

Colourful stripes send mixed messages to safe and risky partners in a diffuse cleaning mutualism

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Abstract

The steps by which neutral, random and/or negative biological interactions evolve into mutualistic ones remain poorly understood. Here, we study *Elacatinus* gobies and the 'client' fishes they clean. Colourful stripes are common to mutualist cleaners and noncleaning sister species. Blue stripes are unique to cleaners and are more conspicuous to predators than are basal yellow or green stripes. In turn, we focused on the role of colour as a potentially specialized signal. We show that cleaners may possess a chemical defence and demonstrate that stripes are sufficient to elicit client posing behaviour and to deter attack, corroborating the putative role of chemistry. Analysis of previously published records shows that yellow cleaners interact with predatory clients less often compared to green and blue cleaners. Our results highlight evolution from predator resistance to advertising with conspicuous signals. Similar trajectories, via recognizable signals to risky partners, may be common in other diffuse mutualisms.

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 are difficult to reconcile with classical theory: mutualist genotypes are prone to infiltration by cheaters (Trivers, 1971). Work has concentrated on modelling the conditions under which mutualism might persist. For instance, iterated interactions (Axelrod & Hamilton, 1981), image scoring (Nowak & Sigmund, 1998) and spatial population structure (Nowak & May, 1992; Doebeli & Knowlton, 1998) can sustain cooperative behaviour, 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 & Knowlton, 1998).

Recent conceptual models suggest that antagonistic interactions (e.g. predation and parasitism) might shift to mutualism as resistance traits like chemical defence are modified or traded for partner tolerance (Edwards, 2009; Oliver *et al.*, 2009). For instance, *Acacia* plants produce extrafloral nectars that are more palatable to mutualist than nonmutualist ant species (Kautz *et al.*, 2009), and aphids moderate interactions with predatory ants using honeydew (Fischer *et al.*, 2001; Oliver *et al.*, 2009). 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 (colours, chemicals and behaviours) of intent. Such signals are under strong selection to communicate relevant information to the chosen partner while avoiding cheating or exploitation by eavesdroppers

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(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 colours 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 colour 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 & Hellberg, 2005). Despite similarities between the cleaner–client mutualisms of wrasses and gobies, there are striking differences. Wrasse cleaners invest in preconflict management strategies with predators and are subject to partner switching and punishment by clients (Bshary & Würth, 2001; Bshary & Grutter, 2002, 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 nonparasitized skin – whereas gobies do not preferentially refrain from cheating potential predators (Soares *et al.*, 2007, 2008c). The implication is that there may be a different mechanism by which Caribbean gobies mediate conflicts of interest with some clients.

Blue colour 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 (Rüber & Zardoya, 2005; Taylor & Hellberg, 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 & 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; L. Lettieri, observation). In the eyes of many fishes, yellow stripes are cryptically coloured 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 colours, 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 behaviour and increased conspicuousness to piscivores, we reasoned that the evolution of stripe colour 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 colour stripes and the evolution of the cleaning mutualism in *Elacatinus* gobies. First, because *Elacatinus* cleaners do not engage in preconflict management behaviours with predators, do not refrain from cheating predators and are rarely eaten by predators (Soares *et al.*, 2007, 2008c), we expected cleaners to exhibit increased survival, perhaps because of chemical defences or toxicity (Colin, 1975), compared to noncleaners, upon close encounters with predators. Second, we expected that stripe colours would be a sufficient signal to elicit 'appropriate' client behaviour (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.

Methods

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

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 1 trials as *naïve* and day 2 trials as *post-exposure*. Although the diet history of the wild-caught hamlets is not known, our day 1 trial results did not suggest that the frequency of attack on treatments differed, suggesting that prior exposure to the treatment phenotypes did not alter the rate of attack. 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 (two 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 neighbouring 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 neighbours' views. Hamlets were acclimated overnight prior to day 1 trials. On day 2, hamlets were offered the same treatment of goby (but not the same goby) as on day 1. Hamlets were not food deprived and were fed thawed scallop two hours prior to feeding trials to confirm that they would eat. All treatments of goby were fed ORAGlo™ pellets (ORA, Fort Pierce, FL, USA) prior to experiments.

Seven goby treatments were chosen to represent the colour patterns, stripe colours and ecological habits of the group – each prey goby was used only once, but each hamlet was offered the same treatment (but not the same goby) on day 1 and 2:

- (1) wild-caught (WC), cryptically coloured *Elacatinus (s.l.) macrodon* ($n = 25$ day 1, $n = 25$ day 2), the closest sister species to *Elacatinus* sponge-dwellers and cleaners (Rüber & Zardoya, 2005) was chosen as a cryptically coloured outgroup to the *Elacatinus (s.s.)* subgenus;
- (2) WC syntopic *Coryphopterus personatus* ($n = 26$ day 1, $n = 26$ day 2) 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. (L. Lettieri, personal observation);
- (3) WC yellow striped, sponge-dweller *Elacatinus horsti* ($n = 8$ day 1, $n = 8$ day 2) from Curaçao was chosen as a basal representative phenotype as yellow stripe colour and sponge-dwelling pre-date cleaning and green or blue colour;
- (4) WC green-striped cleaner *Elacatinus evelynae* ($n = 9$ day 1, $n = 9$ day 2) from Curaçao was chosen as an intermediate phenotype in terms of colour (Lettieri *et al.*, 2009) and evolution (Taylor & Hellberg, 2005);
- (5) tank-raised (TR) green-striped *E. evelynae* ($n = 11$ day 1, $n = 11$ day 2) was chosen as a phenotypic copy of (4);
- (6) WC blue-striped cleaner *Elacatinus oceanops* ($n = 9$ day 1, $n = 9$ day 2) from the Florida Keys, USA, was chosen as a recently evolved, blue-striped cleaner and
- (7) TR blue-striped *E. oceanops* ($n = 11$ day 1, $n = 11$ day 2) served as the phenotypic copy of (6).

WC and TR specimens were jointly evaluated to test the hypothesis that cleaner chemical defences, if

observed, were nonconstitutive. The colour stripes of WC and TR specimens were not statistically different when evaluated with spectrophotometric methods and fish colour vision models (Lettieri *et al.*, 2009). Neither WC nor TR yellow-striped cleaners were available. *Elacatinus evelynae* are less likely to clean on sponges than on corals (Whiteman & 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 min. 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 (time until the goby last entered the hamlet mouth) among treatments, using a right-censored parametric survival model and regressed survival time with a Weibull distribution to test for the effect of cleaning status and possession of stripe on survival, performed in R (version 2.8.1, R Core Development Team, <http://www.r-project.org>). We tested the null hypothesis of equal number of times escaped or rejected from the predator on day 1, among treatments, using a Kruskal–Wallis nonparametric ANOVA, followed by a Tukey's post-test, calculated in GRAPHPAD Prism (version 4.0.1; GraphPad Software, Inc., La Jolla, CA, USA). We tested the null hypothesis of equal percentages of surviving gobies on day 1 and 2, (i) between wild cleaners and noncleaners and (ii) between wild and laboratory-raised cleaners, using Fisher's exact test, performed in R (version 2.8.1).

Field tests with painted models

We monitored approach behaviour 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 5–9 m 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 colour copies of an *E. oceanops* (blue), *Elacatinus randalli* (yellow) and *C. personatus* (unstriped) goby. Each image was standardized to 4 cm and painted with 6–8 coats of clear liquid craft latex. The dried latex was soaked for 20–30 s in warm water, and the paper was gently rubbed away from the latex, leaving the colour image on a clear latex background. The mirror images were then hot-glued together and painted with acrylics to enhance colour saturation. Blue and yellow acrylic paints were mixed to match wavelength reflectance patterns as closely as possible, as measured from



Fig. 1 Unstriped (*Coryphopterus 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 coloured with acrylic paints; these were then deployed in front of coral heads with fishing line and lead fishing weights.

live gobies (Lettieri *et al.*, 2009) with an Ocean Optics USB2000 spectrophotometer (Dunedin, FL, USA). Paint colours fell within the standard deviation of goby stripe colours for two sample dichromat and two sample trichromat fish visual systems (L. Lettieri, unpublished data, 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 10 cm (Fig. 1).

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 5 m 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 h 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 10 m) 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 nongoby 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 behaviour and tested the null hypothesis of equal frequency among approaches to treatments using a multiple comparison of means by

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 min), (ii) one of the treatments was taken by a predatory fish away from the placement site with more than 10 min remaining in the trial or (iii) water visibility prevented identification of fishes for greater than a 10-min period.

Cleaner and client interactions in the literature

We searched the literature for observations of cleaner–client mutualism where the colour of the cleaner stripe was identified. Cleaning interactions were collected from eight articles published in peer-reviewed journals and from one published dissertation (Appendix S1). If a species was listed as a client of a cleaner goby, the colour 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 colours. We hypothesized that green and blue stripes, which are more likely to be of high contrast against dominant reef colours, would be visited more frequently by piscivores, whose visual systems detect these colours more easily (Lettieri *et al.*, 2009). We compared client pools of (i) combined cleaner species segregated into the three divergent colour stripes as well as (ii) three representative *Elacatinus* species with the highest representative client pool within the three colours. 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 colour 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

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. Geographical heterogeneity – in e.g. protection level of reefs – could bias this *post hoc* method if all representative cleaners from a particular colour phenotype tend to be found in areas where there are more predators in general because of protection level. This particular bias is not apparent in our data. Although studies were included from protected (e.g. Barbados) and nonprotected reefs, specific cleaner species and colour types are not restricted to one type of site or another.

Results

Feeding experiments: WC *Elacatinus* 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 5-min naïve day 1 encounter, and a 5-min *post-exposure* encounter on day 2. 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 s to consume prey. There were no significant differences among treatments in percentage eaten on day 1; 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 (see Movie S1) significantly more times on day 1 than all other treatments except TR *E. oceanops* and WC *E. horsti* (Kruskal–Wallis $t_6 = 35.72$, $P < 0.0001$ 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*.

Significantly fewer WC cleaners *E. evelynae* (green) and *E. oceanops* (blue) were eaten in *post-exposure* second encounters compared to all other noncleaners (Fisher's exact, $\chi^2_1 = 13.116$, $P = 0.0004$; Fig. 2). Among all consumed prey on day 2, a smaller proportion than expected belonged to the WC cleaner group (exact binomial goodness-of-fit, $P = 0.0013$). There was a significant difference in Kaplan–Meier survival curves among WC treatments ($\chi^2_4 = 40.8$, $P < 0.00001$). Cleaner status was a significant predictor among WC survival in *post-exposure* trials with right-censored survival and Weibull distribution ($Z = 3.76$, $P < 0.0002$). Possession of a stripe was also a significant predictor of survival time ($Z = 4.07$, $P < 0.00005$), but when possession of stripe was analysed among only noncleaners (as all cleaners possess stripes), then stripe phenotype was no longer a significant predictor of survival time ($Z = 1.67$, $P = 0.10$). TR *E. evelynae* and *E. oceanops* individuals were eaten in

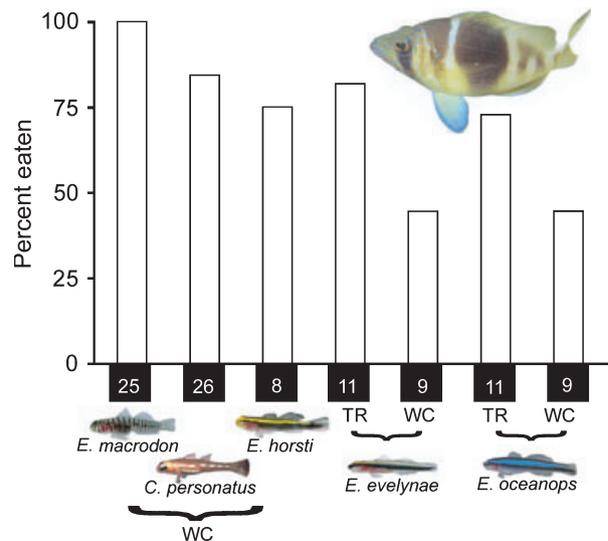


Fig. 2 Wild-caught (WC) cleaners are defended: the percentage of individual prey gobies eaten in day 2 (*post-exposure*) treatments. Significantly fewer WC cleaners *Elacatinus evelynae* (green) and *Elacatinus oceanops* (blue) were eaten compared to all other noncleaners (Fisher's exact, $P = 0.0004$) in day 2 *post-exposure* 5-min 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 2 (Fisher's exact, $P = 0.0503$). Numbers in black boxes indicate the number of individuals tested.

higher proportions than their WC counterparts on day 2 (Fisher's exact, $\chi^2_1 = 3.259$, $P = 0.0503$; Fig. 2), but wild and TR statuses were not significant predictors of survival time among the cleaner treatments ($Z = 1.54$, $P = 0.12$).

Field trials: stripes deter attack and induce posing

We used painted glue models deployed in the field to ask how potential client fishes respond behaviourally to colour 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' $\chi^2_2 = 45.67$, $P < 0.001$; Fig. 3). 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, $F_2 = 0.705$, $P > 0.5$). However, it is difficult to assign motivation to a passing observer from a scored approach. Therefore, we tallied the total number of behaviours 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

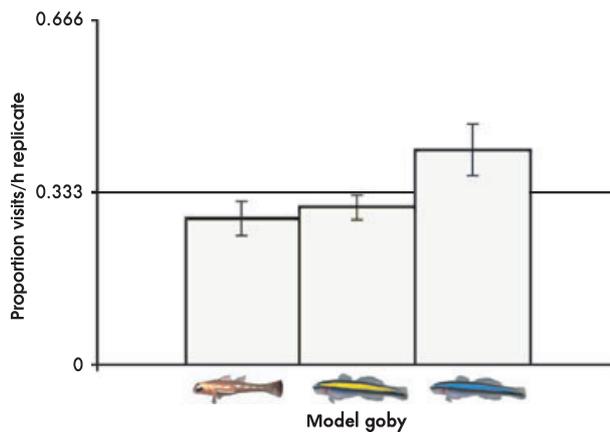


Fig. 3 Proportion of approaches per hour replicate varies among phenotype models. Hourly proportion of total reef fish approaches are significantly different among masked goby (unstriped *Coryphopterus personatus*), yellow- and blue-striped model types as determined by Yates' $\chi^2 = 45.67$, d.f. = 2, $P < 0.001$.

among the three model types (Freeman–Halton extension to the Fisher exact test, $\chi^2_2 = 25.61$, $P < 0.0001$; Fig. 4). *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, whereas only three total attacks occurred on any striped model (< 1% of total approaches in each case; Fig. 4). 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 (compared to blue stripe) = 2.96, $P = 0.008$; Z (compared to yellow stripe) = -2.61, $P = 0.02$, residual deviance < d.f. = 27).

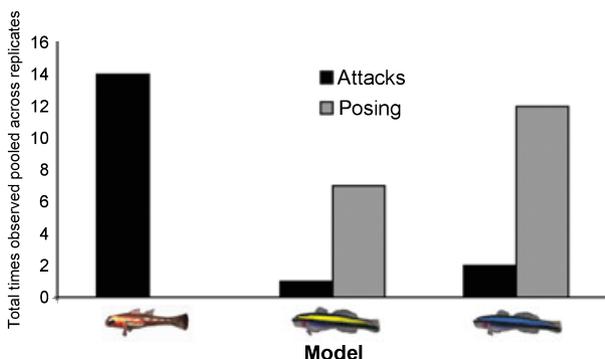


Fig. 4 Colour stripes are sufficient to elicit client behaviour. Attack and stereotypical client 'posing' frequencies are significantly different among masked goby (unstriped *Coryphopterus personatus*), yellow- and blue-striped model types as determined by Freeman–Halton extension to Fisher's exact test, P -value < 0.0001.

Elacatinus 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 colour stripe. Piscivorous species comprise about 31% of the total Caribbean client pool (Fig. 5). Piscivores made up a smaller than expected fraction of the client pool among yellow-striped cleaners based on comparison with a randomized distribution ($Z = -2.81$, $P = 0.005$). For both the combined yellow-striped species (Fig. 5, 19%) and for a representative yellow-striped species with the highest overall number of clients among yellow-striped cleaners (*Elacatinus figaro* data not shown, 9%, $Z = -2.94$, $P = 0.003$), 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%, $Z = 0.29$, $P = 0.771$, blue 32%, $Z = 0.19$, $P = 0.849$). The proportion of herbivorous clients was greater than expected for green-striped cleaners (36%, $Z = 2.66$, $P = 0.008$), and the proportion of invertivore clients was less than expected for blue cleaners (9%, $Z = -2.19$, $P = 0.03$).

Discussion

Positive interspecific interactions between cleaner fishes and their clientele represent a primary example of mutualism (Trivers, 1971; Hammerstein & Hoekstra, 1995; Grutter, 1999; Côté, 2000; Bshary & Noe, 2003). Caribbean gobies exhibit many of the ingredients suggested by theory to sustain mutualism once evolved (Axelrod & Hamilton, 1981; Nowak & May, 1992; Doebeli & Knowlton, 1998). For example, *Elacatinus* population genetic structure (Taylor & Hellberg, 2003) likely leads to geographical 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 colour stripes (Côté, 2000; Taylor & 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) WC *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) yellow-striped cleaners have lower proportions of interactions with risky predatory clients compared to blue- and green-striped cleaners. These data, in combination with our previous visual modelling of stripe colours that shows blue of highest contrast among the three colours (Lettieri *et al.*, 2009), imply that blue-striped gobies are better equipped to attract and survive attacks from

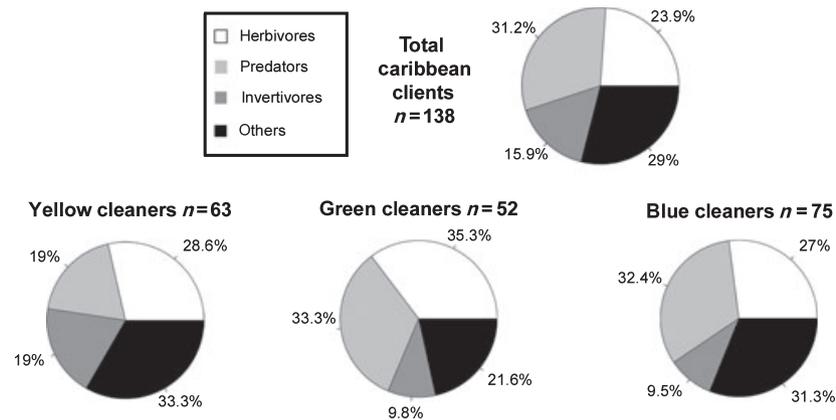


Fig. 5 Cleaner–client interactions differ among three cleaner colour phenotypes. Pooled client species are from all documented cleaning interactions in the literature we surveyed (Appendix S1). Yellow-striped cleaners consisted of *Elacatinus randalli*, *Elacatinus figaro* and *Elacatinus evelynae* (yellow). Green-striped cleaners were *Elacatinus prochilos*, *E. evelynae* (green), *Elacatinus illecebrosus* (green) and *Elacatinus genie*. Blue-striped cleaners consisted of *Elacatinus 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 colour morphs.

predators. Considered together with the natural history and phylogenetic relationships among *Elacatinus* gobies (Rüber & Zardoya, 2005; Taylor & Hellberg, 2005), we suggest that mutualism evolved in this lineage as predator resistance traits were co-opted for advertising and cooperation. We highlight key components of this evolutionary scenario later (Fig. 6).

Colourful stripes pre-date mutualism

The origin of colourful 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 cryptically coloured – against common sponge microhabitats to many fishes (Lettieri *et al.*, 2009) – and are generally reclusive. Colour 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 (Smith & Tyler, 1972; Colin, 1975), as do other gobies (Schubert *et al.*, 2003). In our feeding trials, 25% of the yellow sponge-dweller *E. horsti* survived all 5 min of the day 2 exposure to predators. Whereas *C. personatus* masked gobies survived at comparable levels (15% survival in day 2 feeding trials), field trials revealed that masked goby models were attacked at higher rates than striped models. Aposematic cues – bright colours that work in concert with toxicity or chemical defence to affect predator behaviour – may communicate nutritional unprofitability to potential predators or facilitate learned feeding aversion (Harvey & Paxton, 1981; Speed & Ruxton, 2005; Skelhorn & Rowe, 2007; Franks *et al.*, 2009). When schooling and fast swimming (e.g. defences used by *C. personatus*, L. Lettieri, personal observation) are unprofitable or unfeasible escape strategies, communicating with colour signals may be adaptive (Kuchta, 2005). Stripes therefore likely first evolved in *Elacatinus* gobies as either neutral (e.g. intraspecific communication) and/or negative

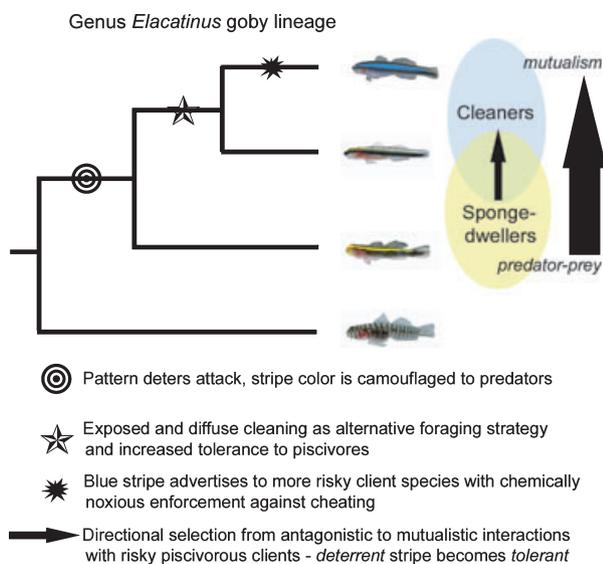


Fig. 6 Colourful stripes mediate the evolution of tolerance in cleaner gobies. Inferred changes in (i) goby stripe colour and the role of this signal, (ii) habitat and behaviour (e.g. cleaning) and (iii) interactions with predators [from resistance to tolerance] during the evolution of mutualism in Caribbean *Elacatinus* gobies. The phylogeny follows Taylor & Hellberg, 2005.

(e.g. camouflage, aposematism) signals in antagonistic interaction with piscivores.

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 & Hellberg, 2005). The evolution of cleaning behaviour as a facultative feeding strategy may be basal to sponge-dwelling, as Pacific sister species *Elacatinus puncticulatus* has been documented to clean (Rüber *et al.*, 2003) and shelters in the spines of *Euclidaris thouarsii* urchins. Cleaning in the Caribbean, however, marks a transition from a reclusive ancestor whose stripes are camouflaged and/or a signal of defence to an exposed advertising descendant whose stripes serve to attract cooperative clientele (Fig. 6). Competition for food resources and microhabitats may be intense for small sponge-dwelling gobies with limited mobility (Smith & Tyler, 1972; Buchheim & Hixon, 1992; Schofield, 2003; Hernaman *et al.*, 2009) and may result in gobies being pushed into less structurally complex habitat (Schofield, 2003). Facultative sponge-dwelling cleaners (Rocha *et al.*, 2000; Whiteman & 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 (Sillen-Tullberg & Leimar, 1988; Whiteman & Côté, 2002, 2004a) and further contributed to high intraspecific competition (Whiteman & 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 & Côté, 2002). Our data support the notion that cleaners have evolved increased unpalatability in response to the additional risk (Fig. 2), 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. 2) and increased exposure to a diverse predator pool further reinforced the evolution of stripes as a weak communication signal (*sensu* Endler & Mappes, 2004). Colourful stripes (i.e. yellow) may have initially conveyed a dual message from cleaners to distinct functional groups of client: they signalled 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 dichromat 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 nonthreatening species. Our analysis of yellow cleaner interactions from the literature supports this idea; yellow-striped cleaners clean proportionally fewer predators (Fig. 5). However, cleaning in isolation on coral heads clearly exposes colourful gobies to unpredictable approaches and threats of predation by risky onlookers.

Blue stripes signal tolerance towards 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 (Fig. 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 (Fig. 5). 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 defence) 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 colour 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 mild feeding deterrence (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 5-min interval. Notably, however, a significant proportion of potentially defended individuals *was* 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 pay-offs to invest in defence has turned a defensive trait combination (colourful 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 signalling 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, 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 signalling to predators (Lettieri *et al.*, 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 defence, signal and tolerance appears to be an important suite of traits contributing to the evolution of the specialized cleaning behaviour.

Tolerance 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 & Krebs, 1979). In other words, it may be too costly or unreliable to invest in a purely defensive strategy if (i) the defence 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 colour vision among florivores has led to selection for red bird-pollinated *Hakeas* (Hanley *et al.*, 2009). The *Hakea* example shows a similar evolutionary trajectory to that observed among Caribbean cleaner gobies: (i) defensive traits with colour signal warnings resist attack by risky individuals, (ii) the signal becomes a cue of reward when the ecological context of colour changes the interaction paradigm, (iii) an arms race is avoided as tolerance traits increase interactions with potentially risky clients, whereas 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 defence, 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.

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Authors' contributions

Both LL and JTS conceived the ideas presented in this paper. LL conducted the experiments and both authors wrote the paper.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 List of cleaner goby and client interaction sources.

Movie S1 Day 1 enclosed feeding trial interaction between a *Hypoplectrus* spp. predator and wild-caught *Elacatinus oceanops* cleaner goby.

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