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Natural selection in novel environments: predation selects for background matching in the body colour of a land fish

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The invasion of a novel habitat often results in a variety of new selective pressures on an individual. One pressure that can severely impact population establishment is predation. The strategies that animals use to minimize predation, especially the extent to which those strategies are habitat or predator specific, will subsequently affect individuals' dispersal abilities. The invasion of land by a fish, the Pacific leaping blenny, *Alticus arnoldorum*, offers a unique opportunity to study natural selection following the colonization of a novel habitat. Various studies have examined adaptations in respiration and locomotion, but how these fish have responded to the predation regime on land was unknown. We studied five replicate populations of this fish around the island of Guam and found their body coloration converged on the terrestrial rocky backgrounds on which the fish were most often found. Subsequent experiments confirmed that this background matching significantly reduced predation. Natural selection has therefore selected for background matching in the body coloration of the Pacific leaping blenny to minimize predation, but it is a strategy that is habitat specific. A subsequent comparative study of closely related blenny species suggested that the evolutionary ancestor of the Pacific leaping blenny might have resembled the rocky backgrounds on land prior to invasion. The ancestors of the Pacific leaping blenny may therefore have already been ideally suited for the predator regime on land. More generally our results imply that animals must either already possess antipredator strategies that will be effective in new environments, or must adapt very quickly to new predation pressures if successful establishment is to occur.

Adaptive divergence among taxa often arises when populations invade new environments (reviewed by Schluter 2001). This is because changes in the selection regime experienced by invaders can lead to changes in phenotype (adaptation). The colonization of novel environments has therefore been of special interest to evolutionary biologists because of the opportunity it brings to study natural selection in the wild (e.g. Losos et al. 1993, 1997; Rundle et al. 2003; Wagner et al. 2012). However, if new habitats are different enough from home environments, invading individuals may not survive long enough to establish a viable population. A key factor that can determine colonization success in this regard is predation, as high predation rates or the presence of different types of predators can prevent population establishment (Lodge 1993; Schoener & Spiller 1995). There are a number of strategies that animals might employ to reduce predation, but the best strategy is often to minimize encounters with predators altogether. This can include behavioural changes in habitat use or activity; for example being arboreal in the presence of terrestrial predators (Losos et al. 2004) or avoiding diurnal predators by becoming nocturnal (Berger & Gotthard 2008). Often, however, the option of shifting habitat use or activity is not viable and animals must resort to other measures.

If an individual is indistinguishable from other aspects of the environment, it is less likely to be attacked than one that is not. Tactics used to reduce detection and recognition by predators in this way include masquerading as an unpalatable food item (Skelhorn et al. 2011) or other adverse stimuli (Clucas et al. 2008), disruptive patterning in heterogeneous environments (Zylinski et al. 2011), or some form of background matching when backgrounds are static or otherwise predictable (Stuart-Fox et al. 2008; Wang & Schaefer 2012). These methods of camouflage are specific to the environment that an individual inhabits or to the predator that prey are trying to avoid (Stuart-Fox et al. 2008). This has obvious ramifications for the ability of animals to disperse into new environments. If an organism can no longer effectively camouflage itself (because the habitat or predators differ too much), predation could prevent population establishment entirely. However, if

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establishment does occur, animals may be restricted to certain areas within that habitat where individuals are less conspicuous. Predation may still be a problem, but selection has the opportunity to drive the evolution of greater crypsis or some other strategy to minimize predation. Species in this latter scenario offer a means to study how predation can affect the colonization process and provide a wonderful opportunity to study the process of natural selection more generally (e.g. Losos et al. 2004; Vervust et al. 2007).

The Pacific leaping blenny, *Alticus arnoldorum*, is a small tropical fish (4–8 cm in length) found on the island of Guam and has made one of the most extreme ecological transitions possible; it is a fish that has successfully colonized land. To achieve this, it has evolved various adaptations including cutaneous respiration capabilities (while still relying on its gills, respiration also occurs through the skin: Martin & Lighton 1989; Brown et al. 1991; Martin 1995) and the ability to move effectively about on land using a unique axial tail twisting behaviour (Hsieh 2010). To avoid desiccation (and subsequent asphyxiation), the fish is limited to the splash zone of intertidal areas around Guam. For all appearances, however, the fish is exclusively terrestrial and almost never returns to water (Ord & Hsieh 2011). The fish spends much of its time feeding on exposed rocks and other areas out of the water (Ord & Hsieh 2011). Furthermore, both sexes display conspicuously using flashes of a conspicuous, red dorsal fin during courtship and aggression (Ord & Hsieh 2011). How the Pacific leaping blenny has coped with the potential change in predation regime that has followed the transition to land has not been studied. It follows from the observation that the Pacific leaping blenny resembles the rocks on which they spend most of their time (see Supplementary material, Figs S1, S2) that camouflage has potentially been a key strategy for this species in predator avoidance. On land, predation of the Pacific leaping blenny is most likely from diurnal predators such as birds, land crabs or lizards (C. L. Morgans, personal observation). In contrast, predation in the ancestral marine environment was probably from other fish (reviewed by Norton & Cook 1999) and to a lesser extent birds (see Cheney 2009). Furthermore, the visual environment is different on land than in the marine environment: on land, fish have an immediate backdrop of rocks, while in water fish have a general background of water and to some extent rocks if marine species occupy the intertidal zone (e.g. Marshall & Jennings 2003).

The primary goal of our study was to test the general hypothesis that visually oriented predators on land have selected for a body coloration in the Pacific leaping blenny that matches the rocky backgrounds against which the fish are typically found. We tested this hypothesis in two ways. First, we examined five populations of blenny around Guam and determined the extent to which the body coloration of each population matched their environments (we measured both the hue (chromatic) and brightness (achromatic) properties of fish and backgrounds). We replicated our study across five populations because initial observations suggested habitat backgrounds varied subtly from location to location. Given this, we predicted that populations would converge on the colour properties specific to their location. To provide a suitable benchmark for this colour comparison, we also quantified the colour of the dorsal fin, which is an important signal in social interactions for both males and females (Ord & Hsieh 2011) and should therefore be conspicuous in the environment (e.g. Stuart-Fox & Moussalli 2009; Zylinski et al. 2011). If predation has selected for crypsis in the Pacific leaping blenny, we expected to find the body of individuals, which is always visible, to match the appearance of habitat backgrounds. Conversely, the dorsal fin, which is only visible when erected during signal bouts, should exhibit high contrast with habitat backgrounds to facilitate its role in communication. Second, we performed an experiment to confirm that cryptic body coloration reduces predation. The experiment compared predation rates on naturalistic model blennies to conspicuously coloured controls in two adjacent, visually different land environments. The Pacific leaping blenny was found in both environments, but differed substantially in density (C. L. Morgans & T. J. Ord, personal observation). If blenny body coloration has been selected for background matching, then predation should be lowest in the environment most often used by blennies and in which blennies appear most cryptic.

To provide some resolution of the evolutionary history of body coloration, and whether it might have facilitated or challenged the invasion of land by the Pacific leaping blenny, we supplemented these two studies with an ad hoc examination of body coloration in representative specimens of several closely related marine species found around the island. This comparative study was not meant to provide a formal phylogenetic reconstruction of ancestor phenotype, which would require detailed and extensive sampling of species across the blenny phylogeny (as well as their environments). Rather, the goal was to reveal the extent to which the Pacific leaping blenny differed or resembled its marine relatives and, by extension, the likelihood that the fish has evolved its present-day body coloration prior to, or following, the colonization of land.

METHODS

Population Colour Analysis

Adult male and female Pacific leaping blennies and specimens of closely related species were captured using hand nets at five locations around Guam between June and August 2011 (overlapping with the probable breeding season of this genus; see Shimizu et al. 2006). The locations were: Pago bay (13°25′39″N, 144°47′56″E), Taga'chang (13°24′16″N, 144°46′53″E), Talofofo (13°20′34″N, 144°46′21″E), Umatac (13°17′40″N, 144°39′29″E) and Adelup Point (13°28′52″N, 144°43′44″E; see Results, Fig. 1). Of the Pacific leaping blenny, a total of 95 males and 95 females were sampled, with a median of 24 males and 25 females per population. All individuals were released at the point of capture after study, except for a small subset from Taga'chang that was euthanized to make prey models (see next section). Of the marine species, a few whole specimens were caught opportunistically as part of tissue collections made for a separate study on the phylogeny of the Blenniidae family. These individuals were ultimately euthanized following procedures outlined in the University of New South Wales Animal Care and Ethics Committee (UNSW ACEC) protocol 11/36B for later molecular analysis. However, before being euthanized, we used this whole specimen collection to examine one representative adult individual for each species. Both the Pacific leaping blennies and its relatives are capable of changing their body coloration from their normal patterning. For example, the colour of the Pacific leaping blenny can change to either a uniform ash grey or charcoal black within minutes (Ord & Hsieh 2011; see also Abel 1993; Heflin et al. 2009). These colour changes seem to be limited to social interactions (e.g. Ord & Hsieh 2011), but to minimize colour variation that might also be induced from the initial stress of capture, individuals were isolated in moist opaque plastic containers and left in a sheltered, shady area for at least 1 h prior to photography. At no time did we observe any noticeable colour change while taking photos of the Pacific leaping blenny or any other species.

Full body photographs were taken of individuals positioned side-on to the camera with the dorsal fin raised against a white standard background (X-Rite ColorChecker White Balance Card) and beside a ruler and a Munsell colour chart (X-Rite mini ColorChecker; Fig. S1). Photographs were taken with a Canon EOS 7D digital SLR using an EFS 15–85 mm, f/3.5–5.6 IS USM zoom lens and stored as high-resolution jpegs. Multiple photographs were

taken of each individual to ensure adequate illumination and positioning of the fish. Photographs were also taken of six backgrounds for each of the five Pacific leaping blenny populations. These background photographs were randomly taken at each site, but were all areas where the Pacific leaping blenny had been caught. Background photographs included the Munsell colour chart in-frame (Fig. S1).

Colour analysis was performed using the inCamera plug-in for Photoshop CS4. The images that were analysed for a given individual were those photographs that exhibited the lowest standard deviation for the red, green and blue (RGB) colour channels of the colour squares of the Munsell colour chart (i.e. those photographs with the least amount of within-image variance in lighting). The colour and brightness of these images were then standardized using the known RGB values of the colour squares in the Munsell chart with the inCamera plug-in (see Bergman & Beehner 2008; Ord & Hsieh 2011). Following this calibration, we used the Marquee Tool in Photoshop to calculate the RGB values for areas of comparable size on the body and dorsal fin (see Fig. S1). We followed previous studies (Bergman & Beehner 2008; Ord & Hsieh 2011) and used the ratio of red to green values to measure chroma (hue). This ratio provided a general measure of the overall colour of an object, while standardizing for differences in brightness between different types of colours. In terms of the specific channels used to compute the ratio, preliminary analyses showed that the choice of channels were not likely to affect the outcome of our analyses (Fig. S3) and, in this sense, focusing on the red/green ratio was arbitrary.

To quantify achromatic properties or the brightness of colour, we took the average value of all three colour channels and divided this number by 255 to compute a value between 0 (corresponding to black or zero brightness) and 1 (corresponding to white or maximum brightness; NB: white has a value of 255 for the R, G and B channels, whereas black has a value of 0 for all three channels).

We quantified the colour of fish for a common section of the dorsal fin and a representative, similarly sized area of the body (Fig. S1a). These measures were replicated across at least two photos per individual, with subsequent R/G ratios and brightness values averaged for each individual prior to statistical analysis. Background photographs were analysed in a similar manner, with the R/G ratios and brightness values computed over six randomly selected areas within each of the six photos taken for each population ($N_{background} = 36$ per population; Fig. S1b).

This type of standardized colour analysis makes several assumptions about the spectral properties of the object being measured and, subsequently, the most relevant spectral sensitivities of the visual system of the organism viewing the object. Like most digital cameras, the one we used relied on image sensors that captured reflected light over a wavelength range of 400 to 700 nm, which is designed to match the wavelengths visible to humans. Actual data on the spectral sensitivity of these sensors for the make and model of the camera we used was not available. However, peak sensitivities of the sensors most likely occur at (or close to) 475, 550 and 625 nm, with sensitivity curves around these peaks overlapping extensively to ensure consistent colour representation across the visible spectrum in captured images (this is based on specifications published by Canon, the colour processing tests of DxO Image Science for the same make and model of camera (www.dxo.com), and the spectral sensitivities of other digital cameras that have been reported by Stevens et al. (2007)). That is, our camera provided reasonable resolution of colour over the 400 to 700 nm range. However, many natural objects reflect in the ultraviolet (UV), between 300 and 400 nm, and many animals can see into this range as well. Our analyses do not provide an estimate of UV reflectance. Instead, our analyses measured colour for the RGB colour space, or reflectance broadly corresponding to short (B; SWL), medium (G; MWL) and long (R; LWL) wavelengths.

Whether or not UV reflectance is relevant is dependent on the visual system of the predator viewing the blenny. We anticipated most predation occurred from birds, land crabs or lizards. Many birds and lizards do see into the UV (Hart & Hunt 2007; Fleishman et al. 2011), but crabs generally do not (Detto 2007). Specifically, a recent review of the visual system of various bird species (Hart & Hunt 2007) showed peak sensitivities in the UV (355–426 nm), SWL (445–480 nm), MWL (530–550 nm) and LWL (600–625) regions. The visual system of crabs ranges from 300 to 650 nm, with peak sensitivities at SWL (430 nm) and MWL (520 nm; Detto 2007). Lizards see from 300 to 700 nm and have very similar spectral sensitivities to birds (e.g. peak sensitivities at: UV, 365 nm; SWL, 455; MWL, 530 nm; and LWL, 570 nm; see Fleishman et al. 2011), but differ from birds in that lizards are more sensitive to wavelengths above 400 (the peak sensitivity of the UV cone is lower than the peak sensitivity of the SWL, MWL and LWL cones; see Fleishman et al. 2011). In short, birds might potentially rely on UV reflectance to localize prey, but this is less likely in lizards and crabs. More generally, as a broad measure of colour, our analyses succeed in covering the widest range of wavelengths likely to be used by all these potential predators (SWL, MWL and LWL).

To compare body, fin and background colour and brightness, we used the degree of overlap between the 95% confidence intervals of means and the magnitude of effect sizes (R/G and brightness ratios were normally distributed for all populations). Means with 95% confidence intervals that did not overlap were considered statistically different from one another (equivalent to $P < 0.05$). For effect size magnitude, we computed effects for two sample comparisons (body versus background colour or brightness; fin versus background colour or brightness) as a standardized mean difference (Cohen's d). This value and its 95% confidence interval were then converted into an r value. A biological effect was interpreted if the confidence intervals of r did not overlap zero. Equations for effect size computations are given in Ord & Stamps (2009) and Ord et al. (2011). See Nakagawa & Cuthill (2007) for a discussion of the advantages of using 95% confidence intervals and effect sizes in place of traditional P-value driven statistical analyses.

Predation Experiment

We used highly realistic plasticine models made from casts of euthanized male and female Pacific leaping blennies to test the relative predation pressure between two environments: intertidal rocks and sandy beach. We chose Taga'chang to perform the experiment because it was a site where both habitat types occurred immediately adjacent to each other and were infrequently used by people. The blenny was found in both habitats, but densities were heavily skewed towards the rocky environment. Observations of blennies on the beach were generally limited to areas of wet sand within several metres of isolated rock outcrops that were within the intertidal zone.

As a method of quantifying predation, the use of plasticine or clay models has been successfully used in a variety of taxa (e.g. Stuart-Fox et al. 2003; McLean et al. 2010; Chouteau & Angers 2011; Linnen et al. 2013). To make models, we created casts of four males and four females caught near Taga'chang. These individuals were euthanized following procedures outlined in the UNSW ACEC protocol 11/36B and frozen in naturalistic postures. Moulds were made of frozen specimens using liquid silicone rubber (CopyFlex™ Culinart Inc., Plainview, NY, U.S.A.). The resulting casts were highly detailed and reusable. Models were created from these casts using malleable plasticine (Colorific, Clayton, Victoria, Australia) with black, white and yellow portions blended by hand to mimic colours

of the blenny (Fig. S2). A black cable tie was embedded into each model to provide an anchor point for securing the model to the substrate in each habitat. Controls were shaped by hand and consisted of a uniform pink plasticine ring (Fig. S2). A yellow cable tie was embedded into each control for anchorage. These controls were designed to be highly visible, novel objects (i.e. non-food-related items).

If attack rates were purely the consequence of the conspicuousness of stimuli against the visual background, and not reflective of blenny-specific predation (e.g. because of the inspection of a novel object in the environment by a nonpredatory animal or if the materials used to make stimuli were an attractant in some way), then attack rates would be higher for controls generally and highest for controls placed on rocks (Fig. 2a, top panel) where the controls were more conspicuous (Fig. S2b, c). Conversely, attack rates on model blennies would be lower overall, and lowest on rocks (Fig. 2a, top panel) where model blennies were less conspicuous (Fig. S2b, c). If attack rates were indicative of blenny-specific predation, but not dependent on background matching (e.g. predators 'sit and wait' in the environment and target any item eventually spotted that looks like a prey item), then attack rates would be higher in both environments on model blennies, with virtually no attacks on the conspicuous controls (Fig. 2a, middle panel). Finally, if attack rates were indicative of blenny-specific predation and detection was dependent on the degree of background matching, then attack rates would be higher on model blennies than on conspicuous controls, and highest on model blennies on the beach (Fig. 2a, bottom panel) where models were more conspicuous (Fig. S2b, c). This latter scenario would confirm that background matching by the blenny functions to reduce predation.

Note that we assumed that predators were equally abundant in both environments. We had no reason to believe that this assumption was not correct prior to conducting the experiment: models and controls were positioned in rocky and beach habitat that were immediately adjacent to each other (e.g. within several metres of each other) and observations of birds, land crabs and lizards (likely predators) were made in both habitats. If anything, predator density may have been slightly skewed towards the rocky habitat: while birds were frequently observed in both habitats, we often observed large land crabs and lizards on the rocks but less so on the beach. Nevertheless, the subsequent frequency of attack rates recorded during the experiment was generally consistent with the notion that predators were equally likely to target stimuli in both habitats (see Results).

Both models and controls were of similar size; photos of both stimuli positioned in the environment are shown in Fig. S2. Colour analysis was conducted on photographs of representative examples of blenny models and controls using the same procedures described in the previous section (Population Colour Analysis). Model blennies were close in colour and brightness to living individuals as well as mimicked their general body patterning (see Fig. S2). In contrast, controls were clearly different in colour, brightness and other characteristics from blennies and were highly conspicuous in the rocky habitat for both colour and brightness, and conspicuously coloured in beach habitats (Fig. S2). However, controls were indistinguishable in brightness to the beach background (Fig. S2), which provided an interesting comparison of the potential interacting effects of colour, brightness and general morphology of stimuli on attack rates (e.g. if predators relied exclusively on a strong brightness contrast for detecting potential prey, then attack rates on controls should be higher on the rocks than on the beach).

Models and controls were placed alternately along transects parallel to the shoreline at intervals of approximately 1.5 m. In the rocky habitat, models and controls were attached to rocks using fishing line and in areas that were above the high tide waterline, but otherwise within the splash zone (i.e. areas frequented by blennies but where stimuli would not be washed away by the tide). In the beach habitat, models and controls were secured by lengths of fishing line to plastic lids that were buried in the sand and positioned above the high tide waterline. The integrity of stimuli was checked daily and evidence of predation was tallied after 3 days, following which all stimuli were removed from the environment. On occasion, unusually large swells buried stimuli on the beach and these were replaced daily.

On day 3, stimuli were categorized on the following criteria: (1) no marks; (2) single or multiple small nicks; (3) large punctures or nicks; (4) entire portions missing; or (5) only the anchor point remaining. For our analyses, we focused on categories 3 and 4 as evidence of predation. Category 2 seemed to reflect the inspection of stimuli by small scavenging organisms rather than predator attacks (e.g. ants nibbling at stimuli). Category 5 was exempt from analysis as it was not possible to exclude wave action or human interference as reasons for model disappearance.

To make monitoring manageable, the experiment was conducted in two halves. Consecutive batches of stimuli were dispatched in nonoverlapping areas of each environment, for a final total of 70 models and 70 controls. The experiment was run over 8 days (2 days were devoted to the removal and positioning of stimuli between batches). Our final tally based on the recovery rates of stimuli was 52 models and 63 controls in the rocky habitat and 58 models and 64 controls from the beach habitat. The experiment was conducted from 6 July to 12 August 2011.

Differences in predation rate between models and controls, and between environments for a given stimulus type, were evaluated through a comparison of 95% CIs of the proportion of stimuli exhibiting predation and relevant effect sizes (see previous section, Population Colour Analysis). Confidence intervals for proportions were computed using formula presented in Zar (2010). Comparisons in which the 95% CIs of proportions did not overlap were considered to be statistical different from one another (i.e. equivalent to $P < 0.05$). We also calculated effect sizes by computing an odds ratio and its associated 95% CI, and then converting this value into an r value using formula presented in Ord et al. (2011).

Comparative Study

To explore the extent to which body coloration might have changed following the colonization of land, we compared the colour morphology of the Pacific leaping blenny to 12 closely related blenny species. These 12 species covered the full diversity of intertidal blenny species around Guam. Species ranged from exclusively marine (seven species) to amphibious (five species). These behavioural categorizations were based on observations made of fish behaviour during collection. Marine species were always observed fully immersed in water (e.g. swimming about in open water), while amphibious species were observed immersed in water and above the waterline in rock holes or (more rarely) out in the open on rocks. The Pacific leaping blenny was the only species that remained exclusively out of the water at all times.

The assessment of species colour morphology relied largely on a qualitative comparison of coloration among species, coupled with a single estimate of the chroma and brightness of the specimen examined for each species. This comparison was made with reference to a preliminary phylogeny created using maximum parsimony and based on two mitochondrial DNA genes (ATPase 6 and 8) and four nuclear DNA genes (RAG1, ZiC, Sreb2 and Ptr; G. M. Cooke & T. J. Ord, unpublished data). A formal ancestor state reconstruction of chroma and hue was inappropriate given that the species found on Guam were only a subset of the species found in the entire

clade (40+ species). To obtain a reliable and quantitative estimate of the ancestral phenotype, a more comprehensive survey of the entire clade would need to be conducted and this was beyond the scope of the current study.

Nevertheless, a general comparison of the colour morphology among our subset of species still provides a reasonable picture of the probable history of colour evolution in the group. For example, if the Pacific leaping blenny appears very similar in colour to its marine and amphibious relatives, then it likely evolved from an ancestor that was also very similar in appearance. Otherwise, the extent to which the Pacific leaping blenny differs in colour morphology from its immediate relatives provides a general indication of the extent to which colour evolution has likely changed following, or in conjunction with, the colonization of land.

RESULTS

Background Matching

The chroma of the rocky environment of the Pacific leaping blenny tended to vary among the five populations, but this variance was largely reflected in differences in heterogeneity rather than in the mean value of chroma among sites. For example, the mean background chroma of Taga'chang and Talofofo was very similar, but the variance in chroma at Taga'chang was much larger than than that of Talofofo, where chroma was more uniform (Fig. 1a). In all five populations, the chromatic properties of the body coloration of the Pacific leaping blenny for both sexes were statistically indistinguishable from the rocky backgrounds of each locality (and subsequently the mean body chroma of fish did not tend to vary among populations). The 95% CIs of the mean R/G ratio of the body always overlapped those of the background (Fig. 1a), and computed effect sizes were not statistically different from zero (Table 1). In contrast, and as predicted, mean R/G ratios and effect sizes of the dorsal fin showed that the fin was highly conspicuous against most backgrounds (Fig. 1a, Table 1). Males from Adelup were the only exception to this trend and possessed dorsal fins that were effectively the same colour as the background. There were also large sex differences in fin coloration for most populations, with females generally possessing more conspicuous fins (fins with more red) than males (Fig. 1a, Table 1).

The brightness of rocky backgrounds also tended to vary among populations, but this was reflected in differences in both the mean and the within-background variance of brightness estimates (Fig. 1b). For example, Taga'chang had the lightest and most heterogeneous background, whereas Umatac had the darkest and most uniform background of the five populations. Although the brightness of the Pacific leaping blenny tended to converge on the brightness of habitat backgrounds, and also varied among populations to an extent, only fish from Taga'chang and to some extent Adelup Point matched the specific brightness level of their backgrounds (Fig. 1b). The remaining populations had 95% CIs that did not overlap those of backgrounds and effect sizes were significantly different from zero (Table 1). In general, fish were lighter than their backgrounds in both body and dorsal fin coloration. There were no discerning differences in brightness between the sexes.

Table 1
The chroma and brightness contrast of the body and dorsal fin against habitat backgrounds in five populations of Pacific leaping blenny, *Alticus arnoldorum*

Sex and population	Comparison with background	$N_{background,\ blenny}$	Effect size, *r* (lower CI, upper CI)
Chroma			
Male			
Pago	Body	36, 25	−0.11 (−0.320, 0.194)
Taga'chang	Body	36, 10	0.13 (−0.213, 0.428)
Talofofo	Body	36, 10	−0.38 (−0.613, −0.055)*
Umatic	Body	36, 25	0.09 (−0.160, 0.330)
Adelup	Body	36, 25	−0.19 (−0.400, 0.058)
Female			
Pago	Body	36, 25	−0.41 (−0.579, −0.173)*
Taga'chang	Body	36, 10	0.29 (−0.049, 0.545)
Talofofo	Body	36, 10	0.09 (−0.406, 0.250)
Umatic	Body	36, 25	−0.19 (−0.409, 0.066)
Adelup	Body	36, 25	0.18 (−0.065, 0.388)
Male			
Pago	Fin	36, 25	0.57 (0.374, 0.696)*
Taga'chang	Fin	36, 10	0.59 (0.336, 0.740)*
Talofofo	Fin	36, 10	0.84 (0.734, 0.897)*
Umatic	Fin	36, 25	0.73 (0.670, 0.814)*
Adelup	Fin	36, 25	0.19 (−0.056, 0.401)
Female			
Pago	Fin	36, 25	0.73 (0.606, 0.814)*
Taga'chang	Fin	36, 10	0.87 (0.784, 0.912)*
Talofofo	Fin	36, 10	0.96 (0.940, 0.975)*
Umatic	Fin	36, 25	0.72 (0.589, 0.806)*
Adelup	Fin	36, 25	0.87 (0.804, 0.902)*
Brightness			
Male			
Pago	Body	36, 25	0.50 (0.278, 0.652)*
Taga'chang	Body	36, 10	0.02 (−0.301. 0.329)
Talofofo	Body	36, 10	0.58 (0.317, 0.738)*
Umatic	Body	36, 25	0.77 (0.659, 0.839)*
Adelup	Body	36, 25	0.24 (−0.004, 0.442)
Female			
Pago	Body	36, 25	0.561 (0.433, 0.727)*
Taga'chang	Body	36, 10	−0.08 (−0.391, 0.257)
Talofofo	Body	36, 10	0.63 (0.375, 0.770)*
Umatic	Body	36, 25	0.91 (0.866, 0.936)*
Adelup	Body	36, 25	0.46 (0.245, 0.611)*
Male			
Pago	Fin	36, 25	0.57 (0.369, 0.702)*
Taga'chang	Fin	36, 10	0.09 (−0.239, 0.386)
Talofofo	Fin	36, 10	0.61 (0.361, 0.756)*
Umatic	Fin	36, 25	0.82 (0.734, 0.874)*
Adelup	Fin	36, 25	0.47 (0.266, 0.623)*
Female			
Pago	Fin	36, 25	0.56 (0.363, 0.690)*
Taga'chang	Fin	36, 10	-0.18 (−0.469, 0.160)
Talofofo	Fin	36, 10	0.41 (0.067, 0.634)*
Umatic	Fin	36, 25	0.87 (0.808, 0.909)*
Adelup	Fin	36, 25	0.28 (0.042, 0.476)*

* 95% confidence intervals (CIs) that did not overlap zero (i.e. were significantly different at $P < 0.05$).

Predation

Puncture wounds and missing body parts were more frequent for model blennies on the beach than on the rocky habitat, whereas this was not the case for controls (Table 2, Fig. 2b). Furthermore, the computed effect sizes of the frequency of puncture wounds and missing body parts between blenny replica and controls on the beach were both large ($r = 0.55$ and 0.74) and statistically different from zero (Table 2). In general, controls received only minimal evidence of predation, despite being generally more conspicuous in both environments than model blennies (Fig. S2). This suggests that attacks on blenny models were from predators believing they had detected a prey item, rather than from nonpredatory animals inspecting a novel addition to the environment. More broadly, these results confirmed that chromatic background matching by the Pacific leaping blenny to its rocky habitat significantly reduces predation.

Comparative Study

Colour analyses of the Pacific leaping blenny showed limited variation in the body coloration among individuals within a population (i.e. confidence intervals were narrow; Fig. 1), and only

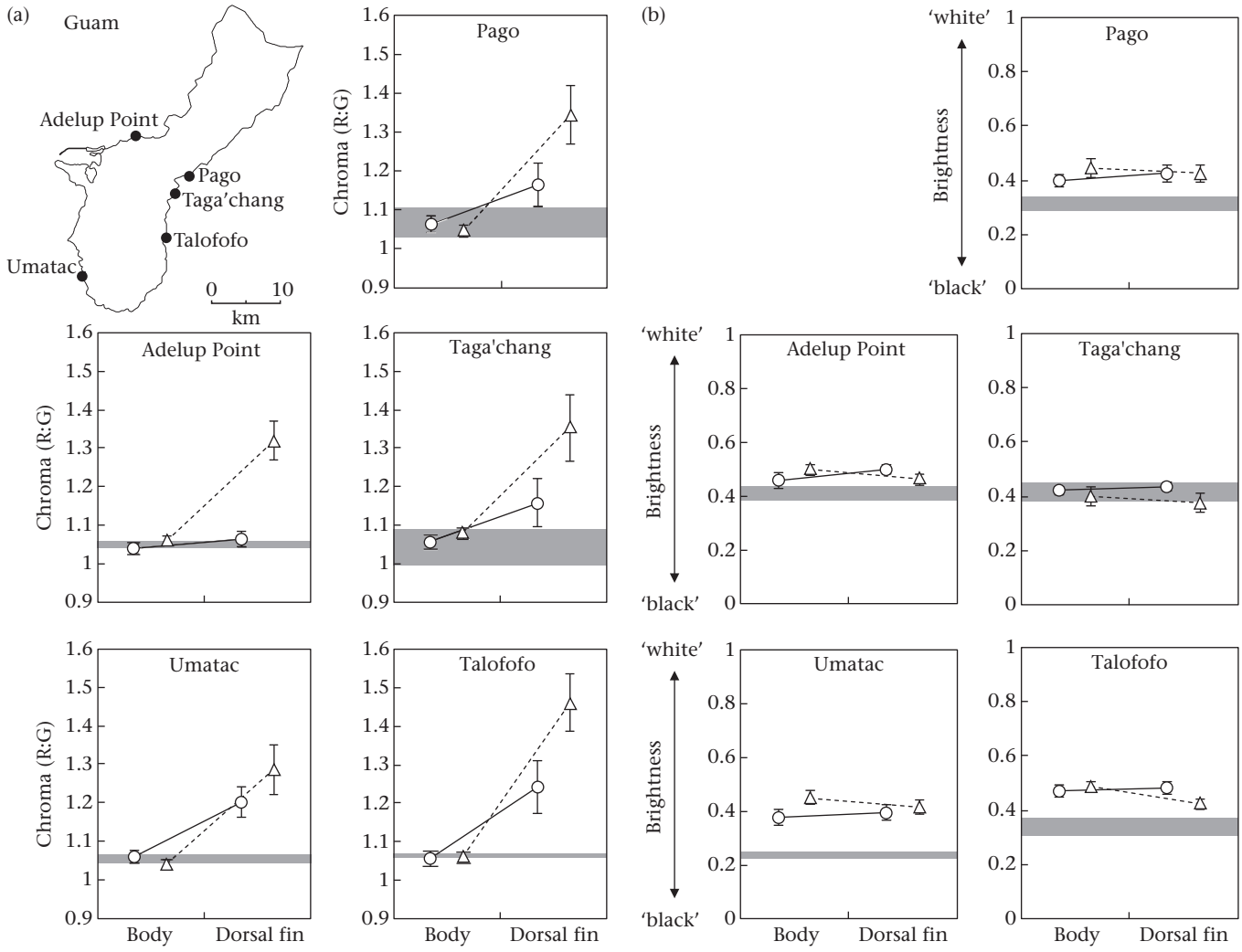

Figure 1. The (a) chromatic and (b) brightness attributes of the body and dorsal fin relative to habitat backgrounds for five populations of the Pacific leaping blenny, *Alticus arnoldorum*, on the island of Guam (a map of Guam in also presented in (a) showing the locations of populations studied). Open triangles: females; open circles: males; shaded regions on the plots correspond to the 95% confidence interval (CI) range of background coloration. Data presented for blennies are means and 95% CIs.

marginal variation among population averages (Fig. 1). This suggests that a single representative specimen would probably provide a reasonable approximation of coloration for the whole species, which further suggests that our qualitative comparison of colour morphology among species provides an informative picture of colour diversity among species.

This comparison among species revealed that the appearance of subtidal and intertidal blennies around Guam were broadly similar and generally within the chroma and brightness range of rocky habitats that were frequented by the Pacific leaping blenny on land (Fig. 3). There were only two exceptions and both were marine subtidal species: both the chroma and brightness of *Istiblennius edentulus* and the brightness of *Blenniella caudolineata* lay outside the range of rocky backgrounds on land. There was some evidence that marine species exhibited greater variation in coloration than species that were partly or exclusively found on land ($CV_{chroma} = 0.032$ versus 0.010; $CV_{brightness} = 0.206$ versus 0.148). However, most of this variation occurred within the marine *Istiblennius* and *Blenniella* clade, so the degree to which this is a general phenomenon of marine species is unclear.

Table 2
Predation rates measured by the proportion of blenny models exhibiting puncture wounds and missing body parts when dispatched in typical (rocky) versus atypical (beach) habitat

Comparison	$N_{model/typical,\ control/atypical}$	*r* (lower CI, upper CI)
Typical vs atypical habitat		
Puncture wounds (category 3)		
Model	52, 58	0.33 (0.111, 0.499)*
Control	63, 64	−0.18 (−0.450, 0.13)
Missing 'body' parts (category 4)		
Model	52, 58	0.35 (0.077, 0.558)*
Control	63, 64	0.00 (−0.737, 0.734)
Model vs control		
Puncture wounds (category 3)		
Typical habitat	52, 63	0.13 (−0.135, 0.370)
Atypical habitat	58, 64	0.55 (0.349, 0.689)*
Missing 'body' parts (category 4)		
Typical habitat	52, 63	0.60 (−0.064, 0.840)
Atypical habitat	58, 64	0.74 (0.304, 0.883)*

* 95% confidence intervals (CIs) that did not overlap zero (i.e. were significantly different at $P < 0.05$).

DISCUSSION

Our results are consistent with the notion that body coloration in the Pacific leaping blenny has been naturally selected to match environmental backgrounds. Population analysis (Fig. 1, Table 1) and predation experiments (Fig. 2, Table 2) confirmed that the

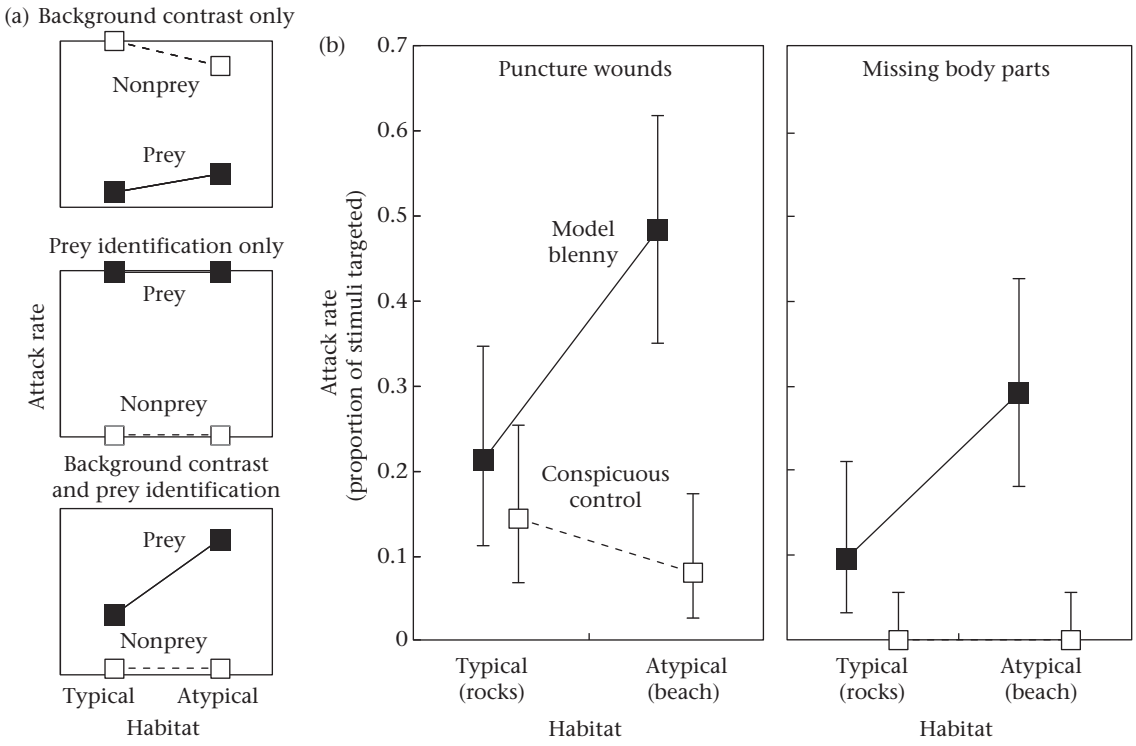

Figure 2. (a) Predicted attack rates under various scenarios and (b) actual levels of predation exhibited on blenny models and controls dispatched in two habitats at Taga'chang. Data shown in (b) are the proportion of models exhibiting puncture wounds or missing body parts and associated 95% confidence intervals.

chromatic properties of the Pacific leaping blenny matched the rocky habitat in which blennies were most commonly found, and that individuals that might happen to move onto the neighbouring beach would be significantly more vulnerable to predation. This was unlikely to be an artefact of higher densities of predators occurring on the beach than in the rocky environment for two reasons. First, prior to conducting the predation experiment, our general observations implied predator densities were either similar between habitats or potentially even skewed towards the rocky habitat. Second, predation on controls during the experiment, while minimal, was largely consistent between habitats, further suggesting comparable predator densities between habitats (although there was a weak tendency for more predatory attacks to occur in rocky habitats; see Fig. 2b).

The role of brightness was less clear, but it appears to have been less influential on predation rates. While the Pacific leaping blenny tended to resemble the brightness of their backgrounds, most populations were lighter than their backgrounds (Fig. 1b, Table 1). Our blenny models also differed in brightness from both the rock and sand backgrounds (Fig. S2), suggesting differences between these environments in predation rate were more dependent on chromatic differences. Either the general brightness heterogeneity of backgrounds has allowed fish to remain inconspicuous despite being lighter than the 'on average' brightness of the background (e.g. see Fig. 3), or predators rely more on the chromatic distinction of prey rather than on the brightness contrast between prey and the background.

Our results also suggest that conspecific discrimination of the dorsal fin was more likely to be dependent on chroma rather than on brightness because the dorsal fin was clearly more conspicuous in chroma (Fig. 1). The general conspicuousness of the dorsal fin was expected because the dorsal fin is used heavily in social communication (Ord & Hsieh 2011) and the ability to effectively communicate to conspecifics depends on a signal being distinct from the environment (Dusenbery 1992; reviewed in Bradbury & Vehrencamp 2011). The prominent sex differences in the chroma of the dorsal fin has been noted previously for the Pacific leaping blenny and attributed to heightened aggression among females relative to males (Ord & Hsieh 2011). Differences among populations in the relative intensity of sexual selection, as well as predation, might also explain the variance in dorsal fin coloration that was evident among populations in our study (Fig. 1). A follow-up study has been conducted to explore this possibility, but at this stage it is unclear why populations vary in fin coloration.

We can say, however, that cryptic body coloration (specifically chroma) does provide an effective means of minimizing predation, but it is also a strategy that is highly habitat specific (Fig. 2). This leads to the question of how the first invaders of these fish might have coped with the change in predation regime when they initially ventured onto land. Our comparative study and predation experiment provide some resolution to this question. There are three possible scenarios. First, cryptic coloration was not initially required because historic predation on land blennies was negligible. This seems unlikely because predation on blennies was clearly evident in our experiment, especially when mismatched with the visual background on the beach (Fig. 2). Note, however, that our models were static. In reality, the Pacific leaping blenny retreat into rock holes and crevices for large portions of the day (at low and hide tide, and at low and high temperatures; see Ord & Hsieh 2011), so our estimates are likely to be the upper bracket of actual predation levels. Nevertheless, our results strongly suggest that predation is an important selection pressure in these fish, and it seems reasonable to expect that it was historically important as well. Second, the invasion of land was followed by a change in body coloration, to a lesser or greater degree depending on the ancestral phenotype, as a result of selection from predation on land. Such a change in colour morphology would have to occur rapidly, because blennies would have presumably suffered heavily from predation if

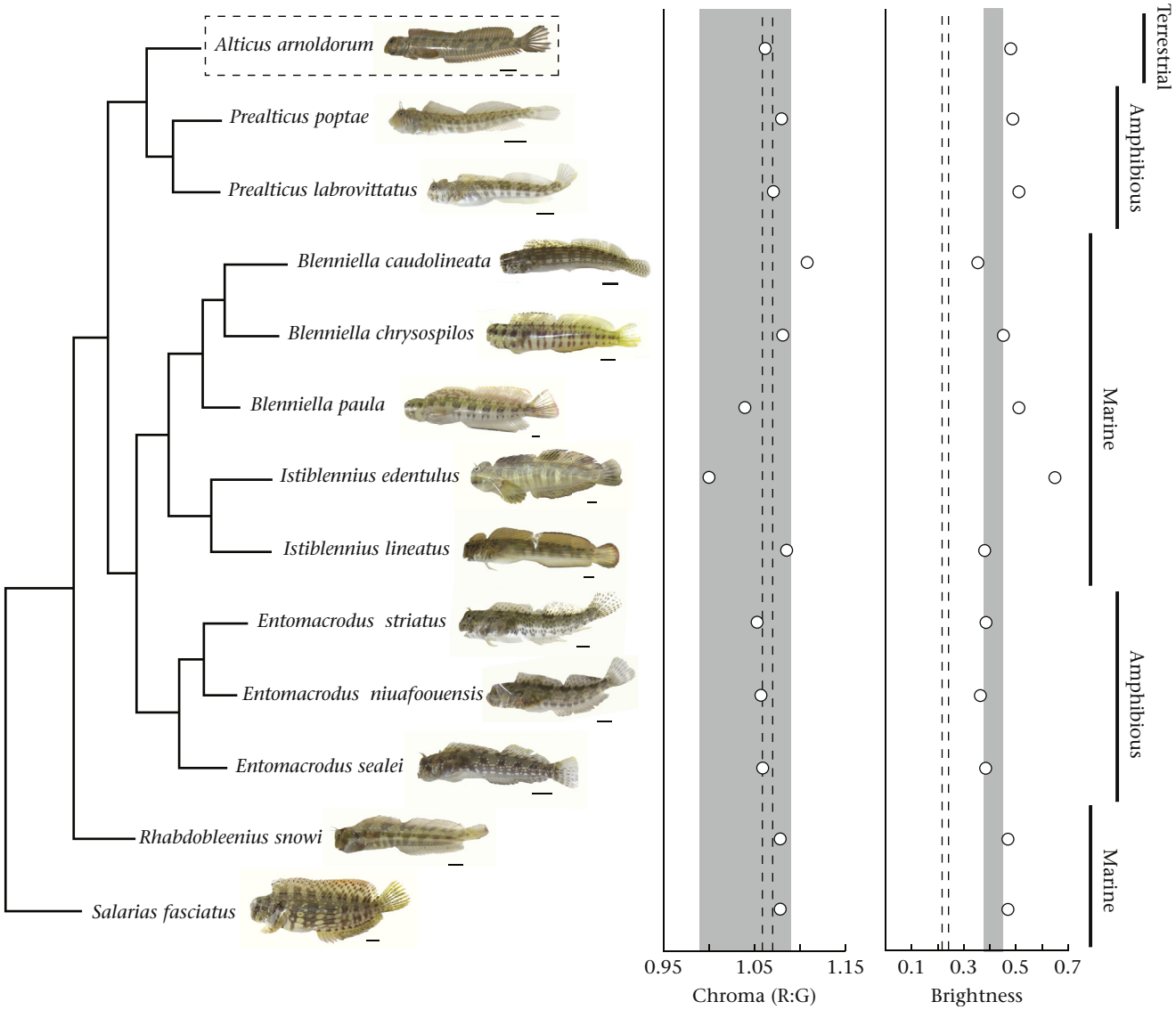

Figure 3. The phylogeny and body coloration of the Pacific leaping blenny (highlighted by the dashed box at the top of the phylogeny) and its intertidal and subtidal relatives around Guam. The dashed lines and shaded regions on the chroma and brightness plots represent estimates of the rocky habitat for Pacific leaping blenny populations with the narrowest and widest background 95% confidence intervals, respectively (see Fig. 2). The branch lengths of the phylogeny reflect substitution rates and a proxy for time since divergence between lineages. The scale bar next to fish equals 1 cm.

mismatched to the background environment (e.g. Fig. 2). Behavioural changes, such as staying close to rock crevices and holes that exclude predators or otherwise minimizing time exposed on rocks, might have also helped. Finally, and more likely according to our comparative study, the Pacific leaping blenny originated from a marine ancestor that was already quite similar in body coloration to the rocky backgrounds on land. In this scenario, the cryptic body coloration of the ancestor facilitated the transition to land by minimizing the impact of predation in its new land environment. Given the similarity in coloration of the Pacific leaping blenny to subtidal and intertidal blenny species around the island (Fig. 3), the marine ancestor of the Pacific leaping blenny probably resembled the rocky backgrounds on land to some extent.

More broadly, our study suggests that animals can only successfully colonize environments with different predation regimes if colonizers already possess an antipredator strategy that is effective in the invaded habitat (as seems to be the case here) or are capable of rapid changes in phenotype that minimize exposure to predation (plasticity is one mechanism by which rapid phenotypic changes might be made; e.g. Losos et al. 2004). Obviously, while predation is an important variable influencing colonization success, the life history and behaviour of invaders can be expected to be influential as well (Losos et al. 2004; Sol et al. 2012). The Pacific leaping blenny, as a species that has made one of the most dramatic ecological transitions possible, offers a unique model system to explore these variables and the adaptations necessary for the invasion of new environments, which is an important first step in the speciation process.

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Supplementary Material

Supplementary material for this article is available, in the online version, at http://dx.doi.org/10.1016/j.anbehav.2013.09.027.

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