phate-containing region of the molecule was complicated by the observation that CNBr digestion of ^{32}P-labeled N-CAM (Fig. 2, lane g) yielded at least six phosphorylated fragments distributed into two zones (19K and 10K) on SDS-PAGE; they did not correspond to any previously identified CNBr fragments. They could have resulted from partial acid hydrolysis of aspartyl-prolyl peptide bonds, but we have no evidence for such bonds in N-CAM; after treatment with 70 percent formic acid without CNBr, the N-CAM polypeptides were not soluble in SDS-PAGE sample buffer (*12*) even after boiling. These peptides probably arise from another source, such as multiple phosphorylation of two regions in one or both N-CAM polypeptides; one zone of three components might be derived from the smaller polypeptide (130K) and the other from the larger one (160K). Differences in the phosphorylation of the N-CAM polypeptides is suggested by their different ratios of phosphoserine to phosphothreonine (Fig. 3) and by preliminary *S. aureus* V8 protease mapping (not shown).

Defining the structural features that distinguish the N-CAM polypeptides is important for establishing the organization of the molecule, its interaction with the cell membrane, and the regulation of its expression. For example, transformation by oncogenic viruses alters the expression of N-CAM (*17*) and may also affect the extent or location of phosphorylation. A description of their structural differences might also help define the mechanism that gives rise to the two N-CAM polypeptides. Both species have the same amino terminal sequence (*6*), yield similar unlabeled peptides when treated with *S. aureus* V8 protease (*3*), and contain the attachment site or sites for polysialic acid (*13*), suggesting that the smaller polypeptide might arise by proteolysis in the carboxyl terminal third of the larger. Consistent with this hypothesis, DNA hybridization experiments have indicated that only one N-CAM gene may exist (*18*). However, filter hybridization of brain messenger RNA to complementary DNA probes suggests that two N-CAM mRNA's may be produced (*18*). The synthesis of N-CAM could thus resemble that of fibronectin, which has similar polypeptides resulting from differential processing of a single heterogeneous nuclear RNA (*19*). Regardless of the origin of the differences in the N-CAM polypeptides, the phosphoamino acids may serve as distinguishing markers.

During neuronal development, N-CAM expression, distribution, and binding are probably controlled by several mechanisms (*1*). Alterations in the sialic acid content of N-CAM (*4*) and the amount of N-CAM on membranes occur in vivo (*20*); both have striking effects on N-CAM binding in vitro (*5*). The relative prevalence of the 170K and 140K forms of N-CAM differs among regions of the central nervous system (*1*, *4*), but the function of this variation remains to be defined. The differences in labeling presented raise additional possibilities for functional control. Sulfation and phosphorylation probably affect N-CAM function indirectly, perhaps participating in the regulation of its prevalence or distribution on the cell surface, its degree of sialylation, or the stability of intermediate forms produced during intracellular or extracellular processing.

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Tactics of Acclimation: Morphological Changes of Sponges in an Unpredictable Environment

Abstract. *Reciprocal transplants of genetically identical fragments of intertidal sponges between environments of high and low wave action exhibit great variability in the timing of their responses to environmental change. Sponges quickly begin production of stiffer and stronger tissues in high wave energy environments but delay formation of new, weak tissues in calm habitats. This may be due to the risks of forming wave-intolerant tissue in a temporally variable, unpredictable environment. These results suggest that the evolution of acclimatory control is linked to environmental predictability and concomitantly to risks of acclimatory errors.*

Although much is known of mechanisms of physiological acclimation and their environmental control (*1–3*), very little is known of the internal developmental changes that lead to acclimatory responses (*2*, *4–6*). By transplanting genetically identical sponge fragments into a range of environments and monitoring the morphology of new tissues produced, I have recorded the rates of response to benign versus stressful environments. Wave-tolerant morphologies are quickly produced in transplants into stressful environments. However, acclimation to wave intolerance is delayed in transplants into benign environments. Separate experiments show that risks of maintaining inappropriate morphologies are asymmetric; the morphology characteristic of benign environments is intolerant of heavy wave action and is at risk because of environmental uncertainty. Thus, asymmetric rates of response to the environment parallel asymmetric risks. This suggests that the process of acclimation to and away from stress tolerance is coupled to the relative risks of stress tolerant and intolerant states.

In the demosponge *Halichondria panicea*, colony morphology varies with

wave intensity (*7*). On the coast of Washington State, sponges in low intertidal surge channels experience pounding surf and high wave energies. In these habitats, colonies are moundlike with strong, stiff tissues. In adjacent mid-to-upper intertidal or subtidal habitats, wave energies are less (*8*), and sponges are thin encrustations with weaker, less stiff tissue (Fig. 1).

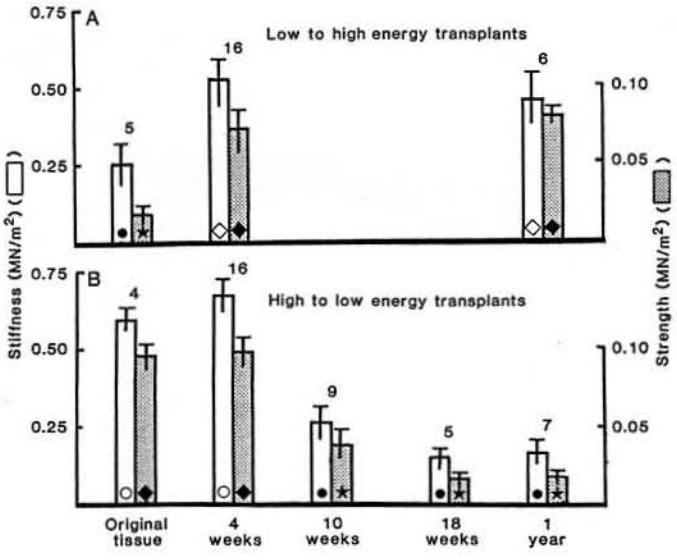
Genetically identical sponge clone fragments were transplanted reciprocally between low and high wave energy environments (*9*). At intervals, new tissues formed by marginal extension of the original fragments were collected and tested for structural properties (*10, 11*). Since only tissues formed subsequent to previous collections were analyzed, the results record the developmental program of the sponges at intervals after transplantation. Within 4 weeks, new growth in sponges transplanted into high wave energy environments was similar to tissue from local, unmanipulated sponges (Fig. 1A). In contrast, after 4 weeks, new tissue from sponges moved into low wave energy environments was structurally indistinguishable from the original (high wave energy) tissue (Student-Newman-Kuels test; $P > 0.05$) (Fig. 1B). Tissue produced between 4 and 10 weeks after transplant, however, was significantly different from the original (Fig. 1B) and was indistinguishable both from tissues of sponges 1 year after transplant and from local, unmanipulated sponges. Local sponges transplanted within their native sites and unmanipulated portions of transplanted clones showed no morphological change (*12*).

Survival of sponges with inappropriately wave-intolerant tissues is low. Sponges in high wave energy environments with morphologies characteristic of lower wave energy areas lost, on average, 41 percent [$n = 10$; standard error (S.E.) = 12 percent] of former tissue mass before morphogenetic switching occurred and showed wave-torn tissues. By contrast, low energy morphotype sponges in these environments protected from wave action by overlying plastic mesh (*9*) gained 23 percent ($n = 4$; S.E. = 15 percent) biomass during the same interval. Sponges native to high wave energy areas concomitantly transplanted into the same high wave energy environments on average showed little change (mean = +1 percent; $n = 7$; S.E. = 36 percent).

High energy morphotype sponges, however, appear to bear an associated energetic cost. The water transport network in low energy sponges is comprised of wider piping elements (0.08 mm, median of 227 radius measurements) than the transport system of stiffer sponges (0.06 mm, median of 207 radius measurements) (Fig. 2). The power required to pump a given fluid volume per unit time through a pipe varies with the inverse of the 4th power of the pipe radius (*13*), suggesting that pumping costs for stiff sponges are severalfold higher than for soft, low wave energy morphotypes (*14*). In high wave energy areas, growth rates of high energy morphotypes are lower than those of low energy morphotypes protected from wave action (see above). Thus, softer morphotypes in high wave energy environments suffer catastrophic tissue loss, but stiff morphotypes suffer persistently increased pumping costs and lower growth rates.

Delay in sponge response to environmental change (Fig. 1) could be due to (i) inherently slow morphogenetic mechanisms in these simple animals or (ii) the existence of a higher level of developmental control than that previously known for sponges. Immediate morphogenetic change in one class of transplants argues strongly against the former and suggests that *H. panicea* has a developmental control system capable of measuring relevant environmental parameters and time since onset of environmental change. Whether the environment is monitored and morphogenesis mediated by individual cells (*15*), or whether "hormonal" controlling substances (*16*) are involved is unknown.

Delay in developmental switching may in fact be advantageous to *H. panicea* in environments of temporally variable wave intensity. In the Pacific northwest, wave energies reach a peak during the winter months but are substantially less during the summer (*17*). Colonies of *H. panicea* that produce tissue appropriate to low wave energy environments during transient summer calms are devastated



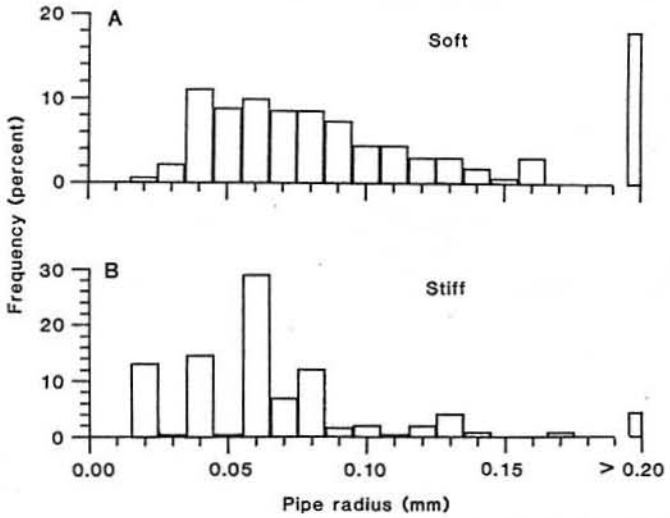


Fig. 1 (left). Stiffness (open bars) and strength (shaded bars) of sponges transplanted (A) from areas of low to high wave energy and (B) areas of high to low wave energy. Units are meganewtons per square meter. Sample sizes are shown above each set of bars; vertical lines are standard errors. Bars with common symbols are statistically indistinguishable ($P > 0.05$, Student-Newman-Kuels test). Bars with different symbols are significantly different ($P < 0.05$). Fig. 2 (right). Size frequency distributions for elements of the aquiferous (water transport) systems of sponges from low energy (soft) and high energy (stiff) environments. Stiff sponges have fewer large radius elements and more numerous narrow elements than do soft sponges. Data were collected by digitizing camera lucida drawings (×26) of pipe cross sections in sections through freshly collected sponges ($n = 227$ pipes from five low energy morphotype sponges; $n = 207$ pipes from four high energy morphotype sponges).

upon the return of severe wave conditions. Transplants switch to wave-intolerant, soft morphologies in normally sponge-free tide pools that are protected from summer but not winter wave action. Survival is high during the summer (32 of 160 transplants) but low during winter (1 of 56) in these pools. In contrast, overwinter transplant survival is high (26 of 33) in habitats exposed to normal wave action patterns.

Morphological or physiological changes in a wide variety of organisms (such as cold hardening in plants and diapause in insects) can be broadly dichotomized into those providing protection from stressful environments and those appropriate for more benign conditions (*2*, *4*, *18*). The verbal model proposed above predicts more complex or slower control mechanisms for transitions away from stress tolerance than transitions to a stress-tolerant state in uncertain environments. The experiments reported here indicate that sponge populations quickly adopt a stress-tolerant morphological tactic but delay establishment of an intolerant tactic. This suggests that acclimatory response to environment and the risks inherent in specific acclimatory tactics are closely linked.

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9. Sponge clone fragments (10 to 20 cm^3) were moved between environments and allowed to attach to the rock surface by immobilizing them for 4 weeks under plastic mesh bolted to the rock surface. Portions of transplanted clones were left at original sites as controls. In addition, sponges were transplanted within their native sites as further controls. All experiments reported here were conducted concurrently on Tatoosh Island (48°23'N, 124°44'W) from September 1981 through September 1982.
10. Tissue samples were gripped with aluminum spring clamps and pulled (tensometer, courtesy Dr. M. LaBarbera) until failure at a strain rate of 0.025 sec^{-1}. Cross-sectional areas and sample lengths were determined by caliper measurements to the nearest 0.1 mm. Stiffness and strength were determined as in (*11*).
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Pargyline Prevents MPTP-Induced Parkinsonism in Primates

Abstract. *1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a neurotoxin which produces permanent parkinsonism in human and nonhuman primates. Treatment of squirrel monkeys with pargyline, a monoamine oxidase (MAO) inhibitor, prevents both clinical and neuropathological evidence of the neurotoxic effects of MPTP. Pargyline also inhibits conversion of MPTP to 1-methyl-4-phenylpyridinium ion (MPP$^+$), a metabolic step that occurs rapidly after administration of MPTP in animals not treated with pargyline. It is proposed that the conversion of MPTP to MPP$^+$, possibly involving MAO, may be important for the neurotoxic effects of MPTP to take place, and MPTP itself may not be the neurotoxic agent.*

The neurotoxic effects of MPTP were recognized when a group of drug addicts in northern California injected this substance under the assumption that it was a new "synthetic heroin" (*1*). Within days of using the drug, these patients exhibited virtually all of the motoric features seen in Parkinson's disease, including the classic triad of bradykinesia, rigidity, and tremor. We are now treating seven humans who were exposed to MPTP and have developed moderate to severe, permanent parkinsonism. They have all responded to dopamine agonist and precursor therapy but are now experiencing many of the typical dose-limiting side effects seen with long-term L-dopa therapy in Parkinson's disease (*2*).

Squirrel monkeys given adequate doses of MPTP intraperitoneally also develop a striking parkinsonian syndrome responsive to dopamine precursor and agonist therapy (*3*). Histopathological examination of these animals has revealed selective loss of neurons in the substantia nigra (*3*), the main site of the pathology in Parkinson's disease (*4*). A profound and prolonged decrease in striatal dopamine has been observed in rhesus monkeys given MPTP intravenously (*5*). However, interest in the compound has centered around the fact that MPTP is a true neurotoxin, that is, causing cell death in the substantia nigra, and thereby inducing a permanent clinical syndrome. In this sense, MPTP-induced parkinsonism is much closer to the naturally occurring disease than reversible syndromes induced by pharmacological agents (such as reserpine).

We now present data showing that the MAO inhibitor pargyline, currently prescribed in humans for moderately severe to severe hypertension (Eutonyl, Abbott), blocks the neurotoxic effects of MPTP. Further, we provide evidence that pargyline inhibits conversion of MPTP to MPP$^+$, a step which we believe is important for this compound to exert its neurotoxicity (*6*). These experiments were carried out after it was noted that pargyline inhibits the conversion of MPTP to MPP$^+$ in vitro (*7*).

Fourteen male squirrel monkeys (*Saimiri sciureus*), aged 1 to 3 years, were used for these experiments; MPTP was