

THE EVOLUTION OF CLEANING MUTUALISM AND PREDATOR COOPERATION IN
A RADIATION OF CARIBBEAN FISHES

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A RADIATION OF CARIBBEAN FISHES

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SUMMARY

The dissertation work presented here tests the main hypothesis that color signals in a specialized mutualism have evolved as a result of natural selection. A species rich lineage of goby fishes distributed throughout the Caribbean and tropical western North Atlantic provide a model system in which to test for evidence of trait adaptation. In the group that has evolved obligate cleaning mutualism as a feeding strategy, traits that may be adaptive to cleaning appear to coincide with increased reliance on this form of survival. In order to understand the potential fitness gains derived from the changes in the signal trait of interest over evolutionary time, we tested the functional capability and functional ecology of color traits in the shared habitats of this diverse group of fishes. We used mathematical modeling of fish visual systems, field experiments with phenotype replicates, feeding assays and literature surveys to understand the changing function of the signal trait in closely related species. The results of the comparative analysis of the focal species revealed a dual role of signals in the diffuse interactions on which the obligate mutualists depend for survival. Advertising color stripes have evolved over time to become increasingly visible to a broad range of species and to communicate non-prey status to approaching reef fishes. Identifying key traits that provide an adaptive advantage to obligate mutualists in diffuse interactions may be one important step toward explaining the underlying genetic and functional architecture of biodiversity. We discuss other natural systems where similar interactions among key traits may facilitate the evolution of mutualisms in species rich groups and suggest a theoretical framework that could be used to further explore the evolutionary dynamics.

CHAPTER 1: INTRODUCTION

Communication signals are highly diverse in nature. The interpretation of the traits that signal the exchange of information is dependent on both the properties of the trait and on the sensory equipment of the receiver. For this reason, the selective pressures that shape communication traits are different from those that influence the evolution of other traits in organisms. Traits that could generate, amplify or differentiate signals in one environment may be unrecognizable, undetectable or undifferentiable in a new habitat or to a new receiver type. Signal traits are therefore influenced by selection on both the focal individual, the one attempting to manipulate the behavior of another, and on the receiver, the one whose behavior is being manipulated. Color signals utilize light to communicate information; reflectance patterns are subject to change depending on light conditions and on variation in the light capturing organs of onlookers. By incorporating light reflectance data collected from the focal species and inputting this information into simulated eyes of onlookers, we can estimate the potential functional differences among signals to form testable hypotheses about their fitness consequences.

In this study, we have focused on signal traits involved in a mutualism. Mutualisms are reciprocally beneficial interactions between unrelated species; the steps leading to their evolution as a result of natural selection are the focus of this research. Despite advances in understanding the conditions that may promote the evolution of mutualisms, that seem to require individuals to oppose their own fitness maximum, the

steps that have led to them over evolutionary time are not well understood. We have focused on the evolution of a cleaning mutualism as a model system. In a species rich radiation of Caribbean fishes that remove parasites from visiting 'clients', the advertising traits that have evolved are convergent with those seen in the Indo-Pacific cleaner wrasses, which are only very distantly related. Because similar ecological conditions can cause similar fitness consequences as a result of natural selection, instances of convergent trait evolution are biological red flags for natural selection on adaptive phenotypes (the physical analogs of the underlying genotypes). Because the Indo-Pacific and Caribbean focal participants share a highly specialized feeding behavior and a specific coloration pattern thought to communicate their mutualist status, we focused on the signal and its function as a potentially adaptive trait.

Although many mutualisms are thought to have arisen as a result of increasing reliance of two species on one another in a coevolutionary coupling of fates, many mutualisms are diffuse, with networks of species interacting with variable strength and dependence. In diffuse networks such as the cleaner and client mutualism, it is more difficult to form predictions about the role of one particular species in shaping the traits of any other, as the ecological context of the interaction may vary over space and time. In Chapter 1, we test the potential signal strength of the range of phenotypes found in the lineage of Caribbean cleaners through the virtual eyes of many different signal receivers using color vision models. We tested whether the trait could be adapted to signaling diverse partners by looking for evidence of increased contrast against typical backgrounds, to many different types of color sensitivities. The most specialized advertising color, blue, was most contrasting to a broad range of potential fish visual systems, most notably to the group containing all of the predator species tested. Thus, in Chapter 2, we focused on determining whether the function of the convergent color signal was duofold, to deter attack and to attract mutualists. We tested the ecological

interactions of phenotypically diverse fishes to measure indicators of the fitness consequences of having these specialized traits. Interactions leading to recognizable behaviors of intent suggested that cleaners may have defenses that predispose them to profit from having signals that communicate to risky individuals or species. We tested for evidence of shifts over evolutionary time to more risky interactions and looked for evidence in other mutualism systems.

In total, the results of this dissertation work begin to implicate signals that communicate dual roles in the evolution of diverse mutualisms. Since mutualisms rely on the link between the genetically heritable traits in one organism and the elicited behavior in an unrelated species, it may follow that communication signals are integral to the architecture of diverse mutualism networks and may be key traits in the evolution of many mutualisms in nature.

CHAPTER 2: COLOR STRIPE COMPARATIVE ANALYSIS

2.1 Introduction

In Caribbean *Elacatinus* (Jordan, 1904) (previously *Gobiosoma*) gobies, evolutionary shifts from non-mutualists to obligate generalist mutualists coincide with shifts in color patterns associated with advertising this derived status. This lineage of species provides a model framework in which to test signal evolution in a mutualism. *Elacatinus* (*E.*) gobies (*sensu lato-s.l.*) are small (approximately 2-4 cm length) and habitat restricted, with limited mobility (Rüber et al., 2003; Colin, 1975). The striped pattern of the subgenus (*sensu stricto-s.s.*) *E.* is notably different from the most closely related sister species' body patterns, which are more disruptive or banded (Rüber et al., 2003). These distinctive stripes are potentially conspicuous to visual detection, which makes it of particular interest that a lineage within the subgenus has evolved highly specialized parasite cleaning behavior.

Molecular phylogenetic analysis shows that *E.* (*s.s.*) gobies diverged into two behaviorally distinct, obligate sponge-dwelling and cleaning, clades (Taylor and Hellberg, 2005; Rüber et al., 2003). Cleaners derive much or all of their nourishment by cleaning parasites from the bodies of visiting "clients." Limited larval dispersal may contribute to the rapid speciation within the clade (Taylor and Hellberg, 2003), and may be an important mechanism by which selection on fitness related traits varies and evolves in the marine environment. Sponge dwellers often associate with chemically-defended sponge species such as *Xestospongia spp.* (Pawlik et al., 1995) and *Agelas spp.* (Assmann et al., 2000) and tend to retreat into sponge cavities if disturbed, while

cleaners tend to be found on corals or on the outside of sponges, where they are both more exposed and more visible to passersby and potential predators (Lettieri, pers. obs.). All species within the clade have distinctive stripe patterns, which set them apart from other *E. (s.l.)* species.

Basal species within the cleaner lineage have a lateral yellow stripe along both sides of the body, while more recently evolved species have derived green (broad spectrum reflectance that appears iridescent white) or blue lateral stripes (Taylor and Hellberg, 2005). In addition, two species of cleaners exhibit color stripe polymorphism, and the order of evolution within these stripes appears to follow this same trend (Taylor and Hellberg, 2005). Whereas bold black dorsal and sublateral stripes with a yellow or green lateral stripe can be found in both cleaning and non-cleaning gobies, the blue lateral stripe is only observed in cleaners and is significantly associated with a morphological change in mouth position that may be adaptive to cleaning (Taylor and Hellberg, 2005). It has been suggested that the black striped pattern is a conspicuous signal evolved to advertise cleaning behavior (Côté, 2000; Arnal et al., 2006; Stummer, 2004) and is a clear example of profile enhancing high contrast edge (Stevens, 2007). Paired with a blue, green, or yellow stripe, the pattern may increase signal contrast against typical backgrounds (Marshall, 2000).

Interactions where fitness may be tied to visual signaling have the potential to exert selective pressure on color and pattern phenotypes (Stevens, 2007). Traits thought to have fitness consequences tied to contrast or chromatic distinctiveness may appear differently to many potential onlookers in the same microenvironment. Recent studies have used visual models to estimate color conspicuousness of fruits or prey to potential observers (Håstad et al., 2005; Schaefer et al., 2007; Siddiqi et al., 2004; Stuart-Fox et al., 2003; Darst et al., 2006). Modeling of goby color stripes across a range of microhabitats, covering the potential diversity of client visual systems, may provide

insight into the nature of signal evolution in a diffuse mutualism (where many potential client species interact with one focal mutualist.)

Mutualisms can be defined as interspecific interactions where both participants gain fitness benefits (Boucher et al., 1982; Cushman and Beattie 1991). Evolution of specialist mutualisms often leads to traits that are coevolved communication signals from one partner class to the senses of a receiver class. In an asymmetric mutualism, partner groups may exert differential selective pressure on traits of the shared mutualist; this could in turn lead to diffuse selection and evolution (Strauss et al., 2005). The ways in which signaling traits mediate interactions between generalist mutualists and their potential partners are not well understood and may hold keys to understanding how asymmetric mutualisms promote diversity (Strauss et al., 2005; Bascombe 2006). For obligate mutualists, especially those spatially restricted to habitat or by mobility, traits that signal to potential partner organisms may become strong arbiters of fitness. Evolutionary adaptations that increase the efficiency of or reduce the cost of signaling to partners are likely to be favored and may exploit pre-existing biases of the intended receivers (Ryan and Rand 1993). They may also increase visibility to potential eavesdroppers, thus affecting the potential risk of engaging in mutualism in an interaction network.

Our main hypothesis is that blue stripes of *Elacatinus* gobies are more conspicuous than other stripe colors to a larger pool of signal receivers. Although many species visit cleaning stations, more abundant Caribbean species such as *Chromis spp.*, *Clepticus spp.*, and *Abudefduf spp.* have been suggested to contribute heavily to selection for traits that are integral to signaling cleaning behavior (Floeter et al., 2007); a general positive correlation between increased client abundance and increased cleaning interactions suggests that the signals indicating cleaner status are effective across broad taxonomic, trophic, and social behavior groups (Floeter et al., 2007). First, we measured

the colors of *E. oceanops* (blue), *E. randalli* (yellow) and their F₁ progeny (green) using spectrophotometry, as these three color classes represent the three phenotypes found across the species in the clade. The hybrid progeny are comparable in spectral reflectance to wild phenotypes often described as “white” striped (e.g. *E. evelynae*, *E. illecebrosus*, and *E. prochilos*) (Lettieri unpublished data), although we will call them green as this most accurately describes the reflectance of the stripes. Next, we used color opponent models of reef fish visual sensitivity (Vorobyev and Osorio, 1998; Vorobyev et al., 2001; Kelber et al., 2003) to assess the likely contrast of these stripe colors against sponge and coral microhabitats. Because cleaners interact with a broad array of species and different species make up the most abundant clients across geographic regions, we modeled chromatic contrast through the eyes of many client visual systems (Losey et al., 2003). This strategy allowed us to evaluate the conspicuousness of color stripes to a variety of model partner organisms. Color stripe may be under selective pressure to be more generally conspicuous to potential mutualist partners, given that evolution has resulted in species utilizing more exposed habitat, engaging in more specialized feeding behavior, and increasing interactions with potential predators (Rüber et al., 2003; Taylor and Hellberg, 2005, White et al., 2007).

2.2 Methods

2.2.1 The model approach

To compare the visibility of goby advertising stripes, we used color vision models (Vorobyev and Osorio 1998) that incorporate three main components: namely, 1) spectral reflectance of the focal object or objects, 2) ambient environmental light, and 3) color vision capabilities of the onlooker.

2.2.1.1 Component 1a: Spectral reflectance of fish color stripes

Color stripe measurements were taken from eight individuals of each representative phenotype: blue-striped *E. oceanops* (Jordan, 1904), yellow-striped *E. randalli* (Böhlke and Robins, 1968), and an F₁ intercross of *E. oceanops* x *E. randalli*. We measured spectral reflectance using an Ocean Optics S2000 spectrophotometer and OOIBASE32 software (Ocean Optics, Inc., Dunedin, FL). The bare end of a 200mm fiber optic UV/VIS cable was placed close to the fish so that it was sampled from the midlateral stripe color region alone and at a 45° angle to prevent specular reflection. Illumination for both the sample and the reference (98% diffuse reflectance standard, Ocean Optics, Dunedin, FL) was provided by a combination of light from a PX-2 xenon illuminator (Ocean Optics, Dunedin, FL) and a Sunray 1000 video light (Light & Motion, Monterey, CA). Fish were immediately returned to the water after measurement. Measurements were boxcarred over 5nm wavelengths and averaged over 10 readings made at 15 millisecond intervals. Two replicate measurements were averaged per individual for further calculations. Quantitative differences between the three stripe phenotypes were determined, first objectively, statistically, and predictively with discriminant function analysis (DFA) and second, subjectively and in an ecologically relevant context for a range of potential fish visual systems. Stripe color reflectances were compared through the eyes of potential fish onlookers in two complementary ways described below.

2.2.1.2 Component 1b: Coral and sponge microhabitat reflectance

In addition to stripe reflectances, we evaluated microhabitat colors either commonly occupied by or commonly available to the focal goby species. Coral and sponge reflectance readings were made in the Florida Keys using a diver-operated DiveSpec spectrophotometer (a self-contained reflectance and fluorescence measurement device using red, blue, and white LEDs to obtain full-spectrum reflectance

calculations without ambient light, NightSea, Andover, Massachusetts). A Spectralon 99% reflectance standard was first used to collect a reference reading, and reflectance was computed as the ratio of the sample to the reference measurement. Reflectances were then smoothed using a one-pass Savitzky-Golay 21-point algorithm and trimmed to show wavelength readings at 5 nm intervals from 400 nm to 750 nm. All coral species were identified to genus or species while sponges were not identified by species. However, Henkel and Pawlik (2005) found that of Florida Keys vase sponge species, *Callyspongia vaginalis* was the most abundant (43%) and this is likely the species used for vase sponge color reflectance in further calculations. Following extrapolation techniques used previously for terrestrial habitats (Endler 1993), we extended the slope of reflectance in the 400-450 nm region down to 350 nm. Reflectance in this region is somewhat variable between coral species and even among colonies of the same species (Hochberg et al., 2004), but on average is fairly achromatic exhibiting a relatively flat reflectance profile (Holden and LeDrew, 1998; Hochberg and Atkinson, 2000; Mazel and Fuchs, 2003). Little spectral reflectance data is available for sponges, but our data show achromatic reflectance if any toward the UV end of the spectrum. Excluding UV sensitive visual systems from our analyses, to avoid extrapolated data, did not change the interpretation of our results.

2.2.1.3 Component 2: Environmental irradiance and sidewelling water background

In order to compare goby stripe and microhabitat colors under ecologically relevant light conditions, we incorporated ambient irradiance collected at depth into the visual models. Irradiance for daylight at open water near reef habitat was collected at several locations using a 1mm diameter fiber optic probe fitted with a cosine collector attached to an Ocean Optics USB2000 spectrophotometer and recorded with a handheld computer with modified Palm-Spec software (Ocean Optics, Dunedin, Florida), encased in an underwater housing (Wills Camera Housings, Victoria, Australia). We

used a representative 6 m depth irradiance (average of 15 technical replicate measurements) collected in October 2006 at West Palm Beach, Florida, USA, mid-day, full sun, in our model as an ecologically relevant depth (all three stripe phenotypes have been documented 1m-40m) (Colin 1975). Sidewelling data toward and away from the sun were also collected at this time. Sidewelling quantum catch collected from the direction toward the sun was averaged with irradiance to represent the adaptive light field and sidewelling data collected facing away from the sun was used as one of the potential background colors.

2.2.1.4 Component 3: Fish visual capabilities

Cone sensitivities of reef fishes, measured using microspectrophotometry, were tested from 25 species (Table 1) representing fourteen different families (Losey et al. 2003). Double cones were assumed to be neurally linked (Marshall and Vorobyev, 2003) and therefore to operate as a single (averaged) chromatic receptor channel. Representative cone sensitivities corrected for ocular media transmission (Siebeck and Marshall, 2001) were chosen to cover a range of ecological and sensitivity classes typical of tropical fish cone sensitivities (Marshall et al., 2006).

2.2.2 Color vision models

Receptor-noise-limited color opponent models of animal color vision (Vorobyev and Osorio, 1998; Vorobyev et al., 2001; Kelber et al., 2003) were used to map *E.* color stripes and coral and sponge microhabitat colors in a scaled coordinate space (Hempel de Ibarra et al., 2001; Marshall and Vorobyev, 2003; Siddiqi et al., 2004) or “chromaticity diagrams.” The exact proportion and number of individual cone types for the fish client visual systems is not known, however if one assumes a 1:1 (dichromat) or a 1:1:1 (trichromat) ratio, the potential for chromatic contrast and color discriminability are maximized overall (relative to e.g. 1:2 or 1:2:2). We present results assuming cone ratios of 1:2 and 1:2:2 for reef fishes (Marshall, unpublished data), and found similar

results for ratios of 0.52: 0.82:1, experimentally shown with “unsaturated blue” light in goldfish (Dörr and Neumeyer 2000). For the purposes of this experiment, we assume that spectral attenuation is negligible, to maximize comparisons among client visual systems, which likely makes our results more conservative as blue should transmit furthest in water (Barry and Hawryshyn 1999). Quantum catches used in the analyses were von Kries transformed by each cone’s von Kries coefficient (Marshall and Vorobyev, 2003) and adapt sensitivities proportional to the illuminant contributing to color constancy which is likely to be used by many reef fish visual systems. We calculated the quantum catch of each cone as the product, integrated from 350-750 nm wavelength, of measured spectral reflectance, sensitivity spectrum, and illuminant.

We plotted stripe colors and microhabitat backgrounds into an onlooker color map (as illustrated in Kelber et al., 2003, Figure 2.5D). This “chromaticity diagram” technique allowed us to compare stripe colors of gobies and to determine how different these and microhabitat colors would appear to representative onlooking client fishes. Distances between color points in the diagram represent chromatic stimulus with respect to individual cone classes, nominally assigned absolute values in the positive (LWS-longer wavelength sensitive cones) or the negative direction (SWS-shorter wavelength sensitive cones). Each unit in the scale free axis denotes a unit of increasing discriminability by the observer at decreased effort. In trichromats, axes are in two dimensions, each corresponding to a color opponent system between two cones with overlapping sensitivities. Colors were plotted following formulas described elsewhere (Vorobyev and Osorio, 1998; Hempel de Ibarra et al., 2001; Kelber et al., 2003). For double cones, we assumed that cone sensitivities were averaged; the opponent system contrasts the single cone and the averaged double cones to discriminate between colors. For all other cases, the axes of the diagrams are opponent contrasts between individual cone class sensitivities. Chromaticity values that plot at absolute distances ΔS

(Vorobyev and Osorio 1998), of less than one scaled unit from one another are assumed to fall below a threshold of discrimination along that axis (i.e., for the cone LWS and MWS opponent discrimination along axis X1). This assumption is a best estimate for fish and could be incorrect for fish color vision, but given that human and fish color constancy responses are highly similar (Neumeyer et al., 2002) and that these estimates have been used as a discrimination cutoff in other visual systems, e.g. primate (Osorio and Vorobyev, 1996) and bird (Schaefer et al., 2007), we argue that they are reliable in this comparative context. In the chromaticity diagram, the origin of the map corresponds to all achromatic reflectances, including white, shades of achromatic gray, and black. As chromatic distances between points increase, colors are distinct and differentiable under a wider range of conditions and at decreased effort to the observer.

Models of client fish visual systems (potential dichromat and trichromat) were also used to calculate the magnitude of color differences (ΔS) between each *E.* stripe color and representative background microhabitat colors. To calculate the dichromatic and trichromatic ΔS between color stripe and microhabitat background (based on noise limited color-opponent cone sensitivities), we employed formulas 3 and 4 from Vorobyev and Osorio (1998). This calculated ΔS gives a sense of how differentiable the stripe is from a specific background color rather than from an arbitrary achromatic value.

2.3 Results

2.3.1 Color stripes are distinctly and categorically different

Figure 2.1 shows the averaged color spectrum of stripes. Blue stripes reflect in a peak waveform around 445 nm. Yellow stripes have a characteristic step-shaped reflectance function, with fifty percent of maximum reflectance reached at around 525 nm and leveling to a flat line at around 625 nm. The hybrid stripe color was uniformly green (Marshall, 2000) with a wide but apparent peak at 500-510nm (Figure 2.1). We

refer to hybrid color as green, and it is comparable in phenotype to wild “white stripe” *E.* species.

The color reflectance functions for blue, yellow, and green are distinctly and categorically different. With *a priori* assignments of *E. randalli*, *E. oceanops*, or hybrid color class, stripes reflectances could be clustered with 100% cumulative accuracy (Figure 2.2) into respective categories, using discriminant function analysis (DFA), with a reduced number of wavelengths (10) in linear combination (Table 2.1). Although standardized reflectance with respect to full spectrum illumination shows the potential for differential chromatic signal, it does not necessarily represent the actual signal variation in an ecologically relevant context. To filter the stripe phenotypes through the spectral window of downwelling and sidewelling irradiance, we next incorporated environmental light (Figure 2.3) to assess ecologically relevant differences among the three stripes, between stripe colors and possible backgrounds, and to compare the effectiveness of each color as a signal against typical microhabitats and water color.

2.3.2 Stripe colors are differentially discernable to fish clients

Trophically and ecologically divergent onlooker visual systems may see stripe and microhabitat colors differently. We wanted to, first, get a sense of how alike or different the stripe colors may be and, second, to evaluate to what extent they differentiate from microhabitat colors. Two dichromats and two trichromats are represented in color vision chromaticity maps (Figure 2.4). Unitless opponency based coordinate distance represents the ease with which colors can be discriminated to the modeled observer. Hybrid stripes are more chromatically similar to blue than yellow *E.* stripes to modeled fish visual systems in all cases. Yellow stripes plot furthest away from blue, and closest to sponge and coral microhabitat colors. Coral and sponge microhabitats are likely chromatically similar in many cases to fish onlookers, as suggested by the overlapping s.d. around the group centroids. Green stripes seem to be

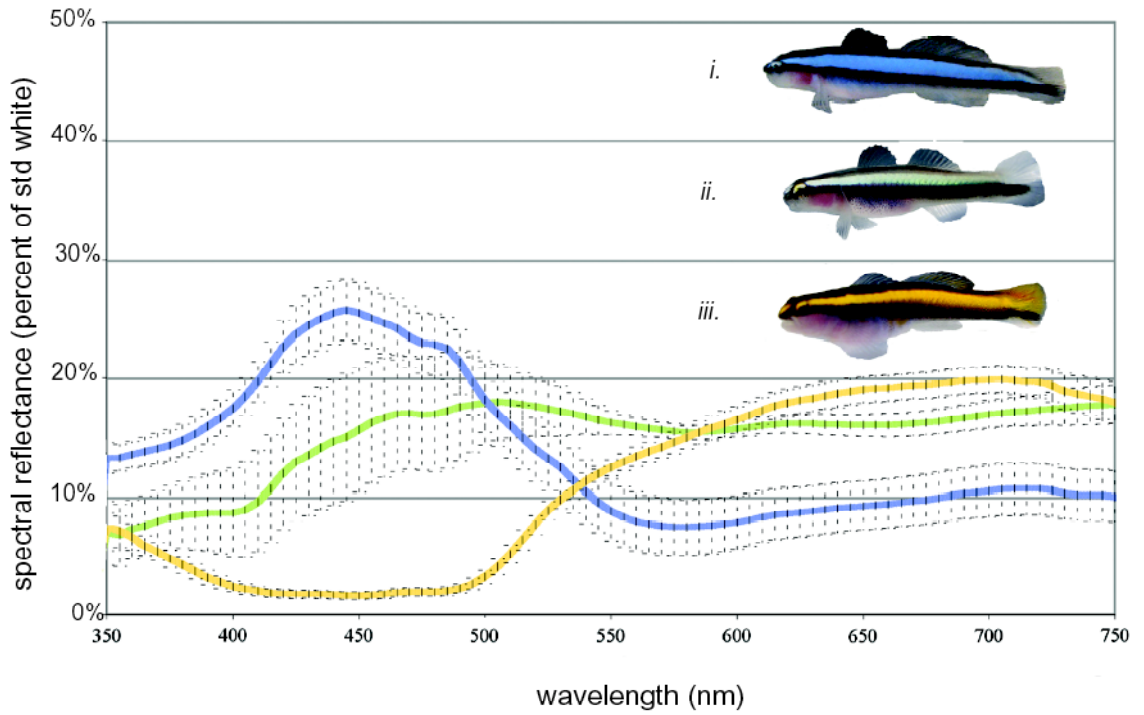


Figure 2.1 Spectral reflectance curves of i.) *Elacatinus oceanops*, ii.) *E. hybrid* and iii.) *E. randalli*.
 Reflectance curves are color coded to match their stripe color. Mean and s.d. for eight individuals per phenotype shown.

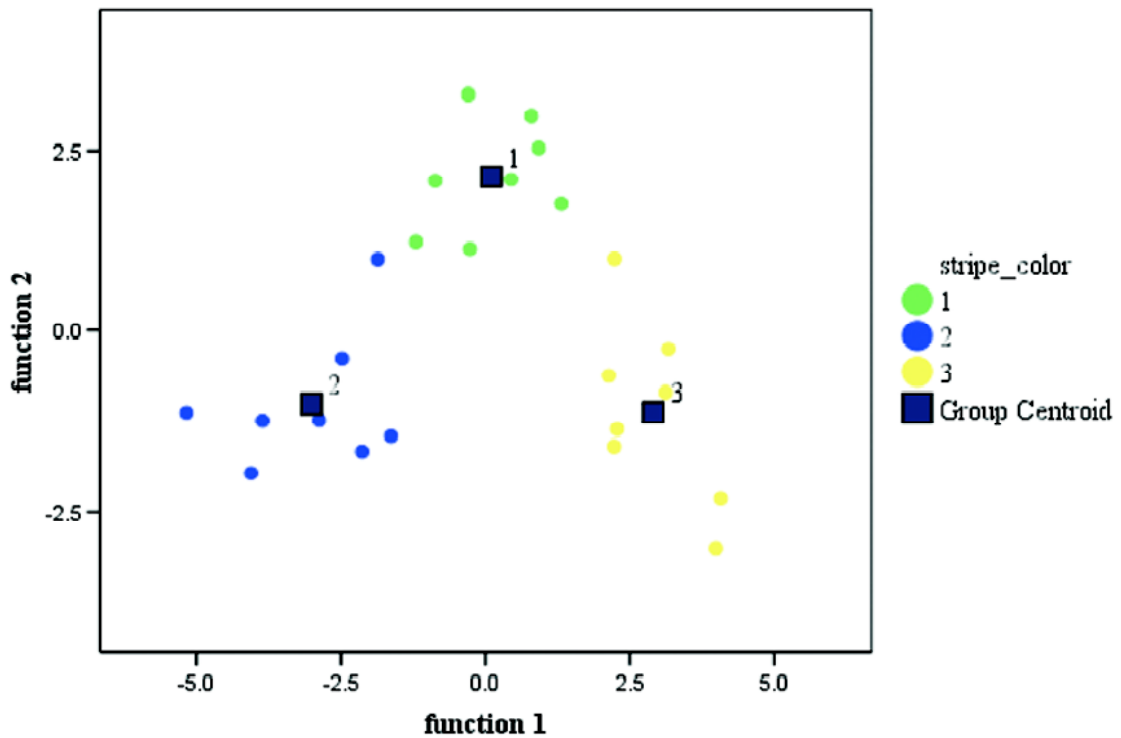


Figure 2. 2 Discriminant function analysis of color stripes.

Stripes show clear clusters of reflectance patterns with ten wavelength comparisons in linear combination (listed in Table 2.1). The stripe colors are categorically different and discriminable without the bias of any particular visual system.

Table 2.1 Discriminant function analysis output and wavelength components.

Linear combinations of wavelength variable measurements in two functions are able to describe 100% of the variation in wavelength reflectance across the three categories of color stripes.

	function		function	eigenvalue	%	cumulative %	canonical correlation
	1	2					
350 nm	-1.262	-4.223	1	6.666	71.9	71.9	0.932
355 nm	5.112	8.432	2	2.609	28.1	100	0.85
360 nm	0.03	-4.179					
365 nm	-6.631	-5.797					
370 nm	6.713	4.498					
375 nm	2.464	-8.42					
385 nm	-15.369	15.243					
400 nm	19.348	-10.973					
410 nm	-11.511	4.799					
550 nm	1.462	0.655					
			test of	Wilks'		degrees of	
			function(s)	lambda	chi-square	freedom	significance
			1 through 2	0.036	54.782	20	0
			2	0.277	21.176	9	0.012

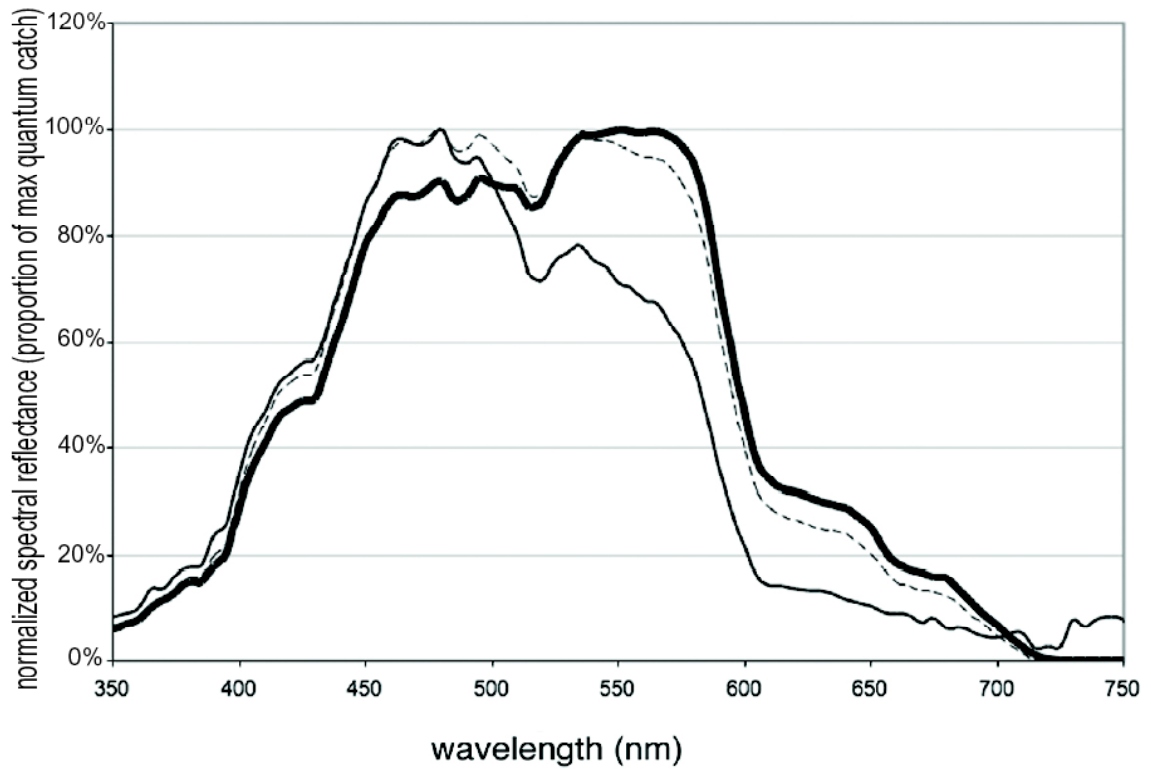


Figure 2.3 Downwelling and sidewelling light spectral envelope at 6m depth.
 Spectral curves normalized to show differences in color transmittance or reflectance. Representative curves, collected at West Palm Beach, FL, USA, midday, full sun are downwelling onto cleaning station (thick line), sidewelling toward the sun and away from coral head (dashed line), and sidewelling away from the sun and away from coral head (thin line).

most achromatic to fish visual systems (as they are to humans) among the three colors, and also seem to be a close match to sidewelling water color among the representative fish onlookers tested. Blue stripes also closely match both the sidewelling water color in three out of four and the achromatic origin in two out of four modeled cases (although see further discussion below for extended absolute chromatic contrast values across all modeled visual systems).

Representative dichromat plots (Figs 2.4A, 2.4B) show blue stripe points furthest offset from coral or sponge microhabitats, while yellow and green may be essentially the same color as some habitats to onlooking fish, if they fall below a threshold of discrimination ability. Dichromats may not chromatically distinguish between yellow stripes and many microhabitat colors, especially sponges. The representative predatory onlooker, *Aulostomus chinensis*, the trumpetfish, may not be able to reliably use chromatic cues to distinguish yellow striped *E. gobies* from many typical backgrounds.

Blue striped gobies, for this same predator, are potentially almost a perfect match to the sidewelling water color. Green stripes fall below discrimination thresholds with a few habitat colors to the modeled trumpetfish and are most achromatic. For the surgeonfish, *Acanthurus triostegus*, green may be more easily distinguishable from microhabitats. In both dichromat cases shown, blue and green stripes are likely more achromatic, plotting closer to the achromatic origin, than is yellow. A potentially ecologically relevant achromatic color for the gobies is their black dorsal and lateral stripes (reflectance is achromatic black). These stripes can vary in saturation, and can fade to dusky achromatic gray if the fish is stressed or during social interactions (Lettieri, pers. obs.)

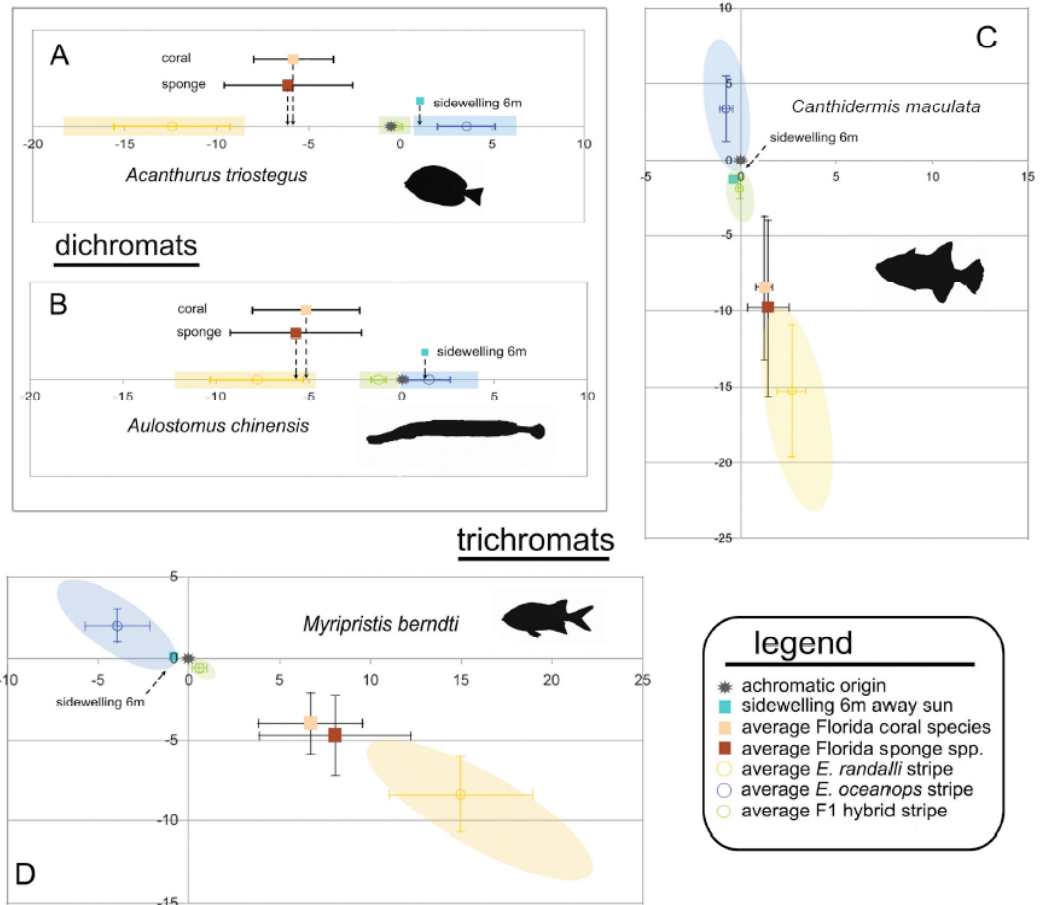


Figure 2.4 A-D Chromaticity diagrams to represent two dichromat (A,B) and trichromat (C,D) visual systems.

Colors plotted at 6 m depth downwelling light spectral envelope adjusted for sidwelling light coming from the direction of the sun. Averaged stripes (n= 8 individuals per phenotype) and microhabitats (n=10 sponges and n= 11 corals) are shown with 1 s.d. whiskers. X and Y-axis coordinates represent unitless color opponent values for dichromat or trichromat models (Kelber et al. 2003) calculated from the two or three (respectively) cone visual systems. Colored clouds encompass the entire span of the stripe color values.

The pairing of a color stripe against achromatic black stripe is likely to enhance signal visibility overall (see Appendix caveat 1 for discussion of brightness and signals.) If we compare yellow to green stripe discrimination potential against background habitats and stripes, models suggest that each maximizes offset from one but not both, while blue accomplishes both chromatic contrast from habitat and the bordering stripes.

In the plots of possible trichromats, the cardinalfish, *Myrpristis berndti*, and the triggerfish, *Canthidermis maculata*, yellow is furthest from green and blue stripe points, which again map more closely to each other and to the achromatic origin than either does to yellow. Colors falling on a straight line through the origin are likely complementary in the visual system represented (Figure 2.4C and 2.4D shown, but this is not *always* the case in trichromats). Both green and yellow are nearly equidistant (on average) from average coral and sponge habitats, but are not chromatically similar; they are different in color, but potentially equally contrasting from habitat colors. The standard deviation of yellow stripes from the group centroid is larger, suggesting that variation in hues of yellow are greater than those of blue and green. In general, trichromats are likely to be better able to distinguish all stripes from possible microhabitat colors than are dichromats.

Overall, there was only slight variation in results between 1m, 6m, and 17m depth (only 6m results shown), which is not surprising as the model assumes von Kries color constancy. All chromaticity points plotted at 17m shifted slightly towards the center (more achromatic) of the plot. To the representative client fishes, sidewelling “blue” water plots within the standard deviation (on at least one opponent axis) of blue stripes and closest to the mean value for green stripes. The color of shallow tropical water is likely categorically different from all goby stripe colors to potential clients at shallow depth, but is likely more similar to blue or green stripes at increased depth. Yellow stripes are likely discernable from most microhabitats at shallow depth, but trend toward

chromatic similarity with more microhabitats at deeper depths, at least for the representative species mapped. All three phenotypes are potentially effective communication colors against most microhabitats (see Lythgoe, 1968 and Marshall, 2000, for discussion of blue and yellow as signals), but blue stripes should operate as a more chromatically distinct color against corals and sponges of many colors.

2.3.3 Blue is higher contrast against microhabitat colors

In order to focus on the potential contrast of each stripe color to relevant microhabitats, we next used a complementary approach. We compared absolute chromatic distances of individual color stripes from selected coral and sponge microhabitats (Figure 2.5). For pairwise comparisons between stripes and microhabitat colors to any particular onlooker, chromatic distance (ΔS) can differ from background color in absolute value or in direction (represented by +/- direction along an opponent system axis, e.g. differential contrast between cones). Contrast results consider only *absolute* color distances and therefore do not reflect the likeness or difference of stripe colors, but the relative ease of discrimination by the observer with respect to the microhabitat reference color (rather than an achromatic origin). This approach allowed us to ask how well each stripe performs in terms of color contrast as a signal against a particular microhabitat across many visual systems. We found that blue stripes overall provide the highest chromatic contrast across a range of possible microhabitat colors to the majority of client visual systems, especially to dichromatic visual systems (Table 2.2). Against the coral and sponge microhabitats examined (Figure 2.5, column 1), possible dichromats see blue in higher contrast to microhabitat background than both yellow and green stripes in all cases. The yellow stripe of *E. randalli* is likely to be of equal contrast compared to hybrid green for dichromats against all focal backgrounds except *Montastrea cavernosa* and sidewelling blue water (higher for both). The blue

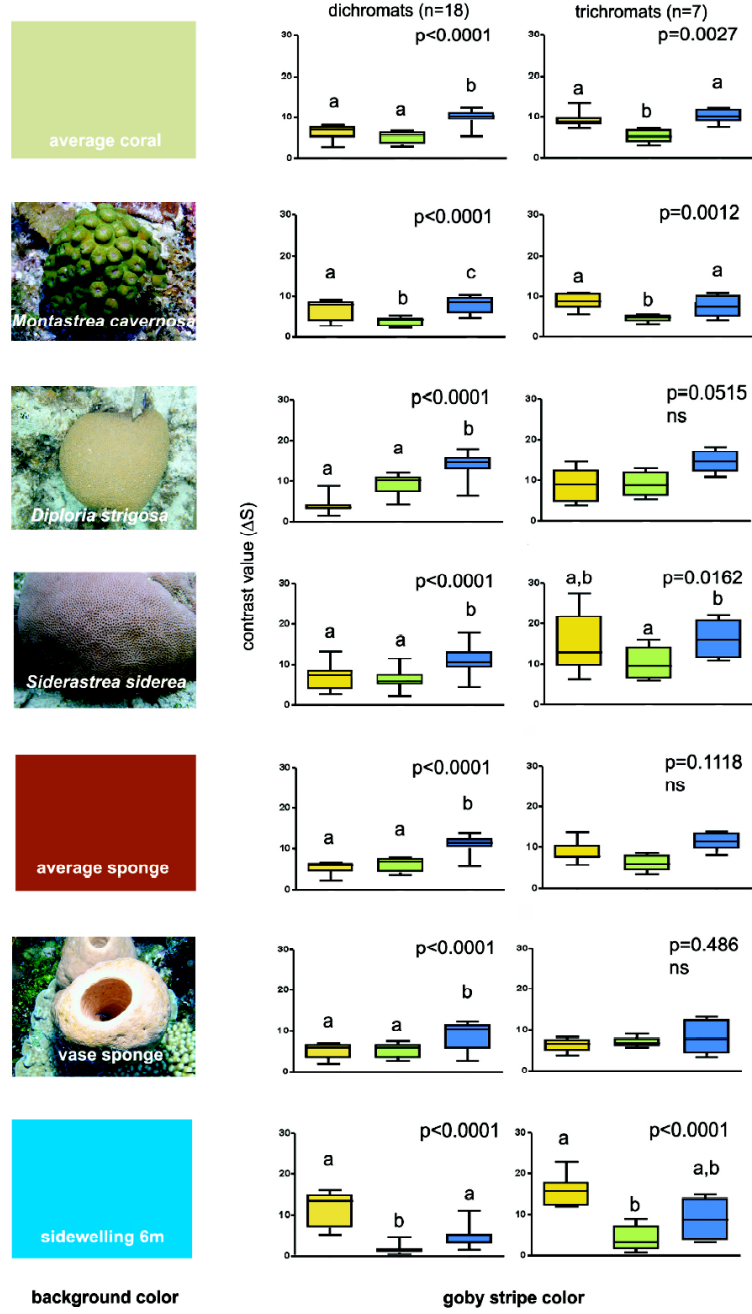


Figure 2.5 Contrast value (ΔS) box-and-whisker plots of stripe colors against each of seven microhabitat background colors (column 1, coral and vase sponge photographs courtesy of R. Ritson-Williams).

Contrasts were calculated within dichromat (column 2) and trichromat (column 3) visual systems (showing median, upper and lower quartile, and least and greatest values). Eight color stripe replicates were used to calculate chromatic contrast values for each stripe color in eighteen dichromat visual systems and seven trichromats using cone sensitivity data from Losey et al. 2003. Friedman non-parametric ANOVA p-values shown. Significant Bonferroni-Dunn post-test differences between stripe contrasts against each microhabitat indicated by different letters above values for each stripe.

stripe color is not significantly different in contrast magnitude from yellow against sidewelling blue in the combined possible dichromat cases tested at the 6m depth. Although contrast potentials of blue compared to yellow differ among dichromats (Table 2.2), for the average sponge and coral microhabitats, blue is higher contrast in all cases. Depending on the visual sensitivity, blue stripes ranged in added contrast potential from 0.3 to 2.5 fold on corals and from 0.8 to over 3 fold on sponges.

For the same potential microhabitat or background colors tested, possible trichromats show a slightly different pattern (Figure 2.5, column 3). Yellow is just as contrasting as blue on average against all microhabitat colors and sidewelling color to the combined trichromats. No significant difference in the absolute magnitude of contrast value among stripes was found for average trichromats against the coral *D. strigosa* color, average sponge, and vase sponge; all three are equally effective. In the cases tested, possible trichromats are better able to distinguish both blue and yellow stripe colors than green stripe from the background colors (see Appendix caveat 2 for further discussion of green stripes and coral fluorescence) with higher overall contrast values compared to dichromats. When ultraviolet (UV)-sensitive trichromats were excluded from the analysis (in order to avoid using any extrapolated data), contrast values mimicked the patterns seen in dichromats, where blue was consistently higher contrast than yellow. In all cases, save vase sponge, both the minimum contrast value and the median contrast value for blue were higher than for yellow. For the vase sponge case, the median yellow contrast was slightly higher, although no significant differences were found among absolute contrast values across stripes (Friedman non-parametric ANOVA test statistic, $p = 0.486$). Average sponges were also equally contrasting as a microhabitat background to all three phenotypes ($p = 0.118$). Blue and yellow appear to be equally effective contrast colors against average coral colors to trichromats. Minimum values for yellow were often below a chromatic distance of 4 (the average

Table 2.2 Blue to yellow contrast ratio for average coral, average sponge, and sidewelling water.

Twenty-five representative visual systems used in chromatic contrast analyses. Species with asterisks are representative predator visual systems. Species underlined represent those from particularly abundant families.

dichromats	ave coral	ave sponge	sidewelling
<i>Acanthurus triostegus</i>	1.3	1.8	0.2
<i>Apogon kallopterus</i>	1.3	1.8	0.2
<i>Arothron melaegris*</i>	1.4	1.9	0.2
<i>Aulostomus chinensis*</i>	1.8	2.4	0.4
<i>Chaetodon kleini</i>	2.0	2.6	0.2
<u><i>Chromis ovalis</i></u>	2.1	2.9	0.4
<u><i>Chromis verater</i></u>	2.0	2.7	0.4
<i>Ctenochaetus strigosus</i>	1.5	2.0	0.2
<i>Forcipiger flavissimus</i>	1.4	1.9	0.2
<i>Lutjanus bohar*</i>	1.5	2.1	0.3
<i>Mulloidichthys flavolineatus</i>	2.0	1.9	2.0
<i>Naso lituratus</i>	1.6	2.2	0.3
<i>Pervagor spilosoma</i>	2.5	3.3	0.6
<i>Saurida variegatus*</i>	1.6	1.5	1.5
<i>Sphyrena helleri*</i>	1.4	1.9	0.2
<i>Stegastes fasciolatus</i>	1.6	1.6	1.5
<i>Zanclus cornutus</i>	1.3	1.8	0.2
<i>Zebrasoma flavescens</i>	1.3	1.8	0.2
trichromats			
<u><i>Abudefduf abdominalis</i></u>	0.8	0.6	0.7
<i>Canthidermis maculata</i>	2.4	1.2	0.3
<u><i>Chromis hanui</i></u>	0.9	0.7	0.9
<i>Dascyllus albisella</i>	1.5	1.0	0.7
<i>Kuhlia sandvicensis</i>	0.9	0.7	0.9
<i>Myripristis berndti</i>	1.8	1.0	0.2
<i>Zebrasoma veliferum</i>	1.8	1.0	0.2

standard deviation of yellow stripe contrast values against the least contrasting background microhabitat), while blue was always above $\Delta S = 5$. Yellow stripes produced the overall highest chromaticity values of all with some ΔS values reaching over 25, suggesting that variation in contrast potential is higher with ancestral yellow stripes. Among possible trichromats analyzed (Table 2.2), contrast potential for yellow compared to blue varies. For some species, blue is potentially more effective, and for others, yellow. A few trichromats see blue and yellow with equal contrast potential against average sponge colors.

In all, twenty-five fish visual systems were modeled in order to ask how the three stripe phenotypes compared against typically used backgrounds. Among predators (Table 2.2, asterisks denote species representing piscivores), blue was on average 0.6 and 2 fold more contrasting against average corals and sponges, respectively. Among representative species from particularly abundant families (underlined), yellow and blue both are potentially effective contrast colors. Cone sensitivities from representative abundant dichromat species seem to have a blue contrast bias, while abundant trichromat species may see yellow more easily compared to blue. Against average sponge color, a total of nineteen species have higher blue contrast than yellow. An additional three have equivalent contrast values for blue and yellow. Overall, twenty-two out of twenty-five species likely perceive blue with higher contrast on coral microhabitats.

2.4 Discussion and conclusions

We compared the signal chromatic contrast of *E. goby* color stripes to twenty-five different modeled fish onlookers. Using a modeling approach, we evaluated three representative phenotypes against many microhabitats on which the cleaners would advertise to passing clients. While spectral reflectance of stripe colors among the three *E. goby* phenotypes measured shows statistically distinct and categorical differences

(Figure 2.2), we considered the colors in the context of the natural surroundings and in the sense-perceived context of trait mediated signal that they communicate. In order to better evaluate the potential in signal variation on which selection could act, we needed to compare the potential signaling ability of the advertising stripes through the eyes of client beholders. We found that variation in signal ability among stripes does exist among different onlooker visual systems and against different microhabitat backgrounds and that derived blue stripes are on average a more robust signal.

We know from previous research that both yellow and blue are potentially good signal colors in the tropical marine environment and are known to be commonly occurring signaling colors in fishes (Lythgoe 1968; Lythgoe 1979; Marshall, 2000; Marshall and Vorobyev, 2003) as the wavelengths reflected are complementary colors to many fishes and effectively exploit the many blue-shifted (and UV-shifted) visual systems found in tropical marine fishes (Bowmaker, 1990; Losey et al., 1999; Lythgoe, 1984; Loew and Lythgoe, 1978; Marshall, 2000). We showed that *E.* blue stripes may be more distinguishable to a larger pool of species or to a particular type of visual system (dichromat) against typical microhabitat backgrounds. Blue is consistently different chromatically from microhabitat hues, although certain yellow striped individuals produced the highest single contrast potentials in both potential di- and trichromats. In the representative client visual system examples we show (Figure 2.4), blue stripes fall below discrimination thresholds with sidewelling water blue (6m) color in a few cases, but overall contrast values across both dichromat and trichromat visual systems (Figure 2.5) suggest that on average reef fish are able to chromatically distinguish yellow and blue equally well (no significant difference between ΔS value) against sidewelling water color. The cleaners and sponge dwellers in this family, however, do not typically advertise against a water backdrop. Cleaner gobies typically rest on sponge and coral surfaces before approaching visiting clients (Lettieri, pers. obs.), and do not engage in

the elaborate dances that characterize their Indo-Pacific cleaner wrasse counterparts, *Labroides dimidiatus*. The use of color signals is likely a reliable communication method (in this case to advertise cleaning status) among fishes (Chiao et al., 2000; Cummings, 2004). It is possible, alternatively, that evolutionary pressure selecting for increased brightness (achromatic signal) of the pattern has led to the observed changes in stripe colors (see Appendix, caveat 1 for further discussion), but we did not explicitly test this hypothesis. Perception of stripe colors against the microhabitat to potential observers will vary depending on the ambient downwelling and sidewelling light, background against which the signal is observed, visual system and sensitivity of the observer, as well as the size, shape, and overall pattern of the color signal perceived (Lythgoe, 1968; Leow and Lythgoe, 1985; Lythgoe and Partridge, 1989; Barry and Hawryshyn 1999; Partridge and Cummings, 1999; Marshall and Vorobyev, 2003). The wide range of visual systems analyzed encompass a variety of tropical reef species and feeding strategies, and most likely are a good representation of potential client visual systems. The vast majority of these species have a blue stripe advertising bias compared to the ancestral yellow phenotype.

2.4.1 Blue signal increases opportunity

Visual cues to attract cooperative partners must be both visible and recognizable to their intended targets, and color stripe traits may respond to selection mediated by the sensory biases of various receivers, both intentional partners and unintentional eavesdroppers (Endler et al., 2005; Cummings, 2007). In the genus *E.* gobies, basal sponge-dwelling as well as facultative and obligate cleaners share black stripe patterns that enhance the visibility of a lateral colored stripe, but only the cleaners use their pattern to recruit a mobile food source. Our results suggest that stripe evolution trends toward more conspicuous signaling to dichromats in particular, a potentially larger pool of onlookers. The black stripes paired with a colored stripe distinguish this entire

subgenus *E.* lineage from other cryptic or disruptively marked congeners. Independent contrasts among both cleaner and non-cleaner species indicate that these long lateral stripes are linked to obligate cleaning (Côté, 2000) and longer stripes (relative to body length) have been experimentally verified to attract more clients (Stummer et al., 2004). Among potential client visual systems, abundant dichromats (Table 2.2) are much more likely to see blue stripes with greater ease than yellow stripes. Many other tropical marine species and trophic groups are potential dichromats with blue signaling more effectively. Many of the potential trichromats we analyzed (including representative abundant family species) showed similar discrimination distance values for yellow and blue, suggesting that among the most abundant reef dwellers color stripe contrast potential varies across species. Evolution of stripe colors in these mutualisms seems to be linked to cleaning, suggesting that within this lineage, the benefits of evolving novel blue advertising stripes may lie in the broader pool of species whose visual senses are likely to see blue with greater ease on typical habitat backgrounds.

2.4.2 Blue signal may increase risk

Cleaning interactions are dependent on the action of the intended client fish, which must approach and accept cleaning from the cleaner. The potential for cheating by clients includes attack and predation on these small, relatively immobile fishes, but they are rarely found in the guts of predatory species (Cheney pers. obs.). The types of clients visiting stations can vary considerably by depth, geography, and habitat (Colin, 1975; Cheney and Côté, 2003; Arnal et al., 2001; Côté, 2000; Floeter et al., 2007) and stripes may communicate different information to different species, and may initiate different responses. The average blue to yellow contrast ratio among all piscivores that we included was 1.6 on average coral color and 2.0 on average sponge color. Our models predict that blue stripes should be more conspicuous than yellow and green stripes to a wide range of potential client visual systems across multiple possible

background colors, and they may also be much more conspicuous to potentially dangerous clients. By driving the mutualism to be more asymmetric in nature (more potential “partner” mutualists) while simultaneously making cleaning more risky (more exposed microhabitats and predator interactions) as a strategy, selective pressure may be increased on traits associated with altering partner behavior. Tradeoffs between attracting partners and deterring predation are likely ubiquitous challenges for immobile mutualists (e.g. Kessler et al., 2008) and may lead to the adaptive evolution of traits designed to manipulate the behavior of partners through various sensory channels. Mechanisms for the increased diversity exhibited in asymmetric mutualisms may lie in the interaction of these traits as they evolve to broadcast to a wider audience and simultaneously deter cheating behavior in mutualist partners.

Distinction from co-occurring fishes (Merilaita and Ruxton, 2007) could have evolved to indicate aposematism, as preliminary feeding tests (Colin, 1975) with predatory reef fish showed avoidance and rejection of several *Elacatinus* gobies, although conclusive evidence that any one species is chemically-defended remains to be tested. Since pattern and defensive chemistry need not coincide (Darst et al., 2006; Endler and Mappes, 2004), the ultimate cause of evolution of the stripe pattern could be different from the proximate selective forces maintaining the pattern and driving color change across the *E.* clade. If mediating risk is indeed a tradeoff for increasing visibility, then it may explain why all cleaners are not blue.

2.4.3 Potential for diffuse evolution in asymmetric mutualisms

Strong local selection pressures have been suggested to influence geographic variation in the color of cleaners among *E. evelynae* (and *E. illecebrosus*) populations (Palumbi and Warner, 2003). Interactions with different combinations of client species in asymmetric mutualisms may alter the evolutionary trajectory of stripe colors, even though, on average, blue is of higher contrast. If diffuse evolution occurs, where traits in

the cleaners are differentially selected in the presence or absence of key consortia (e.g. clients) (Strauss et al., 2005), we might expect that yellow and green could be favored in certain cases. We suggest that inherent sensory biases of clients and local microhabitat availability may also contribute to the maintenance and evolution of color variation and ultimately reinforce mechanisms of speciation among mutualist species.

2.5 Appendix

2.5.1 Caveat 1: Brightness and saturation

All three *E. (s.s.)* stripe colors are paired with the black stripes dorsal and ventral, and there is potential for effective achromatic brightness variation that does not correspond to the patterns we see with chromatic color signal. The visual channels used in detecting achromatic and chromatic signals are likely to be different in fishes (Cummings, 2004) and both systems may contribute to transmitting reliable signals to an intended receiver, whether interspecific mutualists or predators, or intraspecific mates or competitors. We did not specifically test these potential differences in luminance to the fish visual systems. Brightness and saturation are likely also under physiological control by movement of melanophores in the color stripe, in order to highlight or obscure iridophores, similar to color changes described in *Paracanthus* surgeonfish by Goda and Fujii (1998) and may regulate visibility of the overall stripe pattern. Under variable illumination, as is characteristic of coral reef habitats, chromatic signals are often more reliable and may be more important for identifying objects than achromatic (brightness) signals for fishes (Chiao et al., 2000, Cummings 2004). Comparisons of color hue and saturation (chroma) regardless of brightness differences when comparing between color stripes and backgrounds is evolutionarily relevant given the novelty of blue stripes within the *E. cleaners*, but may not fully explain the selective paradigm in which signal evolution is occurring.

2.5.2 Caveat 2: Microhabitat color variation and fluorescence

Reflected and emitted light from corals can include light fluorescing from coral pigments in the coral host tissue (Mazel and Fuchs, 2003), with the most common pigments have fluorescence emission peaks at 486 nm, 515 nm, and 575 nm. The effect of fluorescence on overall *exitance* (total flux per unit area leaving the surface of the coral from emission, reflectance, and transmittance) can vary from coral to coral, across depth and at changing zenith angle of the sun, however in our measurements, no significant contribution to color was noted in the reflectance data. The results of contrast calculations could be affected by increased contribution of the most chromatically saturated pigments, namely p515 and p575, named after the emission wavelength peak, which would likely reduce chromatic contrast values for the hybrid green stripe color.

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CHAPTER 3: PREDATOR AND CLIENT INTERACTIONS

3.1 Introduction

Mutualism is pervasive in natural systems, and may constitute an ecological foundation for highly diverse communities and ecosystems (Stachowicz, 2001; Hay et al., 2004; Bascompte et al., 2006). Positive interactions among species comprise an evolutionary enigma however, because both the origin and maintenance of cooperation is difficult to reconcile with classical theory: mutualist genotypes are prone to infiltration by cheaters (Trivers, 1971). Work has concentrated on modeling the conditions under which mutualism might persist. For instance, iterated interactions (Axelrod and Hamilton, 1981), image scoring (Nowak and Sigmund, 1998), and spatial population structure (Nowak and May, 1992; Doebeli and Knowlton, 1998) can sustain cooperative behavior, even in the absence of 'higher-order' phenomena like memory and reciprocity. In fact, under realistic conditions, mutualisms may be fairly robust to cheating, but the initial origin of positive interactions from neutral and/or negative ones remains poorly understood (Doebeli and Knowlton, 1998).

Recent conceptual models suggest that antagonistic interactions (e.g., predation, parasitism) might shift to mutualism as resistance traits like chemical defense are modified or traded for partner tolerance (Oliver et al., 2009; Edwards, 2009). For instance, *Acacia* plants produce extrafloral nectars that are more palatable to mutualist than non-mutualist ant species (Kautz et al., 2009), and aphids moderate interactions with predatory ants using honeydew (Oliver et al., 2009; Fischer et al., 2001). These phenotypes, by definition, serve to *tolerate* interactions with potentially dangerous species rather than *deter* or *resist* them. Turning deterrence into tolerance has been

observed in ecological time (Oliver et al., 2009) but few examples of evolutionary transitions are known (Edwards, 2009).

Interactions between a focal individual and a partner (i.e., negative encounters like those between predator and prey or positive relationships between mutualists) are often mediated by signals (colors, chemicals, behaviors) of intent. Such signals are under strong selection to communicate relevant information to the chosen partner while avoiding cheating or exploitation by eavesdroppers (Hasson, 1994). As partner and eavesdropper pools become more diverse, both the interaction strategy (attract, deter or tolerate) and the repertoire of signals may evolve (Axen et al., 1996; Hasson, 1997).

Here, we study the evolution of mutualism and the colors signifying mutualism between Caribbean gobies and the diverse clientele they clean. Our goal is to understand how this mutualism originated and evolved. Cooperation between cleaners and clients was one of the first examples of reciprocal altruism studied (Trivers, 1971) and has arisen independently in shrimp and various fish lineages (reviewed in Côté, 2000). A striped pattern is a general signal of cleaning status to potential client fishes (Côté, 2000; Stummer et al., 2004). Furthermore, blue color in stripes is evolutionary correlated with obligate cleaning in Indo-Pacific *Labroides* wrasses (Cheney et al., 2009) and has evolved repeatedly in Caribbean *Elacatinus* gobies (Taylor and Hellberg, 2005). Despite similarities between the cleaner-client mutualisms of wrasses and gobies, there are striking differences. Wrasse cleaners invest in pre-conflict management strategies with predators and are subject to partner switching and punishment by clients (Bshary and Würth, 2001; Bshary and Grutter, 2002; Bshary and Grutter, 2005); these modes of enforcement have not been observed among clients of Caribbean cleaner gobies (Soares et al., 2008c). Wrasse cleaners rarely cheat piscivorous clients — by taking a bite of non-parasitized skin — while gobies do not preferentially refrain from cheating potential predators (Soares et al., 2008c; Soares et al., 2007). The implication is that

there may be a different mechanism by which Caribbean gobies mediate conflicts of interest with some clients.

Blue color in stripes attracts more visitors to wrasse cleaning stations (Cheney et al., 2009), but the Indo-Pacific wrasse and Caribbean gobies may have evolved under different selective pressures. All members of the species rich *Elacatinus* (*sensu stricto*-s.s.) subgenus are striped (Taylor and Hellberg, 2005; Rüber and Zardoya, 2005) and the closest Pacific sister species is a facultative but reclusive cleaner that lacks the ubiquitous Caribbean stripes (Rüber et al., 2003). Basal members of the Caribbean group exhibit yellow and green (intermediate between yellow and blue to a fish's eye) (Taylor and Hellberg, 2005; Lettieri et al., 2009) stripes and live in sponges. Sponge-dwellers often inhabit chemically defended species like *Agelas spp.* and *Neofibrularia spp.*, and typically retreat far into the lumen of the sponge when threatened (Colin, 1975; Lettieri, observation). In the eyes of many fishes, yellow stripes are cryptically colored against typical sponge microhabitats (Lettieri et al., 2009). Green stripes are cryptic against some sponges, but overall are more conspicuous than yellow, and less so than blue (Lettieri et al., 2009). Cleaning gobies, by contrast to their sponge-dwelling relatives, advertise parasite-removal services to potential clients by perching atop the substrata (usually coral heads). Cleaners can be yellow- or green-striped, but most are blue-striped. Among stripe colors, blue is found only in the cleaner lineage and provides the greatest chromatic contrast against coral microhabitats, especially to piscivore visual systems (Lettieri et al., 2009).

Given that the evolution of blue from yellow and green stripes coincides with a transition from reclusive to exposed behavior and increased conspicuousness to piscivores, we reasoned that the evolution of stripe color might signal a change in trait function from *deterrence* to *tolerance* of risky interactions with potential predators. We evaluated three main predictions related to the shifting role of color stripes and the

evolution of the cleaning mutualism in *Elacatinus* gobies. First, because *Elacatinus* cleaners do not engage in pre-conflict management behaviors with predators, do not refrain from cheating predators, and are rarely eaten by predators (Soares et al., 2008c; Soares et al., 2007) we expected cleaners to exhibit chemical defense (Colin, 1975) and increased survival, compared to non-cleaners, upon close encounters with predators. Second, we expected that stripe colors would be a sufficient signal to elicit “appropriate” client behavior (approaches, poses and decreased attack) in nature. Third, because blue and green stripes are more conspicuous to piscivore visual systems than are yellow stripes, we hypothesized that green and blue cleaners might engage in more interactions with potentially risky clients.

3.2 Methods

Feeding experiments were conducted under ethical guidelines approved at the Georgia Institute of Technology (IACUC project A08051).

3.2.1 Laboratory feeding trials with hamlet predators

Feeding trials were conducted on two consecutive days with wild caught *Hypoplectrus spp.* (Serranidae) hamlets — sympatric predators that are known clients (Arnal et al. 2000). We chose hamlets as a representative common, ubiquitous Caribbean predator that is known to ingest fishes and has a generalized diet (Whiteman et al., 2007; Holt et al., 2008). Generalist predators are often used to test the outcomes of ecological interactions that could affect traits deterring ingestion (Pawlik et al., 1995). We refer to day one trials as *naïve* and day two trials as *post-exposure*. Although the diet history of the wild-caught hamlets is not known, our day one trial results did not suggest that the frequency of attack on treatments differed. The feeding assays were intended to elicit an attack on the prey, deprived of the environmental cues that would otherwise inform the predator’s decision to attack. Hamlets were housed in a divided 12-

foot long flow-through tank (14 at a time) with clear plexiglass walls allowing water to pass through the compartments of the entire unit, or in divided 20-gallon tanks (2 at a time) with individual filtration. Ninety-nine hamlets were used in these experiments. Replicates conducted at Dynasty Marine Associates (Marathon, FL, USA) in the single flow-through tank were assigned so that each fish along the length of the tank was separately offered a randomized treatment fish; during each replicate trial, a white sheet of plastic was used to block the view of the neighboring hamlets. Replicates conducted at Georgia Institute of Technology (Atlanta, GA, USA) were assigned so that each member of a divided pair in every 20-gallon tank received a randomized treatment goby, again with a white plastic divider to block neighbors' views. Hamlets were acclimated overnight prior to day one trials. On day two, hamlets were offered the same treatment of goby as on day one. Hamlets were not food deprived and were fed thawed scallop two hours prior to feeding trials, to confirm that they would eat.

Seven goby treatments were chosen to represent the color patterns, stripe colors and ecological habits of the group:

(1) wild-caught (WC), cryptically colored *Elacatinus (s.l.) macrodon*, the closest sister species to *Elacatinus* sponge-dwellers and cleaners (Rüber and Zardoya, 2005) was chosen as a cryptically-colored outgroup to the *Elacatinus (s.s.)* subgenus;

(2) WC syntopic *Coryphopterus personatus* was chosen as a ubiquitous, evasive schooling goby with comparable size and microhabitat usage, likely to encounter similar types of predators to the *Elacatinus spp.* (Lettieri, pers. obs.);

(3) WC yellow-striped, sponge-dweller *Elacatinus horsti* from Curacao was chosen as a basal representative phenotype since yellow stripe color and sponge-dwelling pre-date cleaning and green or blue color;

(4) WC green-striped cleaner *E. evelynae* from Curacao was chosen as an intermediate phenotype in terms of color (Lettieri et al., 2009) and evolution (Taylor and Hellberg, 2005);

(5) tank-raised (TR) green-striped *E. evelynae* was chosen as a phenotypic copy of treatment (4);

(6) WC blue-striped cleaner *E. oceanops* from the Florida Keys, USA, was chosen as a recently evolved, blue-striped cleaner and

(7) TR blue-striped *E. oceanops* served as the phenotypic copy of treatment (6).

Wild caught and tank-raised specimens were jointly evaluated to test the hypothesis that cleaner chemical defenses, if observed, were non-constitutive. The color stripes of WC and TR specimens were not statistically different when evaluated with spectrophotometric methods and fish color vision models (Lettieri et al., 2009). Neither wild caught nor tank-raised yellow-striped cleaners were available. *E. evelynae* are less likely to clean on sponges than on corals (Whiteman and Côté, 2002); the *E. evelynae* from Curaçao were caught from live coral.

Individual trials consisted of one goby offered to one hamlet for a period of 5 minutes. For each trial, we recorded total survival time and the number of times the goby escaped or was spit from the predator's mouth. We tested the null hypothesis of equal survival time (log-transformed, as Bartlett's test for homogeneity showed significantly different variance among treatments, $p < 0.05$) among treatments using a one-way analysis of variance (ANOVA). We tested the null hypothesis of equal number of times escaped or rejected from the predator on day one, among treatments, using a Kruskal-Wallis non-parametric ANOVA, followed by a Tukey's post-test, calculated in GraphPad Prism (version 4.0.1). We tested the null hypothesis of equal percentages of surviving gobies on day one and day two, (i) between wild cleaners and non-cleaners, and (ii)

between wild and lab raised cleaners, using Fisher's exact test, performed in R 2.8.1 (R Core Development Team 2009).

3.2.2 Field tests with painted models

We monitored approach behavior by wild reef fishes towards model gobies on a sloping coral reef at Coco Point, Bocas del Toro, Panama (9°18.019'N, 82°16.350'W) during the months of July and August in 2008. Ten replicate hours of blocked treatments were recorded with PVC mounted video cameras in underwater housings at 5-9m depth, between the hours of 0730 and 1100. Each blocked replicate consisted of a yellow-striped, blue-striped, and unstriped painted glue model placed in separate suitable microhabitats. Because resident *Elacatinus illecebrosus* at Coco Point were green-striped, both striped models were novel within the habitat.

We constructed models by first making two mirror image color-copies of an *Elacatinus oceanops* (blue), *E. randalli* (yellow), and *Coryphopterus personatus* (unstriped) goby. Each image was standardized to 4cm, and painted with 6-8 coats of clear liquid craft latex. The dried latex was soaked for 20-30 seconds in warm water and the paper was gently rubbed away from the latex, leaving the color image on a clear latex background. The mirror images were then hot-glued together and painted with acrylics to enhance color stripe saturation. Blue and yellow acrylic paints were mixed to match wavelength reflectance patterns as closely as possible, as measured from live gobies (Lettieri et al., 2009) with an Ocean Optics USB2000 spectrophotometer (Dunedin, FL, USA). Paint colors fell within the standard deviation of goby stripe colors, according to fish visual systems (Lettieri et al., 2009). Achromatic black acrylic paint (as measured by the spectrophotometer) was used to darken dorsal and lateral black body stripes. Monofilament fishing line was tied around the goby model and two lead fishing weights attached so that the final length from model to weights was 10cm (Figure 3.1).



Figure 3.1 Unstriped (*C. personatus*), yellow and blue-striped models deployed in the field.

Model fishes were constructed with latex photographic transfer of goby images bonded with craft hot glue and colored with acrylic paints; these were then deployed in front of coral heads with fishing line and lead fishing weights.

Blocked replicates were deployed in the field by placing a neutrally buoyant goby model in front of a suitable (same species and similar size, depth, and light exposure) but uninhabited coral head within 5m of each of the other treatments. The fishing weights were placed in the sand directly in front of coral heads, facing the camera stand. Camera stands were placed at least 12 hours ahead of data collection, to minimize disturbances caused by the appearance of the PVC structures. Video cameras in underwater housings were attached to PVC stands immediately before placing the goby models. *Elacatinus illecebrosus* (the resident) green-striped goby cleaners were observed within the vicinity (within 10m) of these locations, but were not seen occupying treatment sites. Video recording was started immediately after placing the model in front of the coral.

We recorded the frequency of approaches by all non-goby species and tested the null hypothesis of equal observed approaches to treatments using a generalized linear model with a quasi-Poisson link function, calculated in R. We also counted all observations of attack or recognizable client posing behavior and tested the null hypothesis of equal frequency among approaches to treatments using a multiple comparison of means Tukey contrast, calculated in R, and a Freeman-Halton extension to the Fisher's exact test, calculated in R. We discarded replicates for which (i) an individual fish (e.g., a territorial damselfish) continued to harass a model for an extended period of time (for a total of more than 10 minutes), (ii) one of the treatments was taken by a predatory fish away from the placement site with more than 10 minutes remaining in the trial, or (iii) water visibility prevented identification of fishes for greater than a 10-minute period.

3.2.3 Cleaner and client interactions in the literature

We searched the literature for observations of cleaner-client mutualism where the color of the cleaner stripe was identified. Cleaning interactions were collected from eight

articles published in peer-reviewed journals and from one published dissertation (Table 3.1). If a species was listed as a client of a cleaner goby, the color of the cleaner's stripe and the functional group (e.g., piscivore, herbivore, invertivore, etc.) of the client were tallied. We thus constructed total client species pools of representative cleaner species with different colors. We hypothesized that green and blue stripes, which are more likely to be of high contrast against dominant reef colors, would be visited more frequently by piscivores, whose visual systems detect these colors more easily (Lettieri et al., 2009). We compared client pools of (1) combined cleaner species segregated into the three divergent color stripes as well as 2) three representative *Elacatinus* species with the highest representative client pool within the three colors. We expressed the 'client pool' of a particular cleaner as the fraction of the total number of client species falling into the categories of (i) piscivore, (ii) herbivore, (iii) invertivore and (iv) other (other included planktivores, benthivores, small fish and invertebrate consumers, and omnivores). We tested whether observed cleaner client pools differed from a randomly sampled representative pool of equal number from the overall combined documented client species pool, using a custom randomization program written in Perl. Expected proportions of clients belonging to predator, invertivore, herbivore, and 'other' functional groups were calculated by a random sampling of individuals from the combined pool of all 138 documented client species observed in the Caribbean; the number drawn was equal to the absolute number of clients that have been documented to interact with the cleaner phenotype of interest. This was replicated 1000 times to obtain a distribution of the mean proportion of clients belonging to each functional group. Observed client pools for each stripe color were then compared to this re-sampled distribution. Z-scores were calculated based on deviation from the randomized expectation and p-values were calculated in R. Although this method is not ideal (i.e., we pool possible clients across all species of goby cleaners as our baseline, and do not consider proportion of time spent

Table 3.1 List of cleaner goby and client interaction sources.

Data sets were compiled from published literature with client species documented and goby stripe color identified or inferred.

- Arnal C, Côté IM, Sasal P, Morand S (2000) Cleaner-client interactions on a Caribbean reef: influence of correlates of parasitism. *Behav Ecol Sociobiol* 47: 353-358.
- Böhlke JE, McCosker JE (1973) Two additional West Atlantic gobies (genus *Gobiosoma*) that remove ectoparasites from other fishes. *Copeia* 3: 609-610.
- Colin P (1975) *The neon gobies*. Neptune City, N.J.: TFH Publications, Inc.
- Côté IM, Molloy PP (2003) Temporal variation in cleanerfish and client behaviour: does it reflect ectoparasite availability? *Ethology* 109: 487-499.
- Francini-Filho RB, Sazima I. (2008) A comparative study of cleaning activity of two reef fishes at Fernando de Noronha Archipelago, tropical West Atlantic. *Environ Biol Fish* 83: 213-220.
- Johnson WS, Ruben P (1988) Cleaning behavior of *Bodiansu rufus*, *Thalassoma bifasciatum*, *Gobiosoma evelynae*, and *Periclimenes pedersoni* along a depth gradient at Salt River Submarine Canyon, St. Croix. *Environ Biol Fish* 3: 225-232.
- Sazima I, Sazima C, Francini-Filho, RB, Moura RL (2000) Daily cleaning activity and diversity of clients of the barber goby, *Elacatinus figaro*, on rocky reefs in southeastern Brazil. *Env Biol Fishes* 59: 69-77.
- Whiteman EA, Côté IM (2002) Cleaning activity of two Caribbean cleaning gobies: intra- and interspecific comparisons. *J Fish Biol* 60: 1443-1458.
- Wicksten, MK (1998) Behaviour of cleaners and their client fishes at Bonaire, Netherlands Antilles. *J Nat Hist* 32: 13-30.

cleaning or numbers of individuals within each species cleaned), it serves as a best approximation summarizing results of many studies with varied methods of data collection. Geographic heterogeneity — in *e.g.*, protection level of reefs — could bias this post-hoc method if all representative cleaners from a particular color phenotype tend to be found in areas where there are more predators in general due to protection level. This particular bias is not apparent in our data. Although studies were included from protected (*e.g.* Barbados) and non-protected reefs, specific cleaner species and color types are not restricted to one type of site or another.

3.3 Results

3.3.1 Feeding experiments: wild-caught cleaners elicit predator aversion

Feeding trials were conducted with WC *Hypoplectrus* hamlet predators given close range access to an individual of a single goby treatment during a five-minute *naïve* day one encounter, and a five-minute *post-exposure* encounter on day two. Gobies offered to hamlets do not possess potentially deterrent physical attributes. All are small, immobile, scaleless and lack visible or palpable defensive spines.

Over all trials, survival time varied widely. For instance, among WC and cryptically patterned *E. macrodon* eaten 100% of the time on both days, hamlets took 0.5 – 255 seconds to consume prey. There were no significant differences among treatments in percentage eaten on day one; across treatments, 87.1% of gobies were consumed by hamlets in first day encounters. However, WC blue-striped *E. oceanops* gobies were spit out or escaped (Figure 3.2 movie) significantly more times on day one than all other treatments except TR *E. oceanops* and WC *E. horsti* (Kruskal-Wallis test statistic=35.72, df=6, $p < 0.0001$ with Tukey's post-test between treatments). In all, 77.7% of *naïve* encounters resulted in at least one occurrence of escape or 'spit out' for WC *E. oceanops*.



Figure 3.2 Day 1 enclosed feeding-trial interaction between a *Hypoplectrus spp.* predator and wild-caught *Elacatinus oceanops* cleaner goby. (file [lettieri liliana b 201008 phd fig32 escape.mov](#), 16MB)
Footage shows example of spit out or escape behavior by a blue striped cleaner species.

Significantly fewer WC cleaners *E. evelynae* (green) and *E. oceanops* (blue) were eaten in *post-exposure* second encounters compared to all other non-cleaners (Fisher's exact, $X^2=13.116$, $df=1$, $p=0.0004$; Figure 3.3). Among all consumed prey on day two, a smaller proportion than expected belonged to the WC cleaner group (exact binomial goodness-of-fit, $p=0.0013$). Wild caught cleaners survived longer in *post-exposure* trials (t-statistic=3.81, $df=73$, $p\text{-value}<0.001$) than all other groups. Tank-raised *E. evelynae* and *E. oceanops* individuals were eaten in higher proportions than their WC counterparts on day two (Fisher's exact, $X^2=3.259$, $df=1$, $p=0.0503$; Figure 3.3).

3.3.2 Field trials: stripes deter attack and induce posing

We used painted glue models deployed in the field to ask how potential client fishes respond behaviorally to color stripe patterns. Both the absolute number of visitors (193 for *C. personatus* model; 194 for the yellow-striped *Elacatinus* model and 246 for the blue-striped *Elacatinus* model) and the mean frequency of hourly approaches differed among treatments (Yates' $X^2=45.67$, $df=2$, $p<0.001$; Figure 3.4). There were no significant differences in mean number of approaches, with treatment as the main parameter, in a generalized linear model with counts fitted to a quasi-Poisson distribution (analysis of variance, $F\text{-value}=0.705$, $df=2$, $p>0.5$). However, it is difficult to assign motivation to a passing observer from a scored approach. Therefore, we tallied the total number of behaviors for which we could interpret relevant intent: attacks and stereotypical client posing. These interactions were rare but informative. The total frequency of attacks vs. poses was significantly different among the three model types (Freeman-Halton extension to the Fisher exact test, $X^2=25.61$, $df=2$, $p<0.0001$; Figure 3.5). *Coryphopterus* models were *never* observed to cause client posing, whereas 7 and 12 poses were observed for yellow and blue-striped models, respectively. Fourteen total attacks on *Coryphopterus* models were observed while only 3 *total* occurred on any

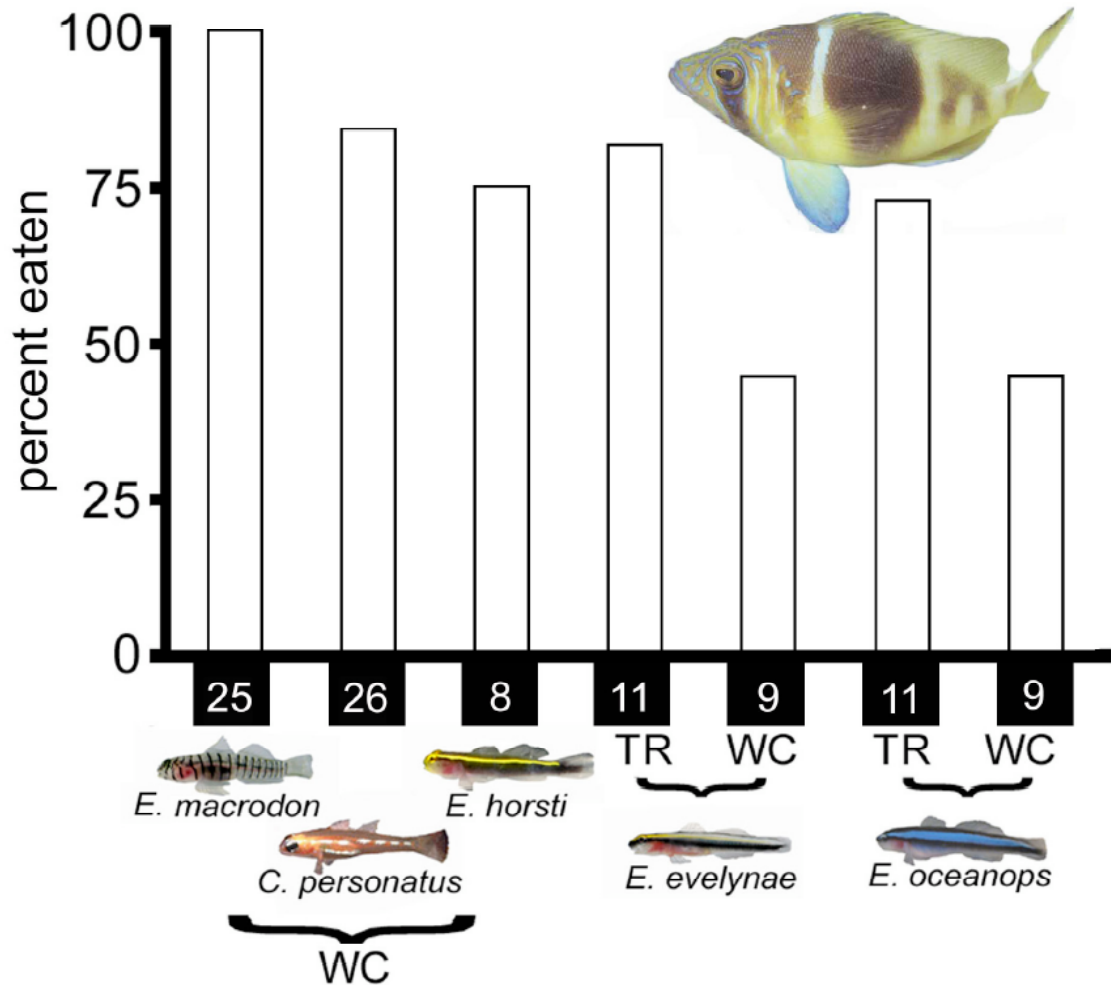


Figure 3.3 Wild caught cleaners are eaten in lower proportion to non-cleaners. The percentage of individual prey gobies eaten in day two (*post-exposure*) treatments. Significantly fewer wild caught (WC) cleaners *E. evelynae* (green) and *E. oceanops* (blue) were eaten compared to all other non-cleaners (Fisher's exact, $p=0.0004$) in day 2 *post-exposure* five-minute feeding trials. Among all consumed prey, a smaller proportion than expected belonged to the WC cleaner group (exact binomial goodness-of-fit, $p=0.0013$). Tank raised (TR) *E. evelynae* and *E. oceanops* cleaner individuals (combined) were eaten in marginally higher proportions than their WC counterparts on day two (Fisher's exact, $p=0.0503$). Numbers in black boxes indicate the number of individuals tested.

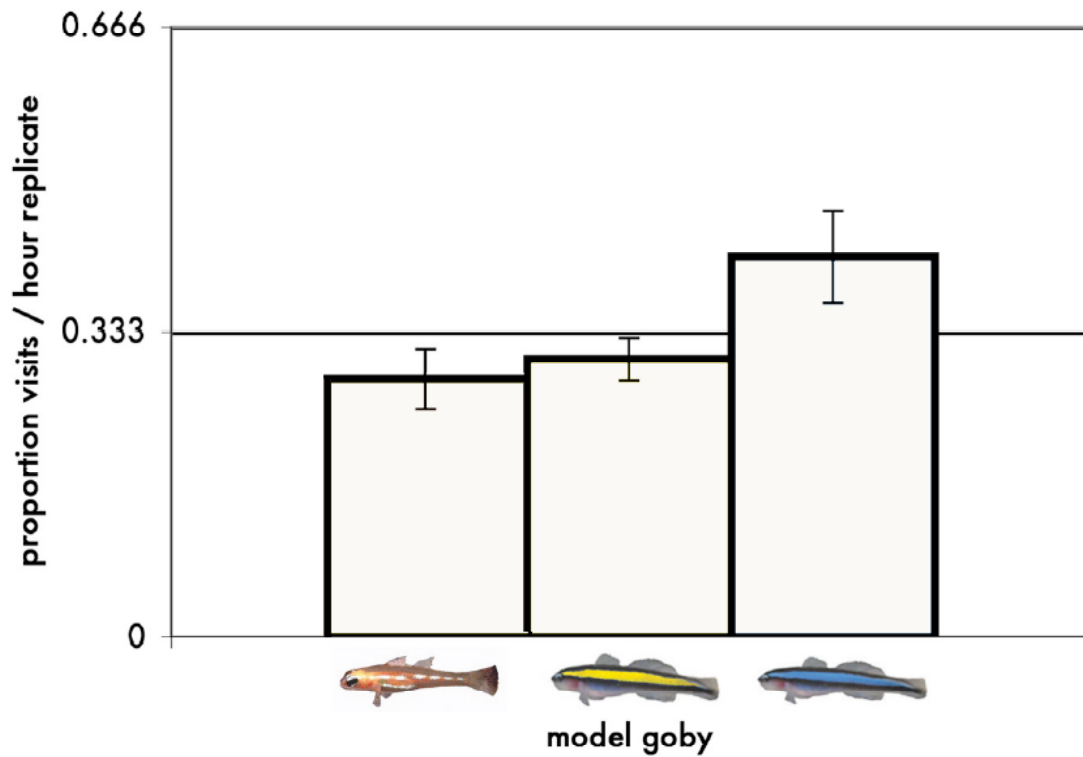


Figure 3.4 Proportion of approaches per hour replicate varies among phenotype models. Hourly proportion of total reef fish approaches are significantly different among masked goby (unstriped *C. personatus*), yellow and blue striped model types as determined by Yates' $X^2=45.67$, $df=2$, $p<0.001$.

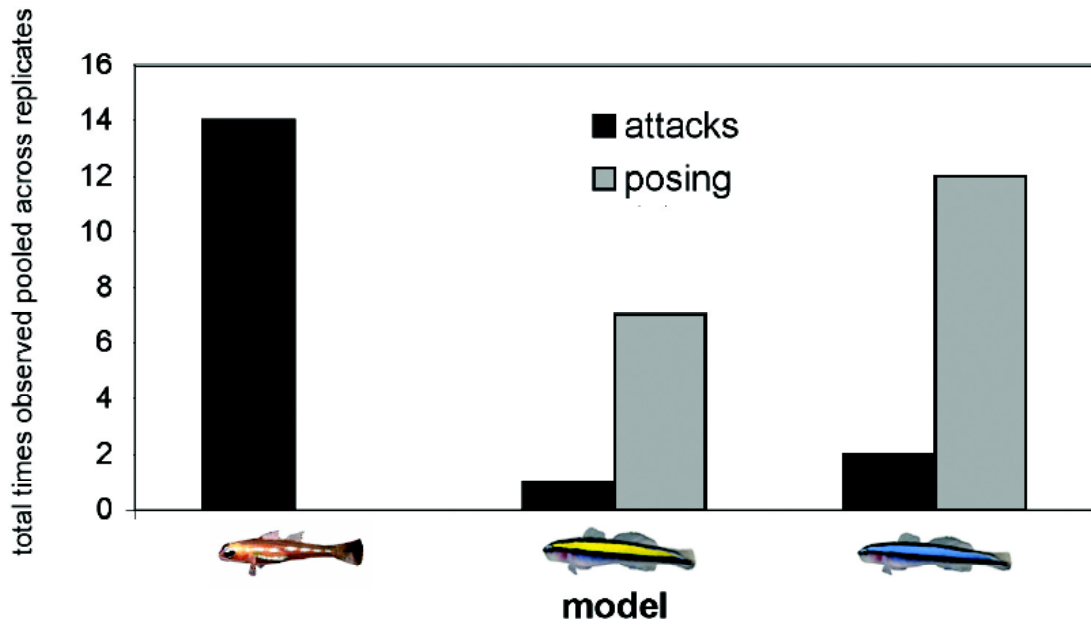


Figure 3.5 Color stripes are sufficient to elicit client behavior.

Attack and stereotypical client “posing” frequencies are significantly different among masked goby (unstriped *C. personatus*), yellow and blue-striped model types as determined by Freeman-Halton extension to Fisher’s exact test, p -value < 0.0001.

striped model (less than 1% of total approaches in each case; Figure 3.5). The mean frequency of attacks as a proportion of approaches was significantly higher in the masked goby treatment, in a generalized linear model with proportion of approaches leading to attack linked by a binomial distribution (Tukey multiple comparison test, z-value compared to blue stripe=2.96, $p=0.008$; z-value compared to yellow stripe=-2.61, $p=0.02$, residual deviance<df = 27).

3.3.3 Cleaner phenotypes differ in interactions with client functional groups

We surveyed the literature to document observed interactions between *Elacatinus* cleaner gobies and clients, and tallied the total client species pools for cleaners of known color stripe. Piscivorous species comprise about 31% of the total Caribbean client pool (Figure 3.6). Piscivores made up a smaller than expected fraction of the client pool among yellow-striped cleaners, based on comparison to a randomized distribution (two-sided p-value = 0.005). For both the combined client pools (Figure 3.6, 19%) and for a representative yellow-striped species with the highest overall number of clients among yellow-striped cleaners (*E. figaro* data not shown, 9%, $p=0.003$), yellow-striped cleaners interacted with predators at proportions lower than expected. Green and blue-striped cleaners service piscivorous client species at proportions equal to that expected from re-sampled pools (green 33% $p=0.771$, blue 32% $p=0.849$). The proportion of herbivorous clients was greater than expected for green striped cleaners (36% $p=0.008$) and the proportion of invertivore clients was less than expected for blue cleaners (9% $p=0.03$).

3.4 Discussion and Conclusions

Positive interspecific interactions between cleaner fishes and their clientele represent a primary example of mutualism (Trivers, 1971; Côté, 2000; Hammerstein and Hoekstra, 1995; Grutter, 1999; Bshary and Noe, 2003). Caribbean gobies exhibit many of the

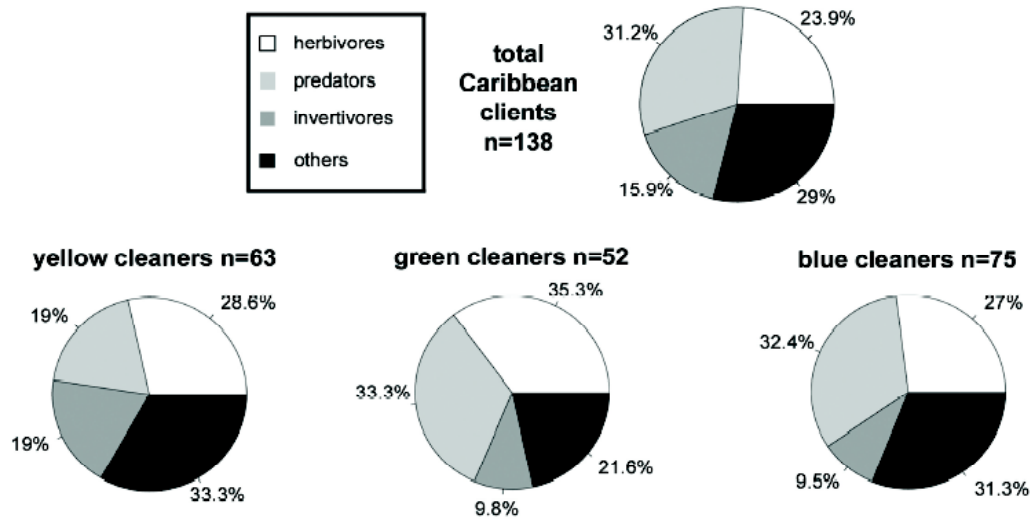


Figure 3.6 Cleaner-client interactions differ among three cleaner color phenotypes.

Pooled client species are from all documented cleaning interactions in the literature we surveyed (Table 3.1). Yellow-striped cleaners consisted of *E. randalli*, *E. figaro* and *E. evelynae* (yellow). Green-striped cleaners were *E. prochilos*, *E. evelynae* (green), *E. illecebrosus* (green) and *E. genie*. Blue-striped cleaners consisted of *E. oceanops*, *E. illecebrosus* (blue) and *E. evelynae* (blue). Total client species belonging to herbivore (white), piscivore (light grey), invertivore (dark grey) and other (black) client functional groups are depicted as a proportion of total documented species that have been observed acting as clients of cleaner goby color morphs.

ingredients suggested by theory to sustain mutualism once evolved (Axelrod and Hamilton, 1981; Nowak and May, 1992; Doebeli and Knowlton, 1998). For example, *Elacatinus* population genetic structure (Taylor and Hellberg, 2003) likely leads to geographic variation in cleaner identity and cleaner-client interactions. *Elacatinus* gobies interact repeatedly with some clients (Soares et al., 2008c), and may signal their status as mutualists with color stripes (Côté, 2000; Taylor and Hellberg, 2005). We investigated the origin of mutualism and the signals signifying mutualism in *Elacatinus* gobies because the transition from neutral, random and/or negative biological interactions to positive ones remains poorly understood.

We found that (i) wild caught *Elacatinus* cleaners have an increased probability of escape and survival after being attacked, (ii) stripes painted on glue models are sufficient to deter attack and elicit client posing in the field, and (iii) blue- and green-striped cleaners do not bias against interactions with risky predatory clients. These data, in combination with our previous visual modeling of stripe colors which shows blue of highest contrast among the three colors (Lettieri et al., 2009), imply that blue-striped gobies are better equipped to attract and survive attacks from predators. Considered together with the natural history and phylogenetic relationships among *Elacatinus* gobies (Taylor and Hellberg, 2005; Ruber and Zardoya, 2005), we suggest that mutualism evolved in this lineage as predator resistance traits were co-opted for tolerance and cooperation. We highlight key components of this evolutionary scenario below (Figure 3.7).

3.4.1 Colorful stripes pre-date mutualism

The origin of colorful stripes in ancestral sponge-dwelling *Elacatinus* gobies poses a theoretical conundrum, because being more conspicuous potentially puts an individual at higher risk to detection by predators (Guilford, 1988; Lindstrom et al., 1999; Broom et al., 2006; Halpin et al., 2008). However, extant yellow-striped sponge-dwellers are

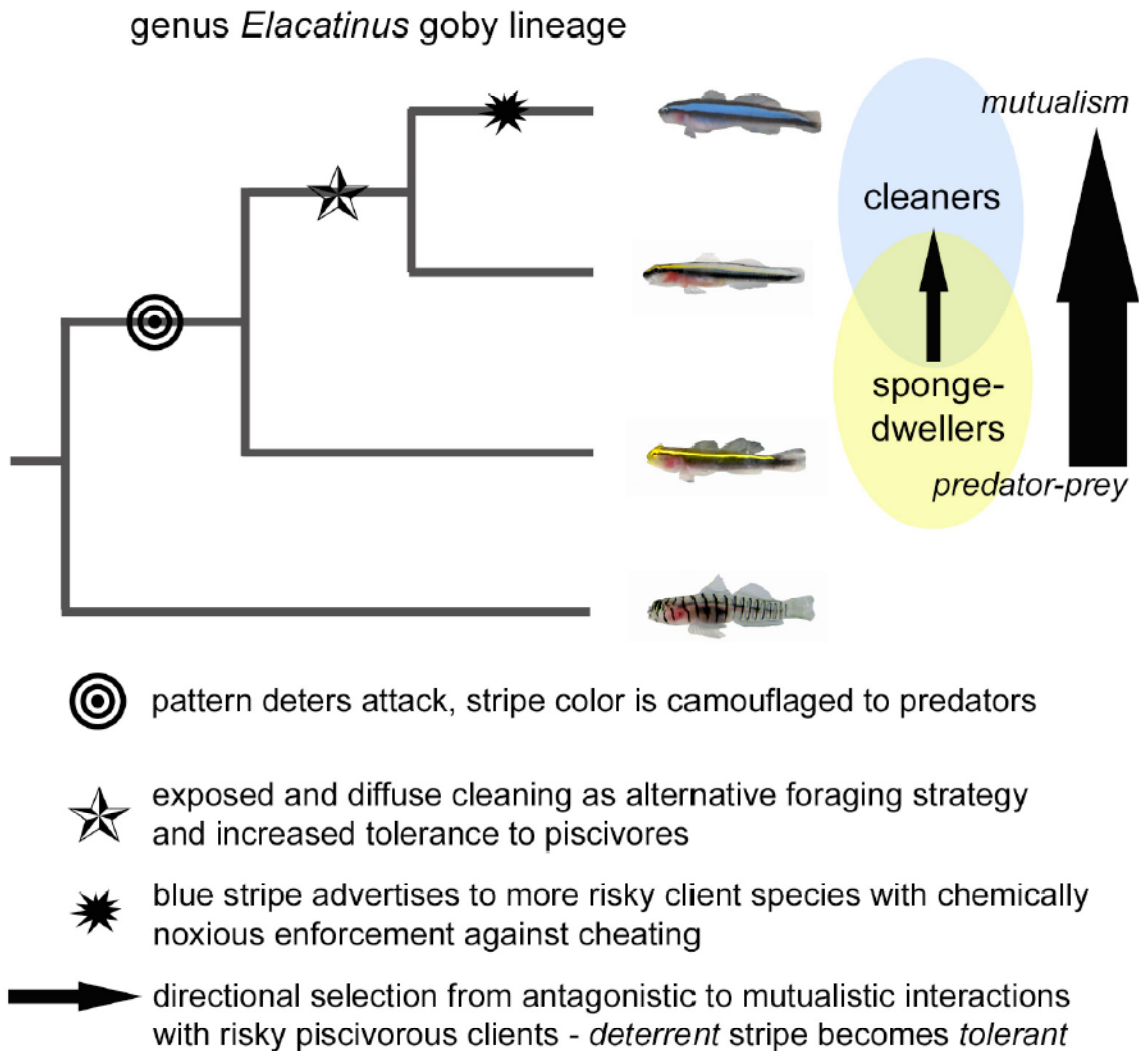


Figure 3.7 Colorful stripes mediate the evolution of mutualism in cleaner gobies.

Inferred changes in (i) goby stripe color and the role of this signal, (ii) habitat and behavior (e.g., cleaning) and (iii) interactions with predators [from *resistance* to *tolerance*] during the evolution of mutualism in Caribbean *Elacatinus* gobies. The phylogeny follows Taylor & Hellberg, 2005.

cryptically colored — against common sponge microhabitats to many fishes (Lettieri et al., 2009) — and are generally reclusive. Color stripes may have first evolved among *Elacatinus* gobies as a means of intraspecific recognition and/or distinction from syntopics, while retaining chromatic crypsis. Sponge-dwellers may possess modest noxiousness (Colin, 1975), as do other gobies (Schubert et al., 2003). In our feeding trials, 25% of the yellow sponge-dweller *E. horsti* survived all five minutes of the day-two exposure to predators. While *C. personatus* masked gobies survived at comparable levels (15% survival in day-two feeding trials), field trials revealed that masked goby models were attacked at higher rates than striped models. Aposematic cues — bright colors that work in concert with toxicity or chemical defense to affect predator behavior — may communicate nutritional unprofitability to potential predators, or facilitate learned feeding aversion (Harvey and Paxton, 1981; Speed and Ruxton, 2005; Skelhorn and Rowe, 2007; Franks et al., 2009). When schooling and fast swimming (e.g., defenses used by *C. personatus*, Lettieri, pers. obs.) are unprofitable or unfeasible escape strategies, communicating with color signals may be adaptive (Kuchta et al., 2005). Stripes therefore likely first evolved in *Elacatinus* gobies as either neutral (e.g., intraspecific communication) and/or negative (e.g., camouflage, aposematism) signals in antagonistic interaction with piscivores.

3.4.2 Coming out of the sponge: stripes signal a new ecological role

Cleaning on open coral habitat in *Elacatinus* gobies likely evolved from sponge-dwelling, and the basal cleaning species (e.g., *E. randalli*, *E. figaro*) in the Caribbean lineage are yellow- and green-striped (Taylor and Hellberg, 2005). The evolution of cleaning behavior as a facultative feeding strategy may be basal to sponge-dwelling, as Pacific sister species *E. puncticulatus* has been documented to clean (Rüber et al., 2003) and shelters in the spines of *Eucidaris thouarsii* urchins. Cleaning in the

Caribbean, however, marks a transition from a reclusive ancestor whose stripes are camouflaged and/or a signal of defense, to an exposed advertising descendant whose stripes serve to attract cooperative clientele (Figure 3.7). Competition for food resources and microhabitats may be intense for small sponge-dwelling gobies with limited mobility (Schofield, 2003; Hernaman et al. 2009; Rocha et al., 2000) and may result in gobies being pushed into less structurally complex habitat (Schofield, 2003). Facultative sponge-dwelling cleaners (Rocha et al., 2000; Whiteman and Côté, 2004a,b; White et al., 2007) tend to assemble in groups (up to 50 or more individuals) and such aggregations may have facilitated the transition from reclusive to more frequent interactions with potential clients (Whiteman and Côté, 2004a, Whiteman and Côté, 2002; Sillen-Tullberg and Leimar, 1988) and further contributed to high intraspecific competition (Whiteman and Côté, 2002).

Coral-dwelling may thus have offered an alternative to food acquisition on sponges. The risk of predation is lower on sponges than on corals (White et al., 2007), but less competitive individuals may be forced out as population sizes increase (Whiteman and Côté, 2002). Our data support the notion that cleaners have evolved increased unpalatability in response to the additional risk (Figure 3.3), perhaps to mitigate, in part, the increased threat of predation on coral microhabitats (White et al., 2007). We suggest that co-occurrence of ancestral cleaning gobies with slightly more palatable alternative prey (e.g. a highly palatable goby such as *E. macrodon*, Fig 3.3), and increased exposure to a diverse predator pool further reinforced the evolution of stripes as a weak communication signal (*sensu* Endler and Mappes, 2004). Colorful stripes (i.e., yellow) may have initially conveyed a dual message from cleaners to distinct functional groups of client: they signaled to predators “I don’t taste good,” and to less risky clients “I can clean you.”

Notably, the yellow stripes of basal cleaners may be conspicuous to species of clients with probable trichromatic visual systems (the most abundant of which are likely planktivores) but yellow is likely to be cryptic on sponge and coral microhabitats to most piscivores, likely possessing dichromatic visual systems (Lettieri et al., 2009); organisms that can chromatically differentiate patterns or objects use either two sets of light sensitive cones (dichromats), three (trichromats), or four (tetrachromats). This suggests to us that extant and basal yellow-striped, and by extension the earliest (yellow-striped) Caribbean cleaners, initially invested in mutualism with non-threatening species. Our analysis of yellow cleaner interactions from the literature supports this idea; yellow-striped cleaners clean proportionally fewer predators (Figure 3.6). However, cleaning in isolation on coral heads clearly exposes colorful gobies to unpredictable approaches and threats of predation by risky onlookers.

3.4.3 Blue stripes signal tolerance toward risky clients

We suggest that blue stripes have evolved in *Elacatinus* cleaners primarily as a way to tolerate approaches by predators — they are more visible to predators but presumably predators are less likely to attack — reducing the risk of advertising (Figure 3.5) which enhances the ability to attract greater numbers of fish species. Both blue-striped and green-striped cleaners seem to interact with predators at frequencies expected from their availability as clients (Figure 3.6). In addition, we have shown that blue-striped cleaners are perhaps most tolerant to predators in several ways: (i) they are most resistant to attacks from predators (via a putative chemical defense) by causing rejection on a first encounter and have subsequent increased survival, (ii) they are most conspicuous to these risky partners (Lettieri et al., 2009), and (iii) they are least attacked among approaching fishes. The suite of reef fishes with probable dichromatic color vision includes predators, and for those species blue is the most chromatically distinct on typical sponge and coral microhabitats (Lettieri et al., 2009), compared to both yellow

and green cleaners. Green-striped cleaners may be more chromatically contrasting than yellow on some sponge and coral habitats, but blue is a particularly effective signal to predators against the coral background where they primarily clean (Lettieri et al., 2009).

With the aid of a mild chemical deterrent (observed in sponge-dwelling and cleaning gobies) in combination with a signal that cheating (eating the goby) is unprofitable (Soares et al., 2008a,b), interactions with piscivorous clients switch from antagonistic to tolerant. In our feeding trials, over two-thirds of WC cleaners were unharmed after being confined to a small area with a predator for a five-minute interval. Notably however, a significant proportion of potentially defended individuals *were* eaten. Thus, it seems unlikely, as has been hypothesized (Soares et al., 2007), that interactions with predators do not pose some level of risk.

Based on our results, we suggest that restricted ability or variable payoffs to invest in defense has turned a defensive trait combination (colorful stripes paired with toxicity or chemical deterrent) into a tolerant one. Both close interactions *with* and potential attacks *by* predatory clients have reduced consequences on the fitness of a goby. To a sponge-dweller, deterrence is the primary goal of signaling to other species, and attraction is necessary for increased fitness of cleaners. The dual role of the stripe trait in either case is to convince the selector to make the “correct” decision (Hasson, 1991; Hasson, 1994), even if that individual poses a risk (i.e., is a potential cheater). When the abundance or diversity of potentially harmful species offering the food source increases, we might expect overt signaling to predators (Lettieri, 2009), tolerance of close encounters with them (Soares et al., 2007), and an antidote to predator error, via repellent taste, to become adaptive. The general and broad evolutionary trend that we describe may have considerable local variance or plasticity, but the interaction of defense, signal and tolerance appears to be an important suite of traits contributing to the evolution of the specialized cleaning behavior.

3.4.4 Defense signal evolves in diffuse mutualism networks

Cleaner gobies interact with a wide variety of potential clients, from small, territorial herbivores like damselfishes to giant roving piscivores like grouper and barracuda. We suggest that tolerance has evolved in cleaner gobies because the diversity of potential predators precludes singular investment in an arms race (Dawkins and Krebs 1979). In other words, it may be too costly or unreliable to invest in a purely defensive strategy if i) the defense is ineffective against some predators, ii) the likelihood of encountering a diversity of predators is high, or iii) the potential benefit of cooperating can be exploited with reliable conflict mediation. Therefore, similar trajectories are likely in other diffuse mutualisms where one partner possesses defensive or deterrent pre-adaptations to avoid exploitation by cheaters or predators, and the diverse partner pool possesses or evolves sensory biases that can select for cooperation. For instance, in *Hakea* plant-bird pollination networks, pre-adaptation to resist florivory, tolerance to interactions with florivorous birds and color vision among florivores has selected for red bird-pollinated *Hakeas* (Hanley et al., 2009). The *Hakea* example shows a similar evolutionary trajectory to that observed among Caribbean cleaner gobies: 1) defensive traits with color signal warnings resist attack by risky individuals, 2) the signal becomes a cue of reward when the ecological context of color changes the interaction paradigm, 3) an arms race is avoided as tolerance traits increase interactions with potentially risky clients while chemistry and signal are enhanced enforcements against cheating. The study of adaptations of communication traits in other diffuse mutualisms, focusing on the evolution of key defense, attraction and signal traits among and within closely related species and the sensory capabilities of the suite of potential partners and predators, will accelerate our understanding of the evolutionary origin of altruism from antagonism, an unresolved problem in biology.

3.5 References

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CHAPTER 4: EVOLUTION OF DEFENSES AND SIGNALS IN MUTUALISMS

4.1 Introduction

Understanding the evolution of mutualisms remains a challenge to biologists; the steps leading from antagonism or neutrality to these mutually beneficial interactions between *unrelated* individuals remain unclear (Herre et al., 1999; Hoeksema and Bruna, 2000, Palmer et al., 2003). Several key factors have been identified that appear to promote the stability and long-term success of mutualistic interactions despite the potential for an individual to increase overall fitness gains by pure exploitation. Historically recognized mechanisms include limited spatial dispersal of one or more players (Nowak et al., 1994), repeated interactions between players (Axelrod and Hamilton, 1981), reciprocity for altruistic exchanges (Trivers, 1971), punishment of cheating or non-cooperative behavior (Bshary and Grutter, 2002; Clutton-Brock and Parker, 1995), sanctions against non-cooperators (Kiers et al., 2004) and partner choice mechanisms (Pellmyr and Huth, 1994; Bull and Rice, 1991); still, the evolutionary steps and key traits leading to mutualisms with diverse partners is not well understood.

Despite the generally recognized fact that many mutualisms are derived from comparatively parasitic interactions— e.g. plant-micorrhiza (Johnson et al., 1987), yucca-yucca moth (Pellmyr and Huth, 1994), fig-fig-wasp (Janzen, 1979, Machado et al. 2001), phylanthaeae-epicephala (Kawakita and Kato, 2008) — the key steps that lead to the evolution of a reciprocally beneficial interaction between individuals that historically interact as predator and prey species are understudied, perhaps because of a paucity of extant examples of the evolutionary relics of the interaction. The study of active

pollination, where pollinators have evolved morphological and behavioral adaptations specifically to acquire and transport pollen, is a notable exception, where combinations of morphological key innovations and behavioral shifts have linked success of antagonists to their hosts. In seed-eating pollinator examples, high host specificity and life history coevolution in several cases points to similar evolutionary steps leading from antagonism to mutualism (Pellmyr, 1997; Holland and Fleming, 1998). However, cases of active pollination are rare and the more general trend of diffuse pollination leading to increased interactions with “predators” is understudied (Holland and Fleming, 1998). This reinforces the need for robust phylogenetic histories and ancestral trait reconstruction (e.g. Armbruster, 2002; Smith et al., 2008) of the focal mutualist lineage that stands to lose the most or is most dependant on the interaction; a thorough investigation into the range of potential interacting partner species and their responses to hidden and externally detectable traits and signals may help inform our understanding of how the balance of power shifts over evolutionary time.

It is clear that many mutualisms are facultative and diffuse (Howe, 1984, Palmer et al., 2003), with interactions ranging from parasitic to mutualistic depending on various environmental (Sakata, 1994; Thompson and Fernandez, 2006) and contextual factors (e.g. the local players - reviews include Ollerton, 1996; Hay et al., 2004; Strauss and Irwin, 2004; Stadler and Dixon, 2005; Gomez et al., 2009). Also, many historically cited coevolutionary examples of tight species coupling are now recognized as more diffuse than originally thought (Herrera, 1987; Marussich and Machado, 2007). Identifying the function of the traits and circumstances leading to the evolution of mutualism in such variable environments and with diverse partners may tell us more the role of natural selection in shaping traits than any tightly coevolved mutualism between two species. Recent attention in the study of mutualisms has turned toward identifying the costs and benefits of interactions (Herre et al., 1999) and on gaining a better understanding of the

rates and types of exploitation across space and time (Bronstein, 2009). Exploitation occurs when a partner obtains the benefits offered by a mutualist partner, but does not hold up its end of the exchange of transport, nutritional goods or protection. In this review, under the umbrella of exploitation, we will take a broad view that includes predation on individuals and physical destruction to their advertising body parts. We will try to identify key traits that may be involved not only in changing the balance of power and the degree of mutualism achieved on an ecological timescale, but over the course of evolution. We take this broad view because we find cases where the most critical interactions in mutualisms are not the most obvious or frequent. Although attracting partners to cooperate may select for traits with a signature for conspicuousness to them, many examples of mutualisms that are diffuse and diverse appear to be shaped by the need to avoid being exploited, rather than an initial ability to invest in a cooperative interaction.

Key sets of traits in combination may lead some mutualisms to shift, over evolutionary time, from interactions with relatively benign potential exploiters (with whom attraction is the dominant driving selective force), to more risky partners (with whom defense or behavioral assortment may also be crucial). With the latter type of partner class, exploitation might have significant, even fatal, costs. In some cases, the risky partners are those most likely to be antagonists, exerting strong selective pressure, perhaps even before a mutualism evolved. For this reason, we argue that many mutualisms evolve as a result of defense signals making organisms more conspicuous in the environment and then becoming a cue for a reward to these same risky individuals. Finding the link between fitness derived from the partner class, behavioral *assortment* into cooperative and non-cooperative actions, and traits in the focal cooperative class may be key to understanding the evolution of mutualisms (Fletcher and Doebeli, 2009). In this review, we outline a framework in which a key trait, defense,

in combination with a signal — which alters the behavior of a potential interactor following detection — may predispose some species to interact with riskier partners and may in fact promote the evolution of mutualisms.

Although many defenses are detectable at a distance, from the point of view of a potential attacker, e.g. horns on a ram, we will highlight examples from both detectable and undetectable defenses, e.g. chemical toxins. When defenses are undetectable, the selective pressure to advertise the trait may lead to new interactions with new partners. In many cases, the deterrent trait is present in closely related species within a lineage. When paired with traits that can become cues for a reward, mutualism may evolve with the potential for signal evolution driven primarily by those partners most likely to pose a threat. In cases where new partner interactions are beneficial to the fitness of the organism, selection on the signal may lead away from an arms race in favor of evolution of a new mutualism. Our aim is twofold; first for experimentalists, we highlight systems that could lead to a better understanding of how widespread this mechanism might be and attempt to identify traits that may lead to a better understanding of assortment in diverse mutualisms. Our second goal is to stimulate theorists to explore the evolutionary dynamics of the proposed mechanism, to gain a better understanding of the role of these types of traits in the evolution of asymmetric and diffuse mutualisms. Progress in both of these camps should help biologists better understand the role of both positive and negative interactions in structuring and creating the biodiversity of communities. There may be key traits that can play a role in relaxing constraints on the types of interactions between sets of players. The phenotypic diversity in some lineages may result from this interaction plasticity spurred by signal traits. Although great strides in cataloguing the taxonomic diversity of the biosphere have been made in the last century, our understanding of biodiversity lacks the same depth of understanding linking key traits with key interactions. By integrating our understanding of key ecological interactions

with the traits that are integral to their evolution, we will be better equipped to understand the mechanisms that drive and maintain biodiversity. We will outline key stages and associated innovated traits that may aid our understanding of evolution in diffuse mutualisms: defense, attraction, tolerance and assortment.

4.1.1 Defenses as a gateway to mutualism?

Phylogenetic analysis suggests that extrafloral nectar first evolved as a defense against herbivores (Blattner et al., 2001; Davies et al., 2001), are invested in when herbivores attack (Heil et al., 2000; Linsenmair et al., 2001), then serve as a reward for mutualist ants. Subsequent alteration of the composition of the nectar and increased investment in food bodies attract ant species that are most beneficial (Heil et al., 1997), while wax coatings deter species that are more costly to the plants (Federle and Rheindt, 2005). The combination of these defensive traits co-opted for rewards and morphological changes has promoted the evolution of mutualism independently in several lineages (Davies et al., 2001). This increased interaction with partners that add to the fitness of the plants has allowed *Macaranga* to expand into new environments (Quek et al., 2004). In many systems, the cost of an exploitative response can range from negligible or slight to fatal (Bronstein, 2009), and the ability of a set of traits to lead to positive assortment may change in new environments. Despite this potential variation in risk among the members of the interaction environment, diffuse mutualistic networks do evolve in nature, more frequently than once believed (Waser et al., 1996; Stanton, 2003; Bascompte and Jordano, 2007; Marussich and Machado, 2007). The mechanisms of evolutionary shifts toward specialization, generalization, or into new interaction networks are not as well understood (Waser et al., 1996; Johnson et al., 2010). A territorially restricted organism stands to gain if it can capitalize on opportunistic

predators that are likely to notice its signals. Even specializing on risky partners could be a byproduct of the necessity to unambiguously signal to them.

Any cue associated with a potential reward or punishment can become a signal to the informed receiver. Any receiver has the potential to act cooperatively or to act defectively, in a way that exploits the focal mutualist to its own gain. As a result, in the face of risk, selection on organisms with physical or chemical defenses that deter cheaters or non-mutualist predators may be substantial enough to change traits over evolutionary time. An arms race could be one trajectory, but if the attack style or damaging mechanisms of predators are diverse, specializing on any one trait may become an Achilles heel rather than a mechanism to “escape-and-radiate” (Ehrlich and Raven, 1964). For example, *Macaranga* plants provide specialized housing structures (domatia), food bodies, and extrafloral nectar for mutualist ants in exchange for protection from herbivores. An increased investment in the rewards (originally a defense) correlates with a tradeoff of constitutive chemical and physical defenses toward attackers, and subsequent increased reliance on ant defense (Nomura and Itino, 2000). In this case, morphological exaptations may have led to increased ant patrolling/inhabitation, capitalizing on ant species that will eat herbivores, regardless of where they are found. *Dalechampia* plants evolved resin defenses, which have been exapted for bee-pollinator rewards (Armbruster et al., 1997) and are positively correlated with honest signals for resin (larger bracts) (Armbruster et al., 2005). *Dalechampia* show increasingly escalated physical defenses against exploitative resin-collecting bees after resin became a reward rather than a defense (Armbruster et al., 1997). The enlarged bracts, likely to have evolved as an attractive signal to pollinators, later became a physical defense, an additional layer of protection along with sharp trichomes and sepal coverings over seeds (Armbruster et al., 1997). *Gossypium* plants contain small needle-like trichomes used as physical defense against herbivores (Rudgers et al., 2004). Both

trichomes and extrafloral nectar are used to deter attack in some species (Rudgers et al., 2004). Some anti-feedant toxins (Wackers and Wunderlin, 1998, Rudgers and Gardner, 2004) are also produced in more derived species (Wu et al., 2007). The extrafloral nectar can attract additional species (Rudgers and Gardner, 2004). Subsequent defense traits seem to be added innovations within the lineage, but how these traits vary among species and to what extent they deter attack and attract new mutualists or herbivores is not clear. The simultaneous effects of mutualists and attackers on traits has begun to be addressed in ecological timescale studies (Adler et al., 2004; Irwin et al., 2004; Johnson et al., 2006) but evolutionary explorations into the key drivers of selection in mutualisms are harder to find.

In the cases we discuss above, most of the defenses invested in by the focal organism are detectable and potentially deterrent from a greater distance than that of a potential physical interaction. In summary, to the extent that a cue for a defense or a reward can become a signal, it serves to assort cooperative behaviors from potential interactors, i.e. to become a method of expanding the potential pool of beneficial partners in the environment, even when some are antagonists. With adequate defenses, an increased frequency of total interactions has the potential to add to the accumulation of positive behaviors. Attracting uninformed partners may be a crucial part of signal evolution. If a defense pre-dates the signal itself or serves as the signal, then mutualisms stand to be affected by evolutionary pressures by predators and antagonists just as much, if not more than, by benign cooperative partners.

4.1.2 Adding beneficial interactions- attraction signals

Potential cooperators can be thought of as participants in a biological market (Noë and Hammerstein, 1995). For many mutualists, the primary means by which cooperative behaviors from the interaction environment (i.e. the surrounding pool of

partner organisms with which interactions may occur) are accumulated is by attraction of partners. In many cases, the production of a reliable signal that alerts potential participants of a reward is a way to achieve competitive advantage over one's neighbors (Schaefer et al., 2004). Environmentally derived costs associated with the production (or lack of production) of such a signal could be measured as an opportunity cost. Individuals in the environment that do not "correctly" interpret or react erroneously to a signal (Wiley, 1994) are primarily a lost opportunity for a beneficial exchange, but there may also be a risk involved in an erroneous response to the signal. The signal should therefore function and be subject to selection in two ways, to attract but also to warn against exploitation. In this case, the balance of the costs and benefits are derived from the cost of making the signal and the added benefit of attracting cooperators, to the extent that exploitation can be deterred. This way of thinking about interspecific mutualisms aligns most closely with that of Doebeli and Knowlton's 1998 theoretical model, where evolution of mutualism easily occurs and is sustained with increasing investments among partner classes. In this model, partner classes compete with members of their own kind and can evolve to increase investments in exchanges with the trading class. Although signaling was not explicitly included in their model, investment in a signal that was indicative of a potential mutualistic reward would have a net return as long as an increase in partner interactions yields higher fitness compared to one's neighbors. Organisms are bound to vary in the quality of the return yielded by partners chosen (Roberts et al., 1999, Fenster et al., 2004). Mobile mutualist partners are likely to use search strategies to attempt to interact with individuals with a higher yield (Hanley et al., 2008). Signals may play a role in this search, and if they attract partners with the cooperative behavior, will have a potential fitness benefit; without a signal, there is a potential opportunity cost. There is certainly variation in the quality and types of partners that the signal can attract (Schemske and Horvitz, 1984; Palmer et al.,

2003), which could place selective pressure on the signaler to tailor the signal for higher pay-offs, or lower-risk. Some signals operate by mimicking a cue that a particular partner group uses for a completely separate function — intraspecific communication for instance (Edwards et al., 2007). This has been termed a sensory trap (Edwards and Yu, 2007). The benefits of such a signal are high partner fidelity, but the costs could include lost opportunities for additional cooperative partners. Organisms use various modes of communication, which can become signals to interspecific partners. Treehoppers use vibrational signals to signal the location of attack when disturbed by predatory ladybeetles. In exchange for the honeydew that treehoppers produce as waste, ants respond to the same vibrational cue produced by the homopterans with increased patrolling and attacking of predatory beetles (Morales et al., 2008). These vibrational signals are likely to be exaptations of intraspecific communication (Cocroft, 2001; Cocroft, 2005; Ramaswamy and Cocroft, 2009), and have been coopted in an interspecific mutualism. Figs use volatile chemical cues to attract specific fig wasp partners (Grison-Pigé, 2002). Volatile chemicals are also used by many plants to respond to herbivore threats. Intraspecific and interspecific signals allow them to produce added defensive chemicals (Dicke and van Loon, 2000). Fungi produce anti-feedant chemicals that attract gamete dispersers (Schiestl et al., 2006). Enhancement or amplification of a signal can increase the behavioral response of available partners or increase the types of partners that could detect the signal, a subset of which may pose potential risks (Schaefer et al., 2004). Added investment could increase detection by possible predators or attackers (Adler and Bronstein, 2004; Schaefer et al., 2004), so it is perhaps not surprising that some of the attractive cues mentioned are likely evolved from deterrent ones (Grison-Pigé et al., 2002; Pellmyr and Thien, 1986). Even relatively longer range visual cues like color may evolve initially for deterrent reasons.

Dalechampia and *Acer* flowers may exhibit ranges of colors that are a byproduct of

genetic pleiotropy with vegetative tissues that produce anthocyanins for protective reasons (Armbruster, 2002). We know surprisingly little about the signals that attract mutualist partners, to what extent they serve multiple purposes (but see Gronquist et al., 2001 and Irwin et al., 2004), and to what degree they increase the risk of detection or change the behavior of non-mutualists.

Attempts to correlate attraction and mutualistic behavior from the major or most abundant partner group with specific traits thought to be evolved to be most conspicuous to them are limited (but see Bradshaw and Schemkse, 2003) and often inconclusive (Dyer et al., 2007, Martin et al., 2008). Indeed, breaking potential interactors into functional groups based on their potential for detection of signals and for damage to the focal species may give greater power to predict pathways between attraction traits and partner behavior (Baack et al., 2008). Further phylogenetic and genetic explorations of signals of attraction, and how mutualist partners react to them, would help to better understand the evolution of signals and what role they play in speciation (Sapir, 2009). We highlight the role of attraction because much attention on ecological interactions and trait evolution focuses on how mutualist traits may be shaped by potential partners, specifically the most abundant ones (e.g. Floeter et al., 2007). These may or may not be the most critical interactions for the focal mutualist, but attention on these partners as the most important may be biased simply as a matter of their frequency. Perhaps, this attention is leading us to only see part of the story; because signals, by virtue of being conspicuous, alert not only intended but unintended receivers, they may be under selection by less abundant but more dangerous individuals within the environment.

4.1.3 Coping with attacks when detection occurs – tolerance and the evolution of mutualism

Despite the fact that some potential attackers will be deterred by defensive traits, still others may find ways around a defense or can become specialists on them. For example, to cope with attack, *Ipomopsis* and *Polemonium* plants (Polemonaceae) have tolerance — capacity to fully compensate for lost tissue by regrowing it and maintaining fitness at undamaged levels — to herbivory (Juenger and Bergelson, 1997). In other words, they do not escape from or deter herbivory but they are able to cope with the damage inflicted in a way that lessens the impact on fitness (Rosenthal and Kotanen, 1994). Organisms with perfect tolerance to their attackers show no fitness costs even after damage (Strauss and Agrawal, 1999). They compensate for herbivore damage by growing additional flowers (Edwards and Yu, 2008) or by reallocating resources (Juenger and Bergelson, 1997). Tolerance has received some attention as a possible key trait in the ecological stability (Oliver et al., 2009) and evolution (Edwards, 2009) of mutualisms. Although compensation after attack is key to survival, we will argue that without defenses, a mutualism is unlikely to evolve.

Sphaerosystis schroeteri phytoplankton suffer an average of 10% mortality when consumed by *Daphnia spp.* grazers and other zooplankton, but the 90% surviving gut passage grow much faster than if not eaten, and more than make-up for this 10% loss (Porter, 1976). The primary reason for the increased growth is accumulation of nutrients from the gut of the grazer. The reason that 90% are able to survive the passage through the gut is by production of a dense polysaccharide matrix surrounding the phytoplankton cells (Porter, 1976). This species increases in density the more it is eaten, which for clonally reproducing organism means increased fitness. The tolerance is only achieved with a defense and added nutrients. In some contexts, this association between grazer

and phytoplankton could be mutually beneficial, but only with an external defense that makes the plant prey less vulnerable.

Experimentally damaged (clipped) *Ipomopsis* plants have overall lower seed-set than both pollen-limited and nutrient limited plants; additionally, tolerance to flower damage in one study was only achieved in plants that benefited from supplemental pollination (Juenger and Bergelson, 1997), suggesting that this method of fitness compensation depends on additional resources or cooperative partners from the interaction environment. Without any active recruitment of new partners or a resulting mutualistic outcome with some subset of them, averting an arms race may be possible but imperfect (Strauss and Agrawal, 1999; Stowe et al., 2000; Weis and Franks, 2006) and mutualism dependent on additional interactions.

Aphids in subgenus *Aphis* are able to sequester toxins from the plants on which they feed (Pasteels, 2007), yet tolerance to predation has been suggested as a key mechanism promoting the shift to mutualism (Edwards, 2009); as clonal organisms, they can lose individuals to predation but remain genetically viable. They may turn potential predators into mutualistic partners with the honeydew rewards they produce (Edwards and Yu, 2008) and are immune to a level of exploitation or cheating among potential partners. Many species in this lineage are facultative or obligate mutualists with ant species, which provide protection from predators in exchange for the nutrient-rich honeydew excreted by the aphids as a waste product (Stadler and Dixon, 2005). Aphids that are protected by ants must also compete for ant partners (Fischer et al., 2001) and honeydew is altered by the aphids to induce cooperative rather than predatory behavior in the ants (Völkl et al., 1999). It is true that ants may prey on their farmed partners when excess sugar is available (Offenberg, 2001), but the fact that this behavior is only induced when an alternative sugar source is available suggests that the aphids are not a typically preferred protein source and have turned a predatory interaction into a

mutualism by virtue of an associated reward. It has previously been suggested that tolerance to some predation in these clonal organisms can stabilize (Edwards and Yu, 2008; Oliver et al., 2009) and promote the evolution of cooperative behavior between potential foes (Edwards, 2009). We suggest that a more fundamental mechanism is a defense against exploitation that has shifted to become a reward to risky interactors. In the case of ants and aphids, the mutualism has become the new defense.

Morphological adaptations for feeding that increase the chances of being attacked can increase the chance that a waste product could become the reward leading to facultative mutualism (Shingleton et al., 2005); without the honeydew and some characteristic that makes them an otherwise less palatable choice for ants, there is no reason that ants would refrain from eating an otherwise available food source.

Datura plants (Solanaceae) are hawkmoth pollinated (Alarcon et al., 2008). *Sphingidae* hawkmoths have herbivorous, damaging, larvae and are the major pollinator of this lineage. *Datura* spp. have tolerance to grazing (Espinosa and Fornoni, 2006) and are pollinated by several different species of hawkmoths (Alarcon et al., 2008). Adult variation in pollination of plant species covaries with the level of diet use, and all species used as hosts by moths are *Solanaceae* (Alarcon et al., 2008; Hodges, 1971). This suggests that those moth species most damaging to the plants are also their most specialized pollinators. The hawkmoth, *Manduca sexta*, actually gain more nectar reward for their effort in co-occurring *Agave palmeri*, but *Datura* attract enough hawkmoths with an odor signal to ensure reproduction and nearly exclusively rely on this herbivorous species for pollination (Riffell et al., 2008). The intertwining of risk of detection, tolerance to damage and attraction of added cooperators may drive the evolution of attractive traits like nectar to lead to higher specialization on risky partners that can also impart added cooperative behaviors. Experimentally enhanced nectar levels in *D. stramonium* caused increased oviposition by *M. sexta* (Adler and Bronstein,

2004) but it is not known how the nectar affects oviposition by other potential herbivores. The roles of defense against and tolerance to increased risks in new environments or with new types of partners may play a central role in the evolution of mutualisms and the traits contributing most to their evolutionary success. In many of the cases discussed, a particularly risky or damaging interaction becomes a mutualism because a trait is able to increase cooperation as compensation or supplemental defense. Those individuals able to prevent damage and accumulate cooperative behavior from a more risk-prone pool of partners could further increase mutualism-derived fitness. We will return to the signal as a potential mechanism to both attract cooperation and to deter exploitation, rather than tolerate it.

4.1.4 Mutualism as the last defense- assortment in a risky world

As seen in many examples already described, the consequence of risky interactions may lead to avoidance/reversal of an arms race and increased reliance on cooperative interactions under certain sets of circumstances. Many particularly successful and species rich mutualist lineages share the possession of a hidden defense (i.e. a defensive trait that is not readily detectable without a physical interaction); it is often but need not be chemical. This undetectable defense can make cheating or attacking a less profitable course of action with these species *relative to their neighbors*. The existence of this undetectable defense can be advertised with a cue or signal. It is likely to promote mutualisms with a more diverse interaction network if that same cue can also be associated with a reward. Table 4.1 summarizes examples of lineages that seem to capitalize particularly well on this strategy; they include representatives from different classes of mutualisms where the interaction involves exchange of services or goods, including protection, nutrition, and transportation (Bronstein, 2009). Within these.

Table 4.1 Diverse lineages of mutualists with defense traits and advertising signals likely to be particularly visible to risky partners.

Lineages:	Traits:	References:
<i>Asclepias</i>	Cardenolide toxins, increased contrast to herbivores	Agrawal and Fishbein 2006, Farrell & Mitter 1998, Zehnder & Hunter 2007, Kephart & Theiss 2003
<i>Aphids</i>	Tolerance, sequestration of toxic alkaloids, dark pigmentation	Coeur d'acier et al. 2006, Winke et al. 1982, Witte et al. 1990
<i>Elacatinus</i>	Putative chemical distastefulness, stripes with conspicuous coloration	Taylor and Helberg 2005, Lettieri et al. 2009, Lettieri and Strelman in review
<i>Hakeas</i>	Cyanide production, increased contrast to vertebrates	Hanley et al. 2008
<i>lochroma, Dunalia, Nicotiana</i> (Solanaceae)	Withanolide immunosuppressants, increased contrast to vertebrates	Smith, Ane, Baum 2007, Knapp 2010

categories, we may expect slightly varied evolutionary trajectories with respect to defense and signal.

As mentioned in part 4.1.3, some aphids may have adaptations that make them particularly able to turn potential ant predators into protectors. *Aphis jacobaeae* (Toxoptera) sequester quinolozidine alkaloids (Witte et al., 1990), which are able to deter predators (Wink and Römer, 1986). They also sequester high concentrations of pyrrolizidine alkaloids (Witte et al., 1990). *A. cytisorum* (Wink et al., 1982) and *Acraccivora* (Mendel et al., 1992) also sequester toxins from their host plants. The species in this subgenus share black coloration patterns, which presumably make them more conspicuous on their plant hosts than green counterparts. This dark and conspicuous coloration is shared by two monophyletic lineages more derived than the chemically-defended, but non-mutualistic *Aphis nerii* (Coeur d'acier et al., 2006); *Aphis nerii* sequester cardenolides (Malcolm, 1990), are non-mutualists and have bright yellow coloration, from which the black phenotypes have evolved. In fact, the combination of some potential for chemical sequestration and conspicuous coloration may have allowed aphids to colonize new plant microhabitats; polyphenic clonal colonies produce winged alatae that can fly to new locations (Mondor et al., 2007). This lineage of aphids (Coeur d'acier et al., 2006) appears to be species-rich and has many representative species that engage in facultative mutualism with ant species.

A lineage of Australian plants have shifted from pollination by insects (basally) to, more recently, passerine (and potentially florivorous) birds (Hanley et al., 2008). Among the 51 species of *Hakeas* analyzed, the 9 most basal species contain no detectable levels of cyanide, a feeding deterrent to vertebrate florivores (Rafferty et al., 2005). The remaining species do contain cyanogens at varying concentrations, show reduced physical defenses, such as spines, and are more accessible with large, conspicuous flowers. Within the cyanide containing descendants, bird pollination has arisen and has

subsumed insect pollination in 20 species. Bird-pollinated groups show elevated levels of cyanide along with morphological adaptations (influencing accessibility) that facilitate pollination by birds (Hanley et al., 2008). Cyanogen levels were predictive of both bird pollination and possession of red-colored inflorescences. Increased investment in red color that conveys unpalatability most effectively to florivorous species is correlated with increased frequency of bird partners (Hanley et al., 2008). Contingent evolution tests showed bird pollination occurred more frequently in lineages of insect-pollinated plants with a private chemical defense (Hanley et al., 2008). Although red may be more attractive to birds, it is noticeably less attractive to bees (Chittka and Waser, 1997). This suggests that this signal has been subject to selective pressure to minimize confusion with non-defended syntopics rather than to expand its suite of potential pollinators.

In mutualists fishes visited by “clients” seeking ectoparasite removal services from territorially restricted cleaners, a novel stripe color has arisen among obligate species (Taylor and Hellberg, 2005). In Caribbean representatives of this mutualism, genus *Elacatinus*, a notable stripe pattern pre-dates the cleaning behavior in basal sponge-dwelling species (Colin, 1975; Ruber et al., 2003). The color of the stripe is basically camouflaged in the microhabitats inhabited by basal species (Lettieri et al., 2009). While the primary means of escape from predation by these sponge-dwellers is quick retreat into the holes of their sponge microhabitat, there is evidence to suggest that they are not as profitable a prey item as some of their highly camouflaged sister species (Lettieri and Streelman in review). Within non-cleaning sponge-dwellers, a more contrasting color stripe has also evolved, suggesting that the signal could be subject to selective pressure to alert predators that they are not a profitable prey. Cleaning evolves within the lineage, first facultatively, then obligately (Taylor and Hellberg, 2005). Microhabitat shifts to exposed coral, lacking the structural complexity offered by the typical sponge habitat, mean that interaction with riskier fishes are more likely (White et

al., 2007). There is evidence for evolution of a distasteful chemical defense that causes predators to reject the cleaners as prey several times on an initial enclosed encounter. A second encounter by the predator with a cleaner leads to longer survival and reduced consumption of the otherwise exposed prey (Chapter 3), suggesting increased investment in defense in these focal mutualists with diverse clients. These more conspicuous phenotypes (Lettieri et al., 2009) are more likely to interact with predators as clients compared to basal stripe morphs (Chapter 3) and have stripe colors that are most contrasting to them against the range of microhabitats utilized in the wild (Lettieri et al., 2009). The most conspicuous coloration occurs in species that interact with more species of predators, seem to have a means of deterring consumption even if attacked, and elicit reduced attacks in proportion to approaches by partners (Chapter 3).

Lochroma spp. plants (Solanaceae), have a diverse pollination pool and are derived from bee-pollinated ancestors (Knapp, 2010). Members are diverse in form, are a species-rich lineage of the Solanaceae, and are known to possess immunosuppressant and potentially feeding deterrent withanolides or withanines (Mann, 2001; Kaufmann et al., 2002). Withaferin A has been isolated from *Lochroma gesnerioides* (Kaufmann et al., 2002) and *I. fuchsioides* possess withaferin D and others (Raffauf et al., 1991); both share red coloration, which could be more conspicuous to larger florivores. Of fifteen species included in a recent molecular phylogeny, ten are more recently evolved (Smith et al., 2008) than these two species, and they vary in shape, coloration, and pollinator frequency. The content of chemical defense is not known for these. Withanolides were first isolated from *Withania*, members of a monophyletic insect-pollinated sister group, and are likely basal. Sister to *Lochroma*, *Dunalia* species are monophyletic, primarily bird-pollinated, and have deep purple flower coloration; at least three of seven species contain steroidal lactone withanolides (Silva et al., 1999, Lischewski et al., 1992, Luis et al., 1994). Both in the *Lochroma* (Smith et al.,

2008) and in the *Solanaceae* as a whole, no correlation between coloration and pollinator group is found (Knapp, 2010). The potential for coloration corresponding to greatest risk of florivory has not been tested, although ecological factors seem to be the most likely drivers of variation in flower conspicuousness (Smith et al., 2008). *Lochroma* flowers are not consumed by herbivores (Smith et al., 2008). Although nectar reward and flower size correlate with pollinator group, flower color and corolla length do not (Knapp, 2010). The distantly related *Nicotiana* are known to use nicotine as a potent herbivore deterrent (Euler and Baldwin, 1996) and likewise, are diverse in form and coloration, with many species pollinated by bats (Knapp, 2010). In the lineages mentioned, possession of potent chemicals pre-dates a shift to vertebrate, potentially less abundant but longer distance pollinators. Large vertebrates have the potential to exert damage on floral displays that can affect pollinator attraction (Strauss et al., 1996), but if they are turned into pollinators via recognizable signals that are honest indicators of non-food status and a reward, then the potential for increased pollen transfer and longer distance gamete dispersal is exploited to the plants benefit.

Large vertebrate sunbirds are also perching bird pollinators of milkweeds in South Africa (Pauw, 1998), where there is an incredible diversity of both species and pollinators (Shuttleworth and Johnson, 2009). Little is known of the levels of the potential chemical defenses in this particular lineage, but *Asclepia* plants, commonly called milkweeds, are a diverse, chemically-defended and species rich lineage (Agrawal et al., 2009). They possess several layers of defenses against potential attackers, including possession of spiny trichomes, latex and tissue toughness (Agrawal and Fishbein, 2006). Major herbivores on the plants include beetles and juvenile caterpillars of the adult Monarch pollinating butterflies, which are negatively affected by these physical defenses (Agrawal and Fishbein, 2006). Some types of defense traits in this lineage are correlated, for instance trichomes and latex production (Agrawal and

Fishbein, 2006) and may work in concert against particularly damaging herbivores, like caterpillars. The major chemical defenses possessed by many in this lineage are forms of a cardenolide steroid that is toxic to most animals including the caterpillar juveniles of the diverse pollinators (Dussourd and Hoyle, 2000; Malcolm, 1991). This private defense has wide variation in concentrations, ranging from undetectable to highly toxic. Color cues could aid in both attraction and defense. Monarchs, the main pollinator of the most toxic species, learn to avoid and attract to color signals (Rodrigues et al., 2010), which could aid in turning damaging larvae into adult pollinators. *A. tuberosa*, the butterflybush, however, is pollinated more by bees than by butterflies, suggesting that traits thought to be most attractive to butterflies do not in fact attract more of them (Fishbein and Venable, 1996). It is also of interest that milkweeds diversified early with an adaptive burst, coinciding with a reduced investment in latex and cardenolides in the most species rich groups (Agrawal et al., 2009). It would be informative to see how attraction of pollinators changed with the divestment in defenses, as fitness losses due to increased interactions with herbivores could be mitigated by increased pollination (Strauss, 1997). New World species have a more diverse pool of pollinators, including many more Lepidopteran and Hymenopteran species compared to predominantly fly-pollinated Old World lineages (Ollerton and Liede, 1997).

The primary force driving trajectories of both visible and hidden defenses and signal traits in many systems is likely natural selection; competition for space or resources limit the ability to derive total fitness from individual acquisition, leading to an increase susceptibility to parasites or predators. Since signals are costly, especially in these situations, they are likely to be ecologically pleiotropic, whenever possible. Both attracting aid and avoiding attack become important and signals that can serve dual purposes would be competitively favored. When deterrence by physical, chemical or other means become limited, then alternative strategies will be favored, including

mutualisms if an association between cue and reward can be enforced in some risky partners. Some plants, clearly limited in their ability to avoid mobile grazers, have been shown to be under selective pressure to alter flowering phenology (the time at which flowers open and display) in response to the activity time of herbivores (Pilson, 2000). These avoidance tactics can even drive shifts in the main class of mutualist pollinators (Kessler et al., 2010). Physical defenses can be used to deter exploitation, and when mutualistic interactions are crucial to achieve competitive fitness levels (e.g. self-incompatible plants, fish that need food, aphids that need protection), players that can be behaviorally assorted, even when interactions with them are relatively rare or transitory, will contribute to fitness (e.g. Fleming et al., 1993; Fleming et al., 2001). Suites of enhanced defenses can and do exist (Armbruster et al., 2009; Agrawal and Fishbein, 2006). When a hidden one evolves, there is the potential for asymmetric information within this biological market to select for a signal that allows potential attackers to distinguish between those that are likely to have the defense and those that are a potentially safer prey choice, before deciding whether to attempt consumption. This is especially true when the focal mutualist must derive some of its overall fitness from interactions with others, and therefore cannot avoid all interactions by means of escape (a purely resistant or defensive strategy). Signals adapted for one reason are likely to be coopted for new uses when the interaction environment changes.

4.2 A theoretical framework

A theoretical framework for signals and defense as key traits in assortment

We have presented a set of circumstances where we expect signaling to be driven more by the potential to avoid negative interactions than to attract positive ones; we suggested types of traits (defensive and signaling) that are likely to contribute to this trajectory, and provided examples where ecological pleiotropy in certain traits may release evolutionary

constraints on species. It is our aim to also present a theoretical model that may aid in exploring the dynamics of these traits in assorting among signal phenotypes, and perhaps leading to speciation.

Recent theoretical work using the public goods game framework (Fletcher and Doebeli, 2009) has highlighted the role of assortment as a fundamental mechanism to explain the evolution of cooperative traits. Assortment puts the focus of selection squarely on the “interaction environment” of the altruistic individual and, by implication, on the altruists’ ability to derive cooperative behaviors via communication. Specifically, net gains are a sum of the average payoffs from the interaction environment plus any additional payoff due to the cooperating, if one has the cooperative trait. Because we will focus on population level variations in interactions, Table 4.2 summarizes the payoffs at the population level, but the parameters are an extension of factors that promote cooperation at the individual level (Fletcher and Doebeli, 2009). The e_C and e_D terms are the average number of cooperative and non-cooperative *behaviors*, respectively, received from the neighborhood of focal individual. In an interspecific mutualism, the cooperative behavior of the individual would be decoupled genetically from the cooperative behaviors of the interaction environment, but the evolution and stability of the mutualism are favored if cooperative *genotypes* gain cooperative *behavioral interactions* from their environment. The benefit of a cooperative trait/behavior is b , and the cost of a cooperative trait/behavior is c . If an individual’s ability to derive benefits received from others in the interaction environment outnumber the costs imposed by altruistic traits then mutualism is evolutionarily favored (Fletcher and Doebeli, 2009).

Altruistic traits can only increase in a population if the net benefit received by cooperative *behaviors* from others outweighs the costs of the helping behavior to the individual, thus assortment is key (Fletcher and Doebeli, 2009). The evolutionary stability of the altruistic trait critically depends on its ability to *elicit* more cooperative than

Table 4.2 (Adapted from Fletcher and Doebeli 2009) The payoff matrix of cooperative and defecting phenotypes in a public goods game.

In an interspecific mutualism, the cooperative behavior of the individual is decoupled genetically from the cooperative behaviors of the interaction environment, but the evolution and stability of the mutualism are favored if cooperative genotypes gain cooperative behavioral interactions from their environment. The benefit of a cooperative trait/behavior is b , and the cost of a cooperative trait/behavior is c . If an individual's ability to derive benefits received from others (N) in the interaction environment outnumber the costs imposed by altruistic traits then mutualism is evolutionarily favored. The e_C and e_D terms are the average number of cooperative and non-cooperative behaviors, respectively, received from the neighborhood of focal individual.

Behavior phenotype	Pay-off received from individual's behavior	Pay-off received from average interaction environment, derived from others' behaviors	Average total pay-off
cooperate	$(b/N) - c$	$e_C b/N$	$(e_C b/N) + (b/N) - c$
defect	0	$e_D b/N$	$e_D b/N$

defective or cheating behaviors from the individuals in its interaction environment. A common constraint among many mutualists is that they are territorially restricted compared to other species in their interaction environment, and must both attract cooperators with signaling traits and simultaneously deter non-cooperative behavior; experimental evidence that traits are simultaneously expressed to perform these functions show that both are key in the assortment process (Kessler et al., 2008). The traits most likely to actively align *cooperator* with *cooperation* in its environment are those involved in signaling.

Selection for honest signals using economic models, as first described in a biological context by Hammerstein and Hagen (2005), can be explored theoretically by comparing groups of individuals with private (or asymmetric) information. Signaling models developed originally to explain investments in education (Spence, 1973; Spence, 1974), can be applied to mutualists with different levels of private defense. We believe that private defenses, primarily chemicals ones, may be key traits in the evolution of some mutualisms. For organisms that evolve signals involved in mutualistic interactions, the cost of producing the honest signal is partly in the production of the signal itself and partly in the *added* risk of being detected. The difference in benefit comes from differential ability of the signal to act also as an attractor for cooperative behaviors or a deterrent to non-cooperative behaviors.

4.2.1 Assortment and selection for signal divergence: an economic model

Adverse selection (Spence, 1973; Spence, 1974) describes an economic phenomenon whereby investment in a signal is likely to have a separating equilibrium between two groups if two assumptions are true: first, the return on incremental investment in the signal for one group has a different “marginal utility function” than for another group in the same local environment (Figure 4.1) and second, the behavioral

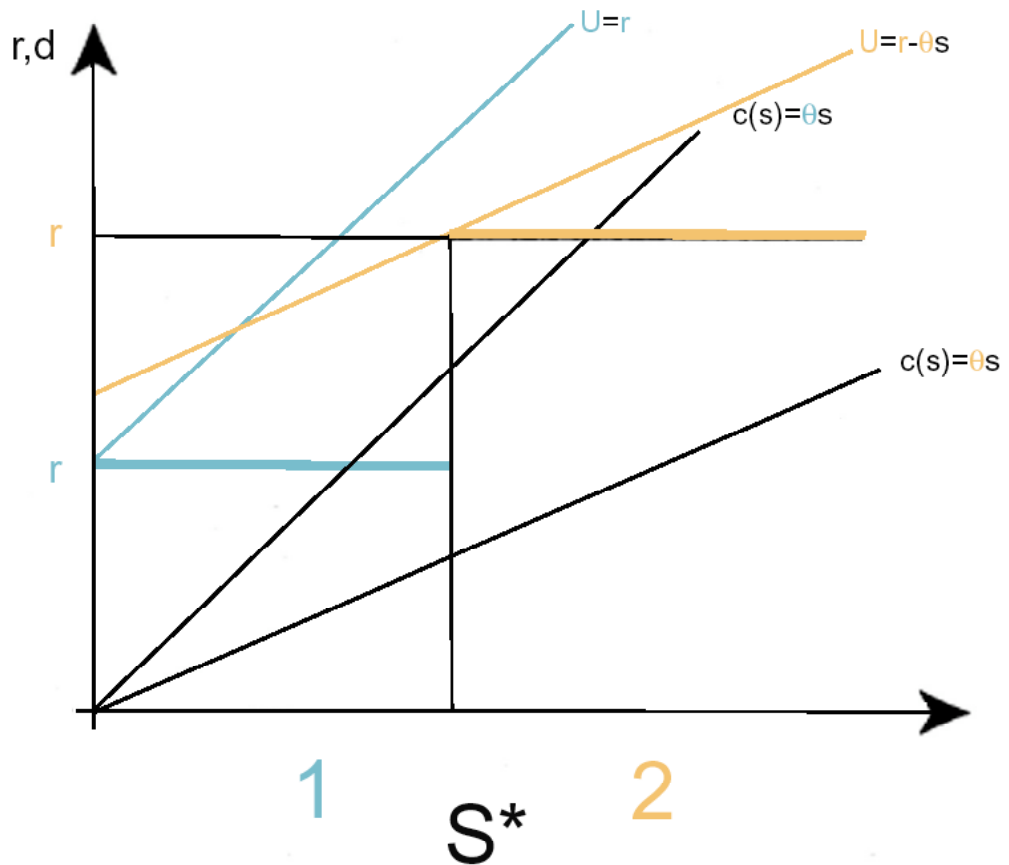


Figure 4.1 Spence model for adverse selection in signal investment.

Differential fitness gains (r) as a result of differential investment in the signal (s) at each level of signal (r_1 and r_2) as it is likely to honestly reflect the unknown information - defense level of potential prey (d_1 and d_2). For a signal to be successful, there has to be differential costs of investing in signal for high and low quality "prey". This cost is a cumulative fitness cost of producing signal plus added detectability to *risky* predators. The total cost of producing signal (c) is the metabolic cost plus increased exploitation as a result of detection. Added fitness (r) will be a result of signal investment's effect on risky partners' behavior. The utility function (U) is the marginal value of investing in signal. Orange group 2 individuals have an increased marginal utility function of investing in the signal because the cost to them is minimized by their ability to accumulate positive interactions from signal receivers. This is likely to happen if the signal serves dual roles, one for defense and one for attraction. The symbol θ denotes "effort"/cost per unit signal gained. In other words the detrimental effects of increased signal investment divided by added beneficial behaviors due to assortment. Orange group 2 individuals have a decreased "effort" for each additional unit of signal as a result of added beneficial interactions.

$$\beta(\theta|s) = \frac{p_i(s)\beta_i}{\sum_{i=1}^2 \beta_i p_i(s)}$$

Figure 4.2 Posterior probability of potential exploiter class differentially assorting according to defense and signal level (i).

Conditional beliefs (B) of potential partners must be differentially influenced by signal (s) between groups. If there are two groups, one with steeper utility function and one with lower cost per unit investment in signal, then selection can favor assortment between the groups. One group will be better equipped to turn signal into added fitness (θ). The posterior probability (p) of the signal influencing behavior of the selecting partner differentially between groups is a key assumption for adverse selection.

action of the partner class, whether to act cooperatively or defectively, is influenced by its belief that the signal honestly conveys information about a private (not externally observable) trait (Figure 4.2 equation) (adapted from Bolton and Dewatripont 2005). More explicitly, the marginal cost of investment in a signal for those belonging to the group with more to offer or less to lose (orange group 2 in figure) must be lower such that the investment is compensated for by the conditional action taken by the partner class (see Figure 4.1 for detailed explanation of parameters). Likewise, the marginal investment must be prohibitively high for those with more to lose and less to offer. For instance, if blue group 1 is a defended population and orange group 2 is also a defended population, a signal investment has a lower overall fitness “effort” if signal both communicates defense and attracts cooperative behaviors. This model can translate into evolutionary scenarios for mutualist and antagonist partner interactions because it shares some key characteristics and players with the signal model: classes of competitors with a similar potential “interaction” pools have externally visible traits (Lázaro et al., 2009), differing net gains to an investment in a signal trait based on interaction outcomes (Schemske and Horwitz, 1984), heritable variation in a private (defense) trait (Memelink et al., 2001).

It is important to note that for signaling to be favored, those individuals that are actually of higher quality need to be better at converting advertising into fitness (Getty 1998a; Getty, 1998b). In this context, pre-adaptations that select for increased positive interactions and a trait signaling defense can be co-opted in an ecologically pleiotropic way. Mutualists that rely primarily on generalist partners may have suites of traits under the strongest selection primarily from less frequent, riskier encounters that could yield added fitness with behavioral assortment, rather than from their most frequent and critical mutualist partners. As an additional example that defense traits may be critical to the evolution and continuation of mutualisms, ant-acacia mutualisms broke down after

experimental removal of large herbivores in the African savanna (Palmer et al., 2008). Paradoxically, this may mean that in many nested mutualism networks, specialized mutualists that interact with the most generalist of partner species in asymmetric mutualisms (Bascompte and Jordan, 2007) have traits that have been driven by selective pressure from rarer but risky predatory species, attackers or non-mutualists.

Although not all mutualisms are diffuse, evolutionary biologists may profit from thinking of mutualisms within the context of a biological market with two sets of competing classes (Doebeli and Knowlton, 1998), since transient interactions likely lead to more repeated and potentially coevolved interactions over evolutionary time. Before traits that tightly couple unrelated species to one another, more transient interactions among species with varying potential to exploit the focal organism must have pre-dated coevolution. Specialized forms of attraction do not necessarily imply specialized partner mutualisms. Generalist partners can be used by different specialists, and can lead to diversification of color type to avoid competition (e.g. Muchhala, 2003; Muchhala, 2008). Likewise, pleiotropy between pigments in vegetative and floral structure or parallel evolution in different directions can also lead to diversification without pollinator specialization (Armbruster, 2002). The idea of correlated traits that best avoid exploitation and attract partners has been discussed and even tested, but identifying the key traits and tracking their evolutionary trajectory has not received much attention (Herrera et al., 2002). Ecological studies suggest that herbivory seems to have little effect of seed set, but when herbivores are present, pollination can have a highly positive effect, suggesting that increased positive interactions with some subset of antagonists can increase fitness (Herrera et al., 2002). Defense and mutualism can interact and result in exploitation (antagonism) having greater selective effect than cumulative effects of mutualist partners (cooperators) (Herrera, 2000; Gomez, 2003; Gomez, 2005). It seems that both private deterrent defenses and coping with risk by

Table 4.3 A list of predictions for mutualist signal evolution when defense is an important driver.

If signal evolution is driven primarily by the ability to deter attacks or cheating, then the trait may respond to selective pressures other than mutualist partner interactions, which are often the focus of studies of trait evolution in diffuse mutualisms.

Predictions:	Factors likely to contribute to signal level:
Signal more detectable when risky partners are more abundant or opportunities to avoid are limited (e.g. due to reduced places to hide)	<ul style="list-style-type: none"> • Level of private defense, competition for predator/exploiter-free space or time • Diversity of possible attackers • Abundance of possible attackers
Signals enhanced where overlap/possible confusion with less defended species occurs	<ul style="list-style-type: none"> • Search territory of exploiters • Percent distribution of defended and less defended species • Errors made by potential attackers
Defended species with an enhanced signal are able to move into new environments	<ul style="list-style-type: none"> • Competition for resources or enemy-free space • Interaction environment of new habitat • Conditional behavior of potential partners in new environment
Defense plus enhanced signal relaxes constraints on interactions and may lead to more diffuse or diverse mutualism	<ul style="list-style-type: none"> • Local interaction environment • Potential for specialization or increased reliance on mutualism • Signal efficacy to broad potential pool of partners

increasing cooperative behaviors among potential attackers may contribute to signals that will ultimately serve as traits of assortment of potential partners into mutualists and non-mutualists. The predictions listed in Table 4.3 summarize some investment in signals for some mutualist species, which could in turn be tested for evidence of adverse selection.

4.3 Further discussion

How likely is it that defenses under selection are a common mechanism leading to mutualism? How can we account for the total fitness from all potential cooperators and cheaters if they are diffuse and diverse? Without phylogenetic histories, we cannot be sure of degree of specialization on riskier types of partners compared to the ancestral state. Without careful analysis of interactors and the added fitness they contribute (or detract), then it is difficult to gauge which signal traits are causing assortment among cooperators. If the primary risk is damage, then a signal that alerts of a defense will have a higher effect on fitness and may be most shaped by risky partners. If the risky species can be rewarded for “good” behavior, then assortment has selected for a mutualism between the former foes. Do chemical defenses and signals that are widely able to assort “good” behavior from uninformed partners cause increased radiations? Could it be that wide-ranging and risky partners are able to specialize heavily on some lineages of mutualists because these are particularly good at assorting good behavior or tolerating bad behavior with compensation? A more synergistic view of traits, specifically those involved in defense and signaling, in the evolution in diffuse mutualisms may help explain why asymmetric mutualisms are correlated with diversity (Bascompte and Jordano, 2006) and why strong interaction strengths in some organisms are correlated with high mutualist to antagonist ratios (Melian et al., 2009). Exploring the effects of interaction neighborhoods on signal evolution may also be

instructive in generating phylogenetic hypotheses. Will greater overlap between groups with different levels of defense or reward select for added investment in an honest signal? Does the presence of riskier potential partners, whose conditional beliefs are subject to error or whose errors have greater fitness consequences, select for enhanced or amplified signal in the defended group and a greater likelihood of mutualism in some cases? Theoretical modeling of adverse selection in public goods games with evolution may help to elucidate the order in which signal and defense are likely to evolve. We hope that further studies, both experimental and theoretical in nature, will focus on the role of signal and defense traits in shaping interactions between mutualists from separate (genetically unrelated) biological market groups and that this will help biologists to gain a better understanding of the role of biological interactions in the generation of biodiversity.

4.4 References

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CHAPTER 5: CONCLUSION

5.1 Discussion

Because organisms must maximize fitness to be evolutionarily viable, mutualistic interactions continue to be an evolutionary conundrum to biologists. In these cooperative interactions between unrelated species, natural selection for fitness maximizing cheating behaviors could be expected to undermine the stability of the relationship, yet mutualisms are pervasive in nature. Recent research indicates that they may also be an especially important type of interaction contributing to the species richness of highly diverse systems (Bascompte et al., 2006). Therefore, identifying key traits involved in causing species to form mutualistic associations over evolutionary time may aid in our understanding of the functional and genomic underpinnings driving the architecture of biodiversity. Such traits may be critical components driving speciation.

The Caribbean *Elacatinus* gobies provided a unique system in which to test the functional role of a key signal trait in a lineage of fishes that have become increasingly specialized mutualists over evolutionary time (Rüber and Zardoya, 2005, Taylor and Hellberg, 2005). We found that the color stripe signal has evolved to become more conspicuous to a wider range of species of reef fishes (Chapter 2) and that the increased contrast potential is particularly notable to predator visual systems (Chapter 2). Because intermediate green stripes are a quantitative chromatic mix between ancestral yellow and more derived blue spectral reflectances (Chapter 2), we propose that this is an adaptive trait regulated by more than one locus in the genome and may respond to natural selection by suites of potential clients at the local scale (Chapter 2 &

3). The resulting trait function serves two important purposes: 1) it alerts predators of non-prey status and unpalatability and 2) it attracts potential clients to the territorial cleaning stations (Chapter 3). The trait function may explain why what may appear to be self-sacrificing predator behavior, refraining from attacking the vulnerable cleaner fish, may actually be self-preserving. The signal may have first evolved in this mutualism as a result of predator and prey interactions. The subsequent habitat shift to more exposed areas then facilitated the evolution of a mutualism, likely first with benign partners.

We analyzed client and cleaner interactions in the literature and experimentally tested recognizable behaviors induced in the wild to assess whether the more recently evolved phenotypes are more likely to be able to interact with riskier partner species (Chapter 3). The results suggest that defense is a primary role of the signal trait that is used as an advertisement in the mutualism, as stripes deter attacks from approaching fishes and more derived colors are more effective in this capacity (Chapter 3). There are similar types of examples in other diffuse mutualisms, like those of plants and pollinators and aphids and their ant bodyguards (Chapter 4). In these disparate systems, conspicuous signals that serve to protect the vulnerable species then allow this species to establish beneficial relationships with a subset of partners that are historically dangerous. Certain signal traits may evolve first to communicate possession of a defense to potential attackers, with subsequent expansion of the range of potential cooperative partners in the interaction environment (Chapter 4). Signal traits that are broadly conspicuous to potentially risky individuals may then be coopted in a mutualism because of their ability to elicit “cooperative” behaviors. To the extent that these visible traits can become a cue for an associated reward, high-risk partners in the mutualism may shape the signal as it subsequently becomes an advertisement to elicit beneficial behaviors from many species.

Defense is not commonly invoked as an important mechanism shaping trait evolution in mutualisms. Our research, however, suggests that in particularly species rich lineages of mutualists, it may be fundamental to the evolution and sustainability of the interactions and may influence trait phenotypes. The efficacy of a signal that provides some immunity from attack may expand the range and variety of habitats available to the organism. Signal traits in mutualisms, therefore, may in some cases be shaped more strongly by risky interactions than they are by the most abundant or effective positive interactions. Whether risk or opportunity drives trait evolution, our understanding of mutualisms and diffuse species interactions stands to gain from a critical assessment of the role of key traits over the phylogenetic history of the more vulnerable species. It is likely that signal traits are an important player in the evolution of mutualisms because of their role in influencing behaviors among the signal receivers across a variable interaction environment.

5.2 References

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