus*, specifically a source of natural divergent selection (crypsis), a mechanism of reproductive isolation (habitat preference and color-based assortative mating) and a link between the two (pleiotropic effect of color). Scenarios where the ecological trait under selection is linked directly with mate choice are considered the most plausible paths to speciation-with-gene-flow. I conclude that color alone (as far as we know) is driving incipient divergence in this species, despite high gene flow and no geographic isolation.

Speciation is a continuum, and based on these data, hawkfish color morphs are in the initial phase of evolutionary divergence. Regardless of its fate, the genetic differentiation between ecologically-specialized phenotypes provides empirical support that partial reproductive isolation can evolve (or at least persist) in the face of continuous gene flow.

Taken more broadly, these results add support to the hypothesis that marine speciation can occur without geographic isolation by outlining how adaptation to ecological gradients can lead to the establishment of reproductive isolation, even in the face of gene flow. This
dissertation makes a strong contribution to the marine speciation debate by showing that the most extreme and most argued form of speciation (divergence-with-continuous-gene-flow) is possible in a wide-ranging marine fish. I argue that the evidence outlined here suggests that this type of divergence is not just possible, but it may be a potentially significant mode of speciation in species-rich coral reefs. I anticipate that the addition of this study to the growing body of evidence supporting the role natural selection plays in initiating speciation should help bring us one step closer to understanding the processes driving high biodiversity in tropical seas.
Figure S2.1. Histograms of the length (TL) distribution of the three color morphotypes of *Paracirrhites arcatus* in this study.
Figure S2.2. Scatterplot illustrating the linear relationship between *Paracirrhites arcatus* total abundance (log) and percent *Poc. meandrina* cover (logit). Shown with a linear smoother and 95% CI (span = 1).

Figure S2.3. Reef profile illustrating spatial segregation of transects dominated by each color morph over a depth/distance to shore gradient. Transects are colored according to which morph was numerically dominant (relative abundance > 0.6), and mixed transects indicate no morph was dominant.
Figure S2.4. Whisker plots showing means with 95% CI of eight environmental variables measured across groups of transects categorized into one of five dominance groupings defined by relative abundance (RA) of the two discrete color morphs. Transects with MEL only (RAMEL = 1) are colored black, MEL-dominated (0.6 > RAPWS < 1) in brown, mixed transects (0.4 > RAboth < 0.6) in green, PWS-dominated (0.6 > RAPWS < 1) in pink and PWS only (RAPWS = 1) in red.
Table S2.1. Summary of environmental variables recorded in habitat surveys with the minimum and maximum values recorded over all sites.

<table>
<thead>
<tr>
<th>Environmental variables</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean bottom depth (m)</td>
<td>0.3</td>
<td>16.8</td>
</tr>
<tr>
<td>Distance from shore (m)</td>
<td>8.0</td>
<td>115.0</td>
</tr>
<tr>
<td>Mean vertical relief (m)</td>
<td>0.4</td>
<td>7.4</td>
</tr>
<tr>
<td>Slope (°)</td>
<td>4.8</td>
<td>87.3</td>
</tr>
<tr>
<td>Wave action (low, mod, high)</td>
<td>low</td>
<td>high</td>
</tr>
<tr>
<td>Total live coral cover (%)</td>
<td>12.2</td>
<td>75.5</td>
</tr>
<tr>
<td><em>Pocillopora meandrina</em> cover (%)</td>
<td>0.2</td>
<td>32.2</td>
</tr>
<tr>
<td><em>Pocillopora meandrina</em> density (#/m²)</td>
<td>0.1</td>
<td>2.4</td>
</tr>
<tr>
<td><em>Porites lobata</em> cover (%)</td>
<td>1.9</td>
<td>68.9</td>
</tr>
<tr>
<td><em>Porites compressa</em> cover (%)</td>
<td>0</td>
<td>37.0</td>
</tr>
<tr>
<td>Rock cover (%)</td>
<td>0</td>
<td>75.0</td>
</tr>
<tr>
<td>Rugosity</td>
<td>1.2</td>
<td>3.8</td>
</tr>
</tbody>
</table>

Table S2.2. Correlation matrix of environmental variables measured during transect surveys. Significant correlations are bolded (Bonferroni adjusted $\alpha = 0.0008$).

<table>
<thead>
<tr>
<th></th>
<th>Depth</th>
<th>Distance to shore</th>
<th>Slope</th>
<th>Rugosity</th>
<th>Total coral cover</th>
<th><em>Poc. meandrina</em> cover</th>
<th><em>Por. lobata</em> cover</th>
<th><em>Por. compressa</em> cover</th>
<th>Rock cover</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth</td>
<td>0.83</td>
<td>-0.49</td>
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<td>-0.47</td>
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<td>0.47</td>
<td>-0.44</td>
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</tr>
<tr>
<td>Slope</td>
<td>-0.49</td>
<td>-0.47</td>
<td>0.10</td>
<td>-0.41</td>
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<tr>
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<td>0.59</td>
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<td>-0.45</td>
<td>-0.57</td>
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<td>Total coral cover</td>
<td>0.43</td>
<td>0.47</td>
<td>-0.41</td>
<td>0.25</td>
<td>-0.38</td>
<td>0.87</td>
<td>0.41</td>
<td>-0.72</td>
<td></td>
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<tr>
<td><em>Poc. meandrina</em> cover</td>
<td>-0.30</td>
<td>-0.44</td>
<td>0.33</td>
<td>-0.45</td>
<td>-0.38</td>
<td>-0.49</td>
<td>-0.48</td>
<td>0.41</td>
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<tr>
<td><em>Por. lobata</em> cover</td>
<td>0.23</td>
<td>0.26</td>
<td>-0.36</td>
<td>0.01</td>
<td>0.87</td>
<td>-0.49</td>
<td>0.08</td>
<td>-0.52</td>
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<tr>
<td><em>Por. compressa</em> cover</td>
<td>0.65</td>
<td>0.74</td>
<td>-0.32</td>
<td>0.71</td>
<td>0.41</td>
<td>-0.48</td>
<td>0.08</td>
<td>-0.60</td>
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<tr>
<td>Rock cover</td>
<td>-0.56</td>
<td>-0.60</td>
<td>0.12</td>
<td>-0.57</td>
<td>-0.72</td>
<td>0.41</td>
<td>-0.52</td>
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Table S2.3. Summary of generalized linear model testing seven environmental variables as fixed effects of total abundance per transect with gamma distribution and a log-link function. Total coral cover was logit-transformed.

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<tr>
<th>Coefficients</th>
<th>Estimate</th>
<th>SE</th>
<th>t</th>
<th>P</th>
<th>R²</th>
</tr>
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<tbody>
<tr>
<td>Pocillopora meandrina % cover</td>
<td>0.582</td>
<td>0.096</td>
<td>6.04</td>
<td>&lt; 0.00001</td>
<td>0.59</td>
</tr>
<tr>
<td>Depth</td>
<td>0.035</td>
<td>0.008</td>
<td>4.57</td>
<td>&lt; 0.00001</td>
<td>0.02</td>
</tr>
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<td>Porites compressa % cover</td>
<td>-0.023</td>
<td>0.015</td>
<td>-1.55</td>
<td>0.12</td>
<td>-0.29</td>
</tr>
<tr>
<td>Slope</td>
<td>0.003</td>
<td>0.003</td>
<td>0.86</td>
<td>0.39</td>
<td>0.06</td>
</tr>
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<td>Total coral % cover</td>
<td>-0.152</td>
<td>0.209</td>
<td>-0.73</td>
<td>0.47</td>
<td>-0.13</td>
</tr>
<tr>
<td>Porites lobata % cover</td>
<td>0.003</td>
<td>0.010</td>
<td>0.29</td>
<td>0.77</td>
<td>-0.11</td>
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<td>Rock % cover</td>
<td>0.000</td>
<td>0.006</td>
<td>-0.04</td>
<td>0.97</td>
<td>0.11</td>
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Table S2.4. Summary of abiotic and biotic benthic community composition of transects categorized by dominant morph (dominance defined by relative abundance > 0.5), and transects with no numerically dominant morph were labeled mixed (n=8).

<table>
<thead>
<tr>
<th>Variable</th>
<th>MEL (n=21)</th>
<th>INT (n=8)</th>
<th>PWS (n=27)</th>
<th>Mixed (n=8)</th>
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<td><strong>Reef Profile</strong></td>
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<tr>
<td>Depth (m)</td>
<td>2.4</td>
<td>4.3</td>
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<tr>
<td>Distance to shore (m)</td>
<td>17.4</td>
<td>29.2</td>
<td>60.2</td>
<td>22.4</td>
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<tr>
<td>Slope (°)</td>
<td>48.9</td>
<td>38.1</td>
<td>21.0</td>
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<tr>
<td><strong>Benthic Cover</strong></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Rock</td>
<td>37.3</td>
<td>39.9</td>
<td>17.3</td>
<td>33.1</td>
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<td>40.3</td>
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<td>19.1</td>
<td>37.1</td>
<td>24.3</td>
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<td>0.1</td>
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<tr>
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<td>1.4</td>
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<tr>
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<td>8.8</td>
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</tr>
<tr>
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Table S4.1. Overall genetic diversity for *Paracirrhites arcatus* color morphs sampled along the West coast of Hawai‘i Island (Total N = 211; melanistic morph, n=107; pink-white-stripe morph, n=104). Shown are the number of fish genotyped (N), number of alleles (N_A), number of effective alleles (N_E), number of private alleles (A_P), observed heterozygosities (H_O), expected heterozygosities (H_E), F_IS and P-values from a Hardy–Weinberg test for heterozygote deficits across all individuals and estimate frequencies of null alleles (EFNA). P-values with an asterisk indicate those that remained significant after sequential Bonferroni adjustments.

<table>
<thead>
<tr>
<th>Locus</th>
<th>N</th>
<th>N_A</th>
<th>N_E</th>
<th>H_O</th>
<th>H_E</th>
<th>F_IS</th>
<th>P</th>
<th>EFNA</th>
</tr>
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<td>0.05</td>
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<td>0.83</td>
<td>0.032</td>
<td>0.047</td>
<td>0.01</td>
</tr>
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<td>6</td>
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<td>0.82</td>
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<td>0.005</td>
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<td>0.90</td>
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Table S4.2. Overall genetic diversity broken down by color morph: M (MEL) and P (PWS). Shown are the # of fish genotyped (N), number of alleles ($N_a$), number of effective alleles ($N_e$), number of private alleles ($A_p$), observed ($H_o$) and expected heterozygosities ($H_e$), $F_{IS}$ and P-values from a Hardy–Weinberg equilibrium test. An asterisk = significant after Bonferroni adjustments.

<table>
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<tr>
<th>Locus</th>
<th>N</th>
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<th>$N_e$</th>
<th>$A_p$</th>
<th>$H_o$</th>
<th>$H_e$</th>
<th>$F_{IS}$</th>
<th>P-value (HWE)</th>
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Mean 103.3 102.1 14.8 15.2 8.3 8.3 1.1 1.5 0.74 0.74 0.83 0.83 0.11 0.11 0.203 0.237
Table S4.3. Pairs of loci that showed significant ($P < 0.05$) linkage disequilibrium across all loci tested on 212 individuals (107 MEL; 104 PWS). $P$-values with an asterisk indicate those comparisons that remained significant after sequential Bonferroni adjustments.

<table>
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<th>Loci pair</th>
<th>$X^2$</th>
<th>df</th>
<th>$P$</th>
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<tr>
<td>Paa61 &amp; Paa23</td>
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<td>224</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Paa03 &amp; Paa77</td>
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<td>242</td>
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</tr>
<tr>
<td>Paa70 &amp; Paa64</td>
<td>270.17</td>
<td>280</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Paa67 &amp; Paa58</td>
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<tr>
<td>Paa15 &amp; Paa68</td>
<td>62.68</td>
<td>44</td>
<td>0.001</td>
</tr>
<tr>
<td>Paa91 &amp; Paa08</td>
<td>157.26</td>
<td>150</td>
<td>0.004</td>
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<tr>
<td>Paa70 &amp; Paa60</td>
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<td>180</td>
<td>0.005</td>
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<td>150</td>
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Table S4.4. Genetic differentiation between melanistic and pink-white-stripe color morphs of *P. arcatus*. Genic (*P*<sub>GENIC</sub>) and genotypic (*P*<sub>GENOTYPIC</sub>) differentiation. *P*-values were obtained using the *G* log-likelihood statistic (Goudet et al. 1996) in GENEPOP, and an asterisk indicates those loci with significant *P*-values (< 0.05) that are below the FDR threshold of 0.05.

<table>
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<th><em>F</em>&lt;sub&gt;ST&lt;/sub&gt;</th>
<th><em>F</em>&lt;sub&gt;ST'&lt;/sub&gt;</th>
<th><em>R</em>&lt;sub&gt;ST&lt;/sub&gt;</th>
<th><em>G</em>&lt;sub&gt;ST&lt;/sub&gt;</th>
<th><em>G</em>&lt;sub&gt;ST'&lt;/sub&gt;</th>
<th>DEST</th>
<th><em>P</em>&lt;sub&gt;GENIC&lt;/sub&gt;</th>
<th><em>P</em>&lt;sub&gt;GENOTYPIC&lt;/sub&gt;</th>
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<td>0.015*</td>
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Table S4.5. Locus selection ranking using BELS to reduce loci set for two groupings: color morph (MEL v. PWS) and sampling location (north versus south), with number of alleles ($N_A$), heterozygosity ($H_E$), as well as $F'_{ST}$ and ranking of loci based on power in assignment tests.

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<th>$F'_{ST}$</th>
<th>BELS rank</th>
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Table S4.6. Summary of analysis of molecular variance (AMOVA) of Mc1r gene.

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