of the order Chordariales, is not closely related to *Ascophyllum*. The identity of the *Spermatochnus* gamete product with the *Ascophyllum* attractant is probably only a coincidence. This interpretation is supported by the occurrence of the same substance in vegetative parts of *Dictyopteris*, a member of yet another order, Dictyotales. Whether *Spermatochnus* interferes with fertilization in *Ascophyllum*, however, is not known. Nor is it known to what concentration finavarrene may build up in a densely populated *Ascophyllum* habitat.

From the few sexual hormones known from marine brown algae, it appears that the marine environment contains numerous substances that function as intraspecific or even interspecific information carriers. The thresholds for biological effects can be extremely low, less than 10^{-11} molar, as has been shown for male gametes of *Cutleria multifida* (*14*). More thorough knowledge of chemical messenger systems in other species is a prerequisite to understanding the interactions in the marine environment.

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Leaf Closure in the Venus Flytrap: An Acid Growth Response

Abstract. *The rapid closure of leaves in the Venus flytrap* (Dionaea muscipula) *involves irreversible cell enlargement, which can be initiated by acidifying the cell walls to* p*H 4.50 and below. Leaves infiltrated with neutral buffers that keep the* p*H above 4.50 to 4.75 will not close in response to stimulation of their trigger hairs even though the action potentials that ordinarily cause closure are produced. During the 1 to 3 seconds required for closure about 29 percent of the cellular adenosine triphosphate is lost. It is likely that this adenosine triphosphate is used in very rapid transport of hydrogen ions from the motor cells and that the movement is due to a mechanism of "acid growth."*

Rapid leaf movements are usually assumed to be the result of changes in turgor pressure following a loss of solute (*1–3*). The best-studied rapid movement, closure of the *Mimosa* leaf, is accomplished by just such a mechanism (*4, 5*). There is no evidence, however, that this mechanism is responsible for rapid movements of leaves that lack pulvini (jointlike thickenings), such as Venus flytrap (*Dionaea muscipula* Ellis) leaves. The limited information available on Venus flytrap leaves indicates that the movements may be due to very rapid growth (*6*).

As early as 1916, Brown (*7*) demonstrated that closure of Venus flytrap leaves results from an irreversible expansion of the outer surfaces of the trap lobes in the lateral direction and that reopening results from an irreversible expansion of the inner surface. More recently it has been demonstrated that the lobes expand longitudinally as well (*8*). While Brown proposed that this movement is due to a rapid gain in turgor by the outer trap surface, analogy with rapid movements of sundew tentacles indicates that the movement may be due to rapid changes in plasticity of the cell walls and a resulting cell expansion, causing a decrease rather than an increase in turgor (*6*).

Many plants exhibit rapid cell expansion when cell wall components are acidified (*9*). This suggests a plausible mechanism for the rapid expansion of trap cells. If hydrogen ions are released from the cells after the action potential that triggers closure, rapid changes in wall plasticity could occur. The release of H^+ would necessarily be active, since the H^+ gradient in nearly all plant cells favors passive movement into the cell, and would most likely occur through the action of the postulated electrogenic H^+ pump in plant cell plasma membranes (*10*). Experiments that show a loss of adenosine triphosphate (ATP) in trap midribs during closure indicate the presence of an energy source for rapid H^+ extrusion in the midribs (*11*). If such activity also occurs in the trap lobes, where movement takes place (*7, 12, 13*), rapid, ATP-dependent H^+ transport may be involved in closure.

We propose that the rapid closure of Venus flytrap leaves is due to acid-stimulated growth triggered by a very rapid H^+ pump. If this hypothesis is true, then (i) Brown's (*7*) observation of cell expansion during closure should be reproducible, (ii) turgor in outer epidermal cells should diminish with closure, (iii) leaves whose intracellular spaces are infiltrated with neutral buffers should not respond to a series of action potentials resulting from stimulation of the trigger hairs, (iv) leaves whose intracellular spaces are infiltrated with acid buffers should close spontaneously, and (v) trap lobes should lose large amounts of ATP or some other high-energy compound during the 1 to 3 seconds required for trap closure.

Cell expansion during closure was directly measured by using a pounce wheel to mark the surface of the lobes with a series of evenly spaced ink dots and measuring the change in distance be-

Table 1. Response of Venus flytrap leaves whose extracellular spaces were perfused with 50 m*M* acetate or 2-(*N*-morpholino)ethanesulfonic acid buffers in the absence of mechanical stimulation and in the presence of stimulation of trigger hairs at 6-second intervals with a camel's hair brush. Sap was expressed from the cut petiole in a pressure chamber and the leaves were rehydrated in the appropriate buffer solution. All buffer solutions were adjusted with sorbitol to approximately 70 milliosmoles per kilogram. Values are means ± standard errors. N.D., not determined.

*p*H*	Number of stimuli necessary to cause trap to close 50 percent	Closure rate of unstimulated traps (percent per minute)
Control†	7 ± 3	0.0 ± 0
3.0	N.D.	0.27 ± 0.01
4.0	N.D.	1.38 ± 0.20
4.5	11 ± 5	0.32 ± 0.16
4.75	60 ± 24	N.D.
5.0	90 ± 7‡	0.0 ± 0
6.0	N.D.	0.0 ± 0

*Buffers had a buffering capacity 30 m*M* above the *p*H listed. †Perfusion with 70 m*M* sorbitol. ‡Traps requiring more than 100 stimuli for 50 percent closure were recorded as >100 and averaged with the data as 100.

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tween the dots after closure. A similar experiment was performed on reopening traps. During closure the outer epidermis of the central portion of the lobes expanded 27.9 ± 2.1 percent (Fig. 1). At the same time, there was no significant change in the distance between dots marked on the central portion of the inner epidermis of the lobes. During 10 hours of reopening the inner epidermis of the central portion expanded 12.3 ± 1.1 percent while the outer epidermis showed no significant change in size. These results closely parallel those of Brown (*7*) and show that an irreversible expansion of the outer epidermis is involved in closure and an irreversible expansion of the inner epidermis is involved in reopening.

Turgidity of the outer surface of the open traps was pronounced, and the points of the pounce wheel rolled easily over the surface. By contrast, the surface of the recently closed traps seemed flaccid, since the points of the pounce wheel tended to dimple it. This loss of turgor is not consistent with the mechanism of turgor gain proposed by Brown, but is expected if expansion is due to an increase in wall plasticity.

The leaf water potential, measured with a pressure chamber (*14*), decreased by 0.51 ± 0.04 bar upon closure and slowly recovered to the initial level over a period of approximately 20 minutes. This result is consistent with a rapid increase in wall plasticity accompanying rapid cell wall acidification, followed by a slow redistribution of solutes in the tissue. Measurements by Kondo (*8*) indicate that, following closure, there is a relatively slow shift in water from leaf areas that are not active in movement to the active parts.

Leaves infiltrated with neutral buffers produced action potentials when their trigger hairs were stimulated. However, leaves infiltrated with 3-(*N*-morpholino)propanesulfonic acid, 2-(*N*-morpholino)ethanesulfonic acid, or acetate buffers (buffering capacity, more than 30 m*M* above *p*H 5.0) showed only slight movement after long and continuous stimulation of their trigger hairs at 6-second intervals. Leaves infiltrated with buffers with a 50-m*M* buffering capacity above *p*H 5.0 were virtually paralyzed (Table 1). Control leaves infiltrated with 70 m*M* sorbitol readily closed after receiving normal stimulation of the trigger hairs. As shown in Table 1, the sorbitol-perfused controls required a mean of seven stimulations to initiate closure. Since all buffers were adjusted to 70 milliosmoles per kilogram, osmotic considerations could not have been a factor in the differences in responsiveness to the various buffers used.

Table 2. Differences in ATP content of matched pairs of trap lobes before and after closure. One lobe of a trap was frozen by dipping it in Freon cooled with liquid nitrogen. The opposing lobe was triggered to close by the cold shock received in the tissue near the midrib and was immediately frozen after this closure by complete submersion of the leaf in Freon. The midrib of the trap was cut away and the lobes were separated, extracted with cold 5 percent trichloroacetic acid, and analyzed for ATP by the luciferin-luciferase assay (*17*). Values are means ± standard errors.

Condition of lobes	ATP		Change in ATP during closure (percent)
	Micromoles per lobe	Micromoles per gram	
Open	8.73 ± 0.75	59.3 ± 2.3	
Closed	6.52 ± 0.54	47.0 ± 4.9	−28.9 ± 3.1

Traps infiltrated with acetate buffers having a 30-m*M* buffering capacity above *p*H 4.75 were only slightly inhibited, while traps infiltrated with buffers with a 30-m*M* buffering capacity above *p*H 4.50 closed nearly as rapidly as the sorbitol-perfused controls on stimulation (Table 1). If it is assumed that the trap is triggered by the release of H^+ from the cells, enough H^+ must be released to overcome a 30 m*M* buffer and still reach the *p*H threshold for wall extension. The enzyme or enzymes involved must have an action threshold near *p*H 4.75 and be quite active at *p*H 4.50 in the leaf tissue. This agrees with observations of wall-loosening enzymes in pea epidermis, where maximum extension rates occurred between *p*H 4.0 and *p*H 3.0 and the response threshold was near *p*H 5.0 (*15*).

Leaves infiltrated with buffers of *p*H 4.50 or lower closed spontaneously (Table 1). Traps infiltrated with acetate buffers of *p*H 4.0 closed more rapidly than those infiltrated with acetate buffers of *p*H 3.0, indicating a *p*H optimum for the response above *p*H 3.0 and considerable activity around *p*H 4.0. Closure was slow and erratic at *p*H 4.5 and nonexistent at *p*H 5.0 (Table 1). Again, this observation is consistent with observations of wall-loosening enzymes in pea epidermis (*15*).

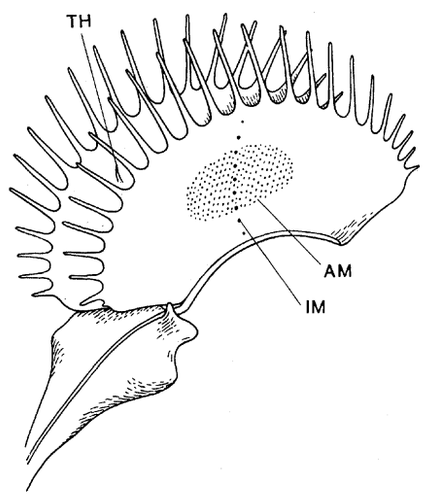

Fig. 1. Diagram of the Venus flytrap leaf, showing a trigger hair (*TH*), which is the sensory structure that detects mechanical stimulation; the area of greatest movement (*AM*), where the largest changes in epidermal length occur during closure; and the location of the ink marks (*IM*) used to measure the change in size of the epidermal cells during closure.

Changes in ATP levels measured during closure indicate that 29 percent of the cellular ATP disappeared during the 1 to 3 seconds required for trap closure (Table 2). This agrees closely with the portion of ATP reported to be lost from the leaf midrib during closure (*11*). Since the lobe must contain a large amount of inactive tissue, the proportion of ATP lost in active cells must have been much higher than 29 percent.

We conclude that very rapid, irreversible cell expansion, which can be stimulated by a cell wall *p*H of 4.5 and lower, results in closure of *Dionaea* traps. These movements can be prevented by neutral buffers, which prevent the cell wall *p*H from reaching 4.5 to 4.75. This *p*H threshold is consistent with the *p*H threshold of enzymes involved in acid growth mechanisms in pea epidermis (*15*). The rapid loss of ATP during trap closure is likely to be the result of ATP-dependent efflux of H^+ from motor cells in the lobes. Thus, the closure movement of Venus flytrap appears to be due to a rapid acid growth mechanism and not to a turgor loss mechanism (*16*) or a turgor gain mechanism (*7*). This tissue could be used to study the chemical and structural changes that occur during plant growth as well as the mechanism controlling H^+ transport in plant cells.

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New Method for Detecting Cellular Transforming Genes

Abstract. *Tumor induction in athymic nude mice can be used to detect dominant transforming genes in cellular DNA. Mouse NIH 3T3 cells freshly transfected with either cloned Moloney sarcoma proviral DNA or cellular DNA's derived from virally transformed cells induced tumors when injected into athymic nu/nu mice. Tumors were also induced by cells transfected with DNA from two tumor-derived and one chemically transformed human cell lines. The mouse tumors induced by human cell line DNA's contained human DNA sequences, and DNA derived from these tumors was capable of inducing both tumors and foci on subsequent transfection. Tumor induction in nude mice represents a useful new method for the detection and selection of cells transformed by cellular oncogenes.*

Recent advances in the technique of DNA-mediated gene transfer have led to the identification (*1–10*) and molecular cloning (*11–13*) of dominant human transforming genes from neoplastic cells. In most cases the genes have been detected by means of focus forming assay on NIH 3T3 cells although in one study the genes were also detected by examining the ability of transformed cells to grow in soft agar (*2*). Since the identification of foci is dependent on cell culture conditions and cell morphology and since the latter can represent a somewhat subjective measure of cell transformation, we sought to develop an alternative screening and selection method for transformed cells. Transformed cells can induce tumors in mice, and in particular, tumorigenicity in athymic nu/nu mice has been used to definitively demonstrate the transformed phenotype (*14*). The potential of the nude mouse as a vehicle for detecting and isolating transformed cells from a mass population of DNA transfected cells is described in this report.

We initially measured the ability of cells transfected with various amounts of the molecularly cloned acute transforming provirus of Moloney murine sarcoma virus (MSV) (*15*) to induce tumors in nude mice (Fig. 1). The results show that mice injected with MSV DNA-transfected cells developed tumors which appeared between 6 to 8 weeks after injection. At the lowest level tested (0.4 ng), one of three mice developed a palpable tumor, while at the highest level tested (400 ng), four of five mice developed tumors. A portion of each transfected cell population was also assayed in the conventional focus induction assay, and transformed foci were counted 14 days later. One focus was observed on cells treated with 0.4 ng of MSV DNA, while cells treated with 400 ng gave approximately 400 foci. It is unclear why only four out of five mice receiving cells transfected with 400 ng of MSV DNA developed tumors, although it may be due to the immune response to tumor cells present in nude mice (*16*). However, the fact that tumors were observed when cells had been transfected with as little as 0.4 ng of MSV DNA indicated that the tumor assay was highly sensitive and able to detect the presence of small numbers of transformed cells. The tumors all arose at the site of injection, grew rapidly, and appeared to be undifferentiated fibrosarcomas (*17*). Superinfection of cell lines derived from tumors with Moloney murine leukemia virus (MuLV) resulted in the rescue of infectious transforming virus from 50 percent of these cultures (four rescued of eight tested). Similar proportions of MSV foci induced by conventional DNA transfection assays are rescuable with MuLV (*18*). Mice injected with untransformed cells or cells transfected with carrier calf thymus DNA did not develop tumors during the 15-week observation period.

We also examined the ability of cells transfected with cellular DNA from virally transformed cells to induce tumors in mice. DNA was isolated from HTMF cells, an MSV-transformed mink cell line containing a single copy of MSV (*15*), and SV80 cells, an SV40-transformed human fibroblast cell line (*19*). These cellular DNA preparations induced tumors in nude mice in 5 to 7 weeks (Table 1), and foci were observed in portions of the HTMF DNA-transfected cells maintained in tissue culture. MSV could be rescued from two of three tumors induced by HTMF DNA-transfected cells. Three tumors induced by SV80 DNA transfection were explanted, and the resulting cell lines were tested for SV40 T antigen expression in an immunofluorescence assay (*20*). From 40 to 80 percent of the cells present in each tumor-derived cell line exhibited nuclear staining characteristics of SV40-transformed cells. These results showed that the assay detected single-copy transforming genes present in cellular DNA and suggested that that assay could be used to detect such sequences in DNA isolated from transformed human cells.

All of the tumors observed in the experiments described above appeared in 5 to 8 weeks, and no tumors were observed in control experiments. However, in experiments not shown, we found that tumors could be induced in mice injected with NIH 3T3 cells transfected with DNA from nonneoplastic tissues, including calf thymus, human placenta, and mouse NIH 3T3 cells. The tumors derived from human placenta DNA transfections lacked detectable human repetitive sequences when analyzed as described below. These tumors developed later (8 to 15 weeks) than tumors induced by cells that had been transfected with DNA containing dominant transforming genes. In addition, the frequency of tumor formation in these experiments was greater when large numbers of NIH 3T3 cells (5×10^6 to 1×10^7 per mouse) were injected and was also influenced by the length of time the cells were maintained in culture prior to injection. Consequently, the incidence of these tumors can be reduced by injecting $< 2 \times 10^6$ cells into each mouse 1 to 5 days after transfection. Cells to be transfected are used either directly after revival from frozen stocks or are passaged no more than twice at subconfluent densities. In addition, our experiences indicate that tumors arising more than 9 weeks after the injection of transfected

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