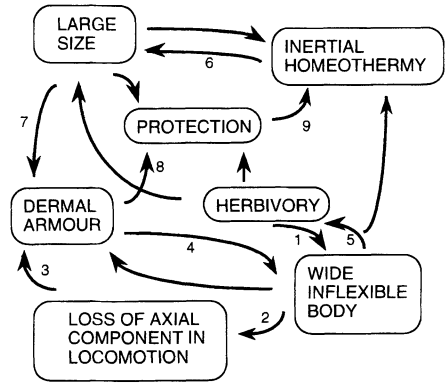

FIG. 3 Postulated selective interactions between various traits in pareiasaurs. These complex interconnections mean that changes in one trait influence the evolution of other traits, leading to a macroevolutionary pattern called 'correlated progression'[7].

limb-driven locomotion (arrow 2). The reduced need for body flexibility during locomotion would have allowed greater development and consolidation of protective dermal armour (arrow 3) which in turn would have further reduced body flexibility (arrow 4). The gradual diminution of agility and speed would have locked the heavily armoured terrestrial pareiasaurs further into a herbivorous niche (arrow 5). It is not hard to see how such a system of positive feedback could have led to the gradual evolution of the totally rigid body of turtles. Similarly, the large body size in early pareiasaurs probably evolved partly in response to a shift towards inertial homeothermy[20] (Fig. 3, arrow 6). This in turn led to support problems alleviated by the development of dermal armour (arrow 7), which later expanded over the body and was co-opted for protection (arrow 8), which, in forming a thick dense insulating layer over the body, might have further enhanced inertial homeothermy as well (arrow 9). The shell of turtles has been shown to confer thermoregulatory advantages[23]. The evolutionary changes within pareiasaurs towards 'turtle-ness' illustrate how an organism adapts to a selective regime composed not of the external surroundings alone, but of an interaction between the external surroundings and its own morphology[24]. □

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Rapid adaptive camouflage in tropical flounders

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DESPITE the commonly held view that flatfish can change their surface markings to match their background pattern[1], there have been few systematic studies[2,3] and it has recently been claimed[4] that their capacity for such adaptive changes is minimal. Here we show that the tropical flatfish *Bothus ocellatus* can achieve pattern-matching with surprising fidelity. By adjusting the contrast of different sets of 'splotches' of different grain size (or spatial frequency) on the skin, the fish can blend into a wide range of background textures in just 2–8 seconds.

Sumner[2] placed flatfish of the genus *Paralichthys* on checkerboard patterns and noticed that after a few days the fish seemed to develop large splotches as if to mimic the checks. These experiments were recently repeated in two genera[4,5], giving rise to claims that their ability to change had been grossly exaggerated and that for the most part the flatfish simply had a 'universal texture' that allowed it to blend into any environment. It was also suggested that many of the photographs of flounders showing apparent camouflage (including those in refs 2 and 3) contained artefacts produced by film nonlinearities that tended to enhance the apparent resemblance between the fish and its background.

Even without any dynamic change, the flatfish can blend surprisingly well with a wide range of variegated backgrounds[4]. But we wondered whether it was also capable of dynamic changes in pattern. Five specimens of the Caribbean shallow-water flatfish *Bothus ocellatus* were used in the experiment. They were initially housed in a tropical salt-water aquarium at 30 °C and transferred to four small 25 × 20 × 30 cm rectangular plastic tanks for testing. Their eyes are mounted on short stalks and move independently (like a chameleon's eyes) through 180 °, and the fish could track and pounce on a thin vertical bar moving horizontally.

The following patterns covered the bottoms of the test tanks: a coarse gravel; printed checkerboards, either 1-cm or 2-mm checks; a homogeneous grey sheet, the luminance of which was identical to the mean luminance of the checks; or fine, yellow beach sand. While in the home tank, all fish appeared almost identical in colour and pattern to the yellowish beige gravel on the floor (similar to that in Fig. 1*a*). When we transferred the fish (one per tank) each changed its surface markings within 2–8 s to resemble closely its new background. Each fish was then moved to a different, randomly selected, tank and once again exhibited a strong change in pattern to match the new background. As long as the fish were healthy and active, such changes could be brought about several times (Fig. 1).

We were concerned that the resemblance between the fish and the various backgrounds might be an optical illusion: that the effect seen in Fig. 1 might be in the eye of the beholder and the fish might not have changed significantly. We therefore took colour photos of the same fish on different backgrounds and then simply cut them out and displayed them on a plain background. It was clear that the pattern on the fish was changing physically. We also took black and white half-tone pictures (Fig. 1*b–d*) and asked 20 naive subjects to choose the background pattern that most closely matched each of the three cut-out fish. They did this correctly in 58 of 60 trials, providing unequivocal evidence that the fish were changing physically in the appropriate direction. The reason that Saidel's results[4,5] were not as clear may be that he used cold-water rather than tropical flatfishes and that the latter may have evolved a greater ability to engage in pattern matching. Indeed, our own

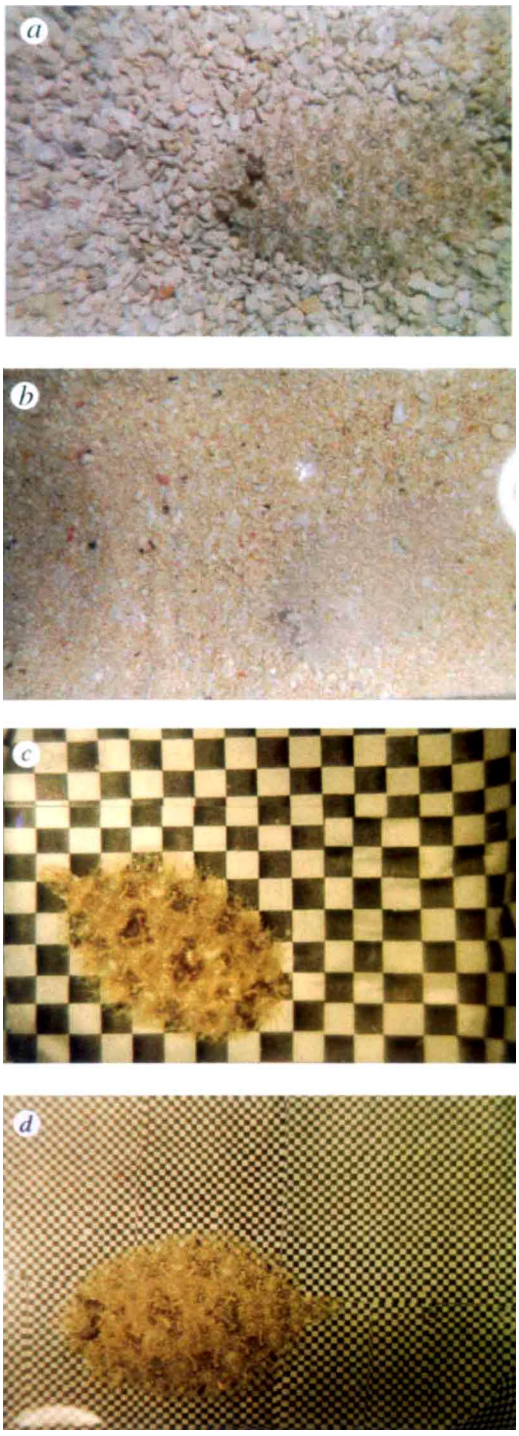

FIG. 1 Photographs of the same fish on 4 different backgrounds. The adaptive change took 2–8 s in each case. We ruled out the possibility that these effects were simply a consequence of the fish being transparent. This was a problem mainly with smaller specimens (<4 cm), but transparency in the specimens studied was also ruled out on the following grounds. First, analysis of the videotapes showed that there was a delay before the fish resembled the background. Second, the blotches on the fish had no spatial coherence with the background squares. Third, after the fish had changed its pattern, we placed an oval piece of grey opaque cardboard between it and the pattern and this did not eliminate the camouflage (photographs were also taken for subsequent analysis). And fourth, when the fish was displaced passively with a ruler, the pattern moved with the fish. The texture changes were unequivocal but emphasis should not be placed on the colour changes as these were not measured objectively (the exact tints seen in these reproductions could be artefacts produced by lighting). We also noted that as the fish were cycled through the tanks repeatedly they seemed to learn to respond faster to the background pattern. It remains to be seen whether this curious form of perceptual learning is specific to the particular pattern to which the fish is exposed.

initial results with cold-water genera (for example, *Paralichthys*) were very similar to those obtained by Saidel.

A convenient way to characterize visual texture is by means of the Fourier transform of the flounder pattern. From photographs of the fish that had had time to adapt to a variety of different background patterns, we obtained the radial power spectrum of the back of the fish, and a corresponding region of the background pattern, averaged over orientation and phase. A principal components analysis of these spectra showed that there were three predominant channels of independent information about the background texture being expressed in the flounder camouflage patterns (Fig. 2). These may be most easily described as a low-frequency/high-frequency opponent channel, a predominantly mid-frequency channel, and a channel with a narrow peak at 8 cycles per fish and a suggestion of higher harmonic structure. The important result from this analysis was that (like human colour vision) flounder patterns have three dimensions of useful control

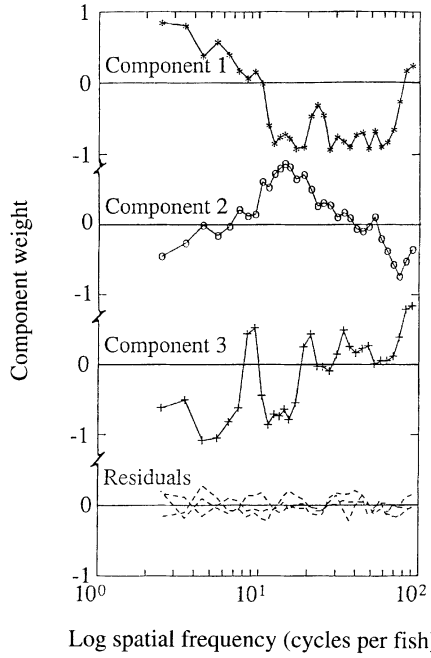

FIG. 2 Correlation analysis of the background texture information mimicked by the flounder patterns. To condense the information, we took a radial average of the 2-D power spectra around successive annular bands with widths of 1/4 octave. This provides a 1-D graph of the spatial-frequency content of the pattern of the fish or the background, averaged over orientation and phase. From photographs of the fish on 23 different background patterns to which they had had time to adapt, we obtained the radial power spectrum of the back of the fish (excluding the motile fringe and tail regions, which were semi-transparent), and an oval region of the background matching the shape of the fish. The degree of adaptive matching of the fish to the background was obtained by means of a cross-correlation matrix of the spectral power at each background frequency with that at each frequency in the flounder pattern. A principal components analysis (singular value decomposition of the cross-correlation matrix of 33 × 33 spatial frequencies) showed that there were three predominant channels of independent information about the background texture being expressed in the flounder camouflage patterns, as depicted by the six curves. (The eigen values of the first six components were 5.68, 5.02, 3.03, 0.94, 0.87 and 0.75.) The three significant components (full curves) may be most easily described as a low-frequency/high-frequency opponent channel, a predominantly mid-frequency channel, and a channel with a narrow peak at 8 cycles per fish and a suggestion of higher harmonic structure. Further components (dashed curves) are of low amplitude and do not appear to contain much structured information. The flounder patterns have three dimensions of useful control by the environmental information. The particular spectral form of the underlying mechanisms may consist of any linear combination of the depicted functions, which correspond to a vector space of the control parameters between the background texture and the fish patterns.

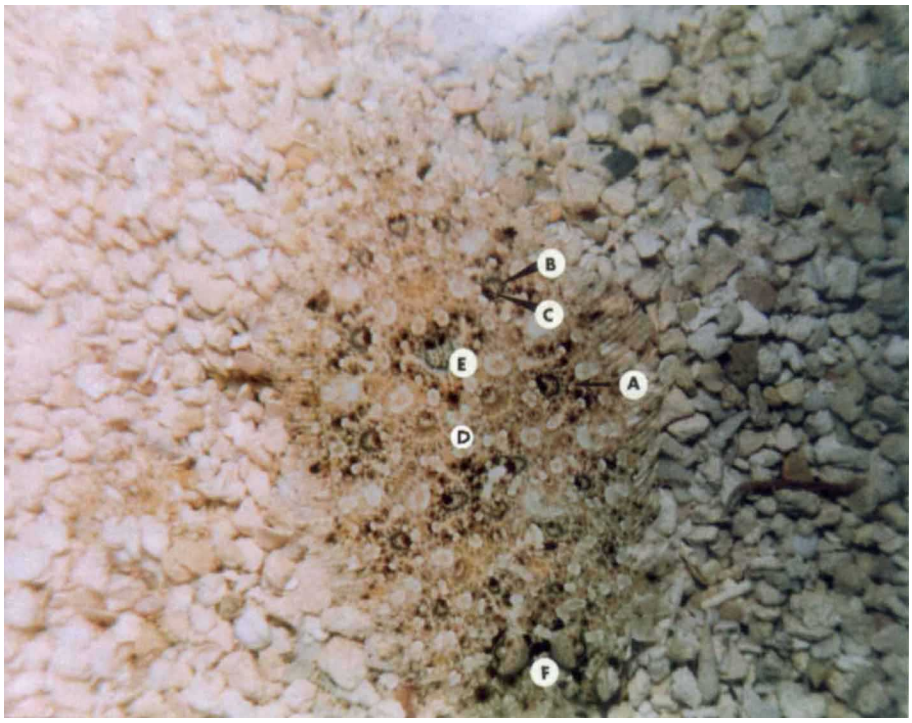

FIG. 3 Different surface markings on *Bothus ocellatus*. The fish seemed to have some degree of independent control over the different types of markings. These markings were *A*, large 'H'-shaped splotches lining the margins of the fish; *B*, small dark rings within each 'H' and elsewhere on the surface, and similar light rings scattered over the surface; *C*, small central black spots, one in the middle of each ring; *D*, stippling over the surface of the fish; *E*, a central 'eye spot' in the middle of the fish which was made up of an 'H' splotch with a ring and central spot in the middle; and *F*, a 'spectacle frame' around the eyes. Additionally, in between each ring and the central black spot was a pale area that was lighter than the yellow-brown colour of the rest of the fish. Each set of markings was essentially a cluster of specific size and shape composed of melanocytes. The luminance gradient seen across the whole photograph is artefactual.

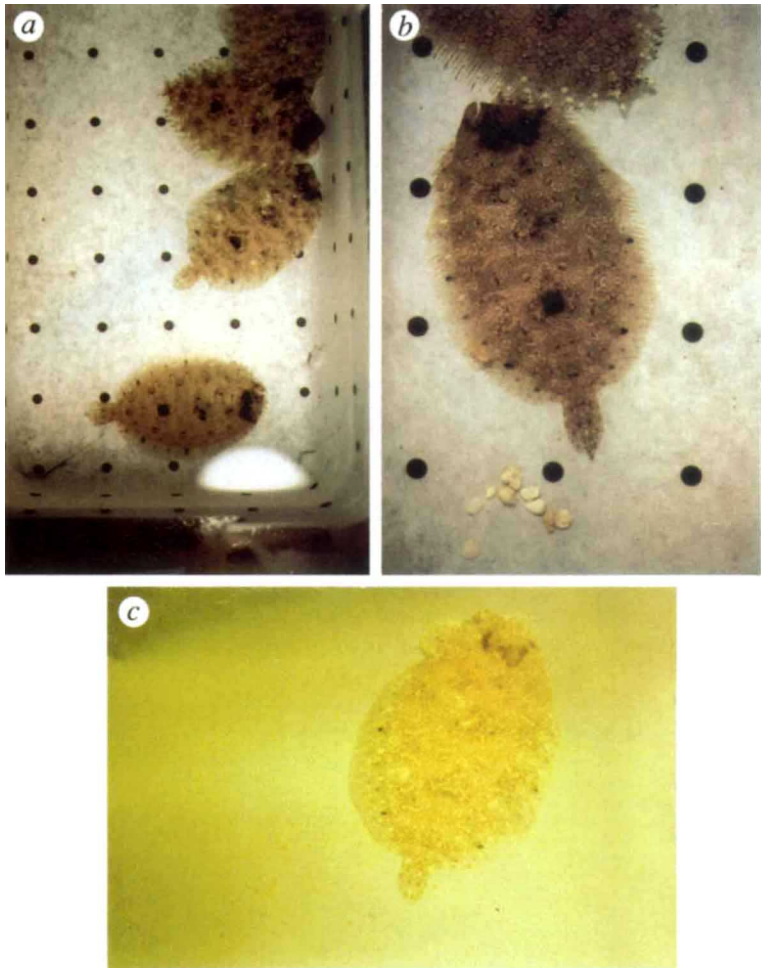

FIG. 4 Response of specimens to sparse black polka dots (*a*, *b*) and a homogeneous light grey (*c*). These adaptive changes took several minutes rather than seconds. In *a* and *b*, the fish increased the contrast of the central 'eye spot' and the dark spectacles around the eyes while the rest of the fish became pale. A grey background (*c*), the mean luminance of which was equal to this pattern, did not produce this effect. Similarly, when the fish was placed on a photonegative of *a* (sparse white polka dots on black) the fish became much darker and developed prominent white spots near its margins, an effect that was not seen on a homogeneous black background. Again, it is difficult to avoid the conclusion that these white spots were made more prominent to mimic the sparse white polka dots in the surround.

by the environmental information over presently unknown combinations of the six categories of elements identified in Fig. 3.

Studies under a dissecting microscope revealed that the fish has at least six categories of surface marking (Fig. 3). Hence the effector response in the skin must be organized in a modular fashion, as noted in refs 4 and 5. By adjusting the contrast of these sets of markings in different ratios, the fish could 'blend' into a wide range of natural backgrounds in much the same way that all spectral colours can be produced by mixing just three primaries in a trichromatic visual system. This argument implies, of course, that the fish must have independent visual control of each set (or subset) of markings, a possibility that requires verification. There may be 'feature detectors' in the fish visual centres that are specialized for detecting different spatial frequencies of textures in the environment and these might exert direct control over the corresponding set of marks on the skin surface. Indeed, there might be a map of effector neurons in (say) the tectum, so that focal electrical stimulation might produce selective contrast enhancement of specific spatial frequency components on the skin.

These experiments show clearly that the flounders' skin patterns were changing in an adaptive manner, but how frequent and reliable are these changes? To explore this we placed our five fish successively for 5 min on each of three types of background (large checks, small checks, and uniform grey of the same mean luminance). They were cycled through this whole sequence six times with each sequence videotaped for analysis using a Sony HiFi Handycam with a timer. Behaviour was considered to be adaptive only if the fish appeared to match the background within the first 20 s and retained the match for the remaining time on that pattern. It was important to use this rigid criterion as the fish also sometimes engaged in non-adaptive pattern changes when disturbed (for example, a blanching took place in less than a second as the fish flipped into a high-speed avoidance reaction). The results showed conclusively that all five animals were capable of adaptive behaviour; they matched their background on 91% of trials. Surprisingly, the adaptation was often completed in 2–8 s, which is considerably less time than quoted by Sumner[2] (several days) or Mast[2] (several hours). Thus a neural, rather than humoral, mechanism must be involved.

In our last experiment we placed two fish on the patterns shown in Fig. 4. Remarkably, the fish increased the contrast of just two spots, the central 'eye spot' and the dark spectacles around the eyes, whereas the rest of the fish became pale. Thus, the fish had obviously made an attempt to match the sparse black polka dots by turning on only two black spots on the skin surface. A grey background (Fig. 4*c*), the mean luminance of which was equal to this pattern, did not produce the effect.

A distraction manoeuvre that we observed also deserves comment. When pursued around a large tank, the fish would disturb the sand in one location while darting back to bury itself quickly in a different position, thereby deceiving us into thinking that it was actually buried in the first location.

In summary, we conclude that tropical flatfishes are indeed capable of adaptive camouflage and that such adaptation can occur with surprising rapidity. The manner in which such precise visual control of melanophores is achieved remains to be determined. Interestingly, squids[6] can also achieve rapid camouflage in a similar manner, a remarkable example of convergent evolution. □

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Vasoactive effects of fibrinogen on saphenous vein

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NORMAL plasma fibrinogen concentrations are critical to haemostasis. Higher fibrinogen concentrations are associated with increasing risk of atherosclerotic disease[1] and with graft stenosis and occlusion after saphenous vein bypass surgery[2,3]. Vein graft stenosis is characterized by the localized proliferation of intimal smooth muscle cells, causing narrowing of the graft with increased risk of thrombotic occlusion. In rabbit arteries, fibrinopeptide B is reported to have both vasoconstrictor and mitogenic properties[4,5]. We report here that fibrinopeptides had no vasoactive effects on saphenous vein rings; however, fibrinogen (0–2 μM) affected an endothelium-dependent relaxation, followed by recontraction at higher concentrations. The fibrinogen-mediated relaxation was inhibited by K^+-channel blockers and antibodies to ICAM-1. Coupled signalling pathways for the synthesis of vasoactive mediators and mitogens could underlie the association between fibrinogen and the development of vein graft pathology.

We studied the changes in isometric tension of saphenous vein rings in response to thrombin, fibrinogen and fibrinopeptides. Vein rings exhibited endothelium-dependent relaxation to increasing doses of thrombin, maximum relaxation $42 \pm 4\%$ at $1\ U\ ml^{-1}$ which increased to $63 \pm 5\%$ in the presence of 0.25 μM fibrinogen, but no response to fibrinopeptides (A + B) alone, at concentrations of up to 5 μM. Unexpectedly, vein rings showed a concentration-dependent relaxation in response to fibrinogen (0–2 μM), this relaxation being reversed at higher concentrations of fibrinogen (Fig. 1). The response to fibrinogen was not observed when vein rings were denuded of endothelium (Fig. 1). However, when intact vein rings were incubated with fibrinogen and the supernatant transferred to a vein ring denuded of endothelium, progressive relaxation occurred (Fig. 1). Other plasma proteins, albumin and transferrin at concentrations of up to 12 μM, did not cause relaxation of vein rings or attenuate the response to fibrinogen. Addition of the fibrinogen fgD.fgE fragment complex (0–15 μM) did not effect relaxation of precontracted vein rings but preincubation of vein rings with the fgD.fgE complex (10 μM) abolished the relaxation in response to fibrinogen (Fig. 2). These experiments suggest that specific fibrinogen receptors on saphenous vein endothelium mediate vasomotor responses.

Nitric oxide and prostacyclin are the two best characterized vasodilators synthesized by endothelium, with nitric oxide stimulating the synthesis of cGMP in the underlying smooth muscle[6]. However, preincubation of vein rings with indomethacin (10 μM), the nitric oxide synthase inhibitor L-NAME (0.1 mM) or the nitric oxide trap haemoglobin ($100\ \mu g\ ml^{-1}$) only effected a small reduction of the fibrinogen-mediated relaxation (data for L-NAME and indomethacin in Fig. 1 and for haemoglobin in Fig. 2). The lipoxygenase inhibitor nordihydroguaiaretic acid (10 μM) did not alter the fibrinogen-mediated relaxation, but inhibited the vascoconstriction observed at higher fibrinogen concentrations (Fig. 1). Preincubation of vein rings with both indomethacin and L-NAME reduced the maximum relaxation in response to fibrinogen from $63 \pm 3\%$ to $40 \pm 3\%$, $P = 0.03$ (Fig. 1). The concentration of cGMP extracted from the vein rings of five patients was measured by radioimmunoassay, at baseline, after treatment with 2 μM fibrinogen or 0.5 μM A23187 (which evokes the synthesis of endothelial nitric oxide): the concentrations of cGMP were 29.9 ± 4.8, 39.0 ± 6.4 and $116.5 \pm 31.6\ fmol\ mg^{-1}$ protein, respectively. The modest increase in cGMP concentration in response to fibrinogen contrasts with the fourfold increase in cGMP concen-