homology between human c-*sis* and v-*sis*, we would expect the woolly monkey c-*sis* locus from which v-*sis* has been derived to be similar to human c-*sis*. If so, the cellular c-*sis* exon could extend to either of two possible splice sites and begin upstream from the region of homology. This allows the possibility that the recombination event that led to v-*sis* formation may have occurred within a cellular exon.

4) A six-base sequence (GGCAGG) is present in SSV, SSAV (*15*), and c-*sis* at the 5′ junction of the v-*sis* substitution (boxed in Fig. 2, A and B). The location of such a homology between helper virus and cellular DNA at the exact site of recombination is not likely to be due to chance (probability $< 3 \times 10^{-4}$). Furthermore, this is a minimal estimate for the homology that may exist between SSV and c-*sis* of the woolly monkey, the host of origin (*6, 7*). For example, the homology of SSAV and c-*sis* may be extended to nine bases (CCTGGCAGG) by allowing a possible change of C to T for the corresponding woolly monkey DNA sequence. This short homology between helper virus and cellular DNA may have played a role in the initial recombination event.

The following sequence of events could have occurred. An SSAV provirus was integrated upstream from c-*sis* in the woolly monkey tumor DNA. Recombination occurred between the homologous regions, deleting out the intermediary sequences. This brought into proximity the defective helper virus genome and c-*sis*. Goldfarb and Weinberg (*14*) proposed that the first step in generation of defective transforming viruses is integration of the helper virus genome adjacent to a cellular *onc* gene followed by read-through transcription. Our result suggests that a legitimate or homologous recombination between the helper virus and the cellular *sis* gene at the DNA level may have occurred first, deleting out a part of the helper virus genome including the transcriptional termination signals. Consequently, the initial read-through transcript was a hybrid molecule of defective helper viral sequences and cellular sequences that was then processed to remove the cellular introns. Further events, which must have occurred to account for the 3′ SSAV sequences of the transforming virus genome, might have taken place at the level of reverse transcription (*13, 14*).

Our studies suggest that v-*sis* may contain only the 3′ portion of the functional c-*sis* gene and that a short sequence homology between SSAV and c-*sis* may have played a role in the initial recombination event leading to the generation of SSV. Although the mechanism of recombination proposed here may not apply to all retroviral *onc* genes, it has been reported that c-*mos* and v-*mos* also share a 4-bp homology at the junction of recombination (*18*). That result would suggest that the homology need not be as extensive as the one we found.

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Leaf Dimorphism in Aquatic Angiosperms: Significance of Turgor Pressure and Cell Expansion

Abstract. *Depending on environmental conditions, the aquatic angiosperm* Callitriche heterophylla *can develop two different leaf types with distinctive morphological characteristics. Cellular turgor pressure seems to act as the biophysical mechanism responsible for the selection of leaf form in control conditions designed to mimic the natural habitat of this plant. The experimental induction of leaf form involves the ability of various treatments to mediate cell expansion through their effects on turgor pressure or wall extensibility.*

Botanists have long been intrigued by the problem of leaf development in aquatic angiosperms (*1*). In the natural environment the leaves of these plants can develop into two alternative forms depending on the position of the shoot meristem relative to the water surface. The elongate, often dissected leaves that originate on submerged apices are called water forms and the shortened, broad leaves from emergent apices are designated land forms. It is possible to force immature leaves to develop into the form atypical for a given environment by applying a wide variety of treatments, including different light exposures, growth regulators, osmotica, and temperature extremes (*2*). This marked sensitivity to environmental conditions and cultural manipulations has made aquatic angiosperms favored experimental organisms in the search to identify the basic mechanisms that control leaf development in all plants. In the present study we attempted to determine whether cellular water relations play a causal role in the developmental choice between alternative leaf forms in aquatic angiosperms.

Clonal specimens of the aquatic *Callitriche heterophylla* Pursh. were grown at 23° ± 2°C in aquariums (submerged apices) or on wet soil (emergent apices) in a growth chamber with a photoperiodic cycle having 16 hours of white light (12 W/m^2) and 8 hours of darkness. Representative shoots grown under the two cultural conditions are shown in Fig. 1, A and B. Water-form leaves on submerged apices exhibit higher ratios of length to width, lower stomatal densities, and fewer vascular bundles than do land forms on emergent apices.

Repeated sprayings with $10^{-5}M$ gibberellic acid caused the growth of typical water-form leaves on emergent shoots, whereas the inclusion of $10^{-5}M$ abscisic

Table 1. Relationship of cell lengths and water relations to the leaf form borne on *Callitriche heterophylla* shoots. For the potential measurements, each treatment was composed of four to five replicates, with water and osmotic potentials being measured for two to three plants per replicate. The lengths of 40 adaxial epidermal cells were measured on the mature leaves of five plants, each of which was treated as a single replicate. Data are means ± standard errors.

Treatment	Apex position	Mature leaf type	Water relations of developing leaves (bars)			Length of epidermal cells in mature leaves (μm)
			Ψ_w*	Ψ_π	Ψ_p	
Control	Submerged	Water form	−3.0 ± 0.2	−6.5 ± 0.4	3.5 ± 0.5	120 ± 6
Control	Emergent	Land form	−8.9 ± 0.5	−8.1 ± 0.6	−0.8 ± 0.7	57 ± 3
Gibberellic acid ($10^{-5}M$)	Emergent	Water form	−5.5 ± 0.2	−8.4 ± 0.8	2.9 ± 0.9	109 ± 4
Mannitol (0.24 mole/kg)	Submerged	Land form	−11.2 ± 1.0	−11.8 ± 0.7	0.6 ± 1.7	56 ± 3
Abscisic acid ($10^{-5}M$)	Submerged	Land form	−3.1 ± 0.2	−7.1 ± 0.6	4.0 ± 0.7	44 ± 2
High temperature (30°C)	Submerged	Land form	−3.3 ± 0.4	−7.7 ± 0.2	4.4 ± 0.3	60 ± 2

*All water potentials were measured in the middle of the photoperiod. In general, readings taken in the dark period were 2 to 4 bars less negative for emergent apices and unchanged for submerged apices.

acid or 0.24 molal mannitol in the aqueous medium led to the formation of land forms on submerged apices. Raising the temperature of the aqueous medium to 30°C effected a similar change to the production of land forms underwater.

Thermocouple psychrometry was used to measure the water potentials (Ψ_w) of developing pairs of leaves exposed to control and experimental treatments. The osmotic potentials (Ψ_π) of immature leaf pairs from plants in the same replicate were obtained by first freezing the leaves with liquid N_2, allowing the leaves to thaw in the sample chamber, and then making similar psychrometric measurements. Turgor pressures (Ψ_p) were calculated from the relation $\Psi_w = \Psi_\pi + \Psi_p$ (*3*).

In nature, water-form leaves arise on *C. heterophylla* plants whose apices are submerged beneath the water's surface, while shoots floating on the surface or growing on muddy shores bear land-form leaves. The most noteworthy distinction between these environments is their marked difference in water availability. Thus, it is not surprising that developing leaves on submerged and emergent control shoots showed consistent differences in cellular water potential (Table 1). The observed water potentials of young leaves on submerged controls were close to the value of −2.3 bars measured for the aqueous medium in which the plants were grown, while immature leaves on emergent controls exhibited much lower water potentials. The low values for the land forms are attributable in part to transpiration losses from numerous stomata, which are fully open in the daylight; the water potentials of these leaves measured in the dark period were 2 to 4 bars less negative. Submerged and emergent control shoots did, however, manifest only a slight difference in the osmotic potentials of their developing leaves. Consequently, the calculated turgor pressure values for water-form controls are more than 4 bars higher than for land-form controls. Associated with the high turgor pressure in developing leaves are the long epidermal cells observed in mature water forms (Fig. 1D).

The effects of various experimental treatments on the water relations of developing *C. heterophylla* leaves are detailed in Table 1. Exposing emergent apices to $10^{-5}M$ gibberellic acid caused the leaves to have higher water potentials and lower osmotic potentials than the emergent controls. Emergent leaves treated with gibberellic acid were thus calculated to have turgor pressures approaching those of the submerged controls. Moreover, the mature leaves assumed all the characteristics of typical water forms, including the long epidermal cells. Growing submerged apices in 0.24 molal mannitol markedly decreased the water and osmotic potentials of the young leaves, resulting in turgor pressures similar to those measured in the emergent controls. In addition, mannitol-treated apices produced land-form leaves with short epidermal cells. Immature leaves on submerged apices subjected to high temperature (30°C) or to $10^{-5}M$ abscisic acid exhibited water and osmotic potentials that did not differ significantly from those measured in the submerged controls. However, even though these treatments caused the developing leaves to have high turgor pressure, the experimental plants produced land-form leaves with short epidermal cells.

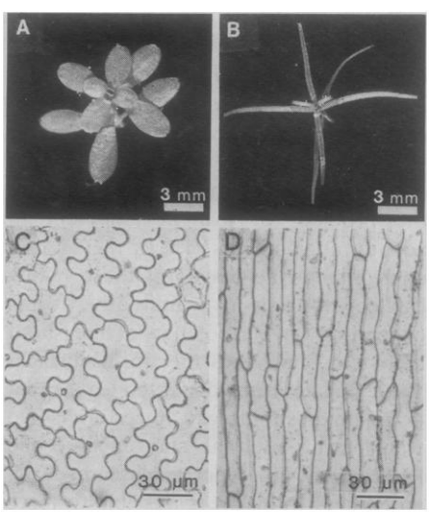

Fig. 1. (A and B) Representative shoot apices of *Callitriche heterophylla* plants. Land-form leaves (A) have developed on an emergent apex from soil culture, while water forms (B) have arisen on a submerged apex from aquarium culture. (C and D) Representative views of the mature abaxial epidermis of land-form (C) and water-form (D) leaves prepared by staining with 1 percent methylene blue. The ordinary epidermal cells of the adaxial epidermis are identical in size and shape to these abaxial cells. Stomates are restricted to the adaxial surface.

The results from the experimental treatments can be understood in terms of the fundamental equation for cell expansion in plants, $r = m(P - Y)$, where r, m, P, and Y represent expansion rate, yielding compliance, cell turgor, and threshold turgor for growth, respectively (*4*). It is presumed that the control treatments exerted their major influence on cell expansion by their ability to regulate turgor pressure: the length of the epidermal cells and hence the overall shape of the mature leaf is directly correlated with the magnitude of cellular turgor. A similar interpretation can be made for leaves produced in the presence of gibberellic acid or mannitol. The limited cell expansion in submerged apices exposed to abscisic acid or high temperature cannot be the result of low turgor; instead, these treatments appeared to alter the yielding compliance or threshold turgor so that even the high turgor failed to promote cell expansion beyond the level characteristic of land-form controls. Figure 1, C and D, shows how cell expansion might act to regulate leaf form. The epidermal cells of land forms from the emergent

controls and from the treated plants are shaped like the pieces of a jigsaw puzzle. It seems that both submergence and gibberellic acid cause these cells to become much more attenuated, and thus the developing leaves become transformed into the long, narrow water forms. The emphasis on cell expansion as the principal control mechanism is further supported by preliminary counts of ordinary epidermal cells: the number of these cells in the two leaf forms in the control conditions differed by only 25 percent or less.

Aquatic angiosperms respond to an environmental change in water status quite differently from other aquatic plants. Almost all estuarine algae studied to date are able to maintain constant turgor pressure in the presence of a wide range of salinities. This regulation is accomplished by exchanging certain ions with the surrounding water or by alternating the level of photosynthate in the cytoplasm (*5*). These mechanisms allow the algae to avoid plasmolysis even when the level of salinity in the estuary approaches that of pure seawater. In contrast, it is the osmotic potential of the angiosperm *C. heterophylla* that exhibits little variation with marked fluctuations in the water status of its environment. Consequently, when a declining water level places the shoot apex of a submerged plant above the water's surface, the newly emergent apex must experience a substantial reduction in cellular turgor, and the pattern of leaf production changes to the land form. Other features associated with this morphological switch are increases in stomatal density and vein number, which help to adapt the leaf for photosynthesis in the aerial environment.

Cell expansion is the essential growth process whereby plant structures increase in size. Internal water relations exert profound effects on the cellular growth rates of such structures as roots, stems, leaves, and coleoptiles (*6*). Furthermore, theoretical studies have shown that the water potentials of cells elongating as a coordinated unit in multicellular structures necessarily decrease with distance from the source of the water supply (*7*). From the present experiments, it appears that the relative amount of cell expansion also determines the overall shape of mature organs, or least in the instance of the leaves of aquatic angiosperms.

In conclusion, the research described here suggests that the relative magnitude of cellular turgor pressure determines leaf form in aquatic angiosperms in their native environment. Moreover, under control and experimental conditions the process of cell expansion affects not only final leaf size but also final leaf shape in aquatic angiosperms. Whether these observations apply to other plants awaits future research.

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A Fossil Noctuid Moth Egg from the Late Cretaceous of Eastern North America

Abstract. *A moth egg assignable to the family Noctuidae (Lepidoptera) is described from 75-million-year-old sediments from Martha's Vineyard, Massachusetts. This sample, which extends the fossil record of this family and modern heteroneuran moths back to the Cretaceous, may provide insight into the coevolution of moths and flowering plants, as well as have implications for the evolution of bats.*

Although the order Lepidoptera is one of the most diverse modern groups, it is underrepresented in the fossil record. The origin and radiation of the Lepidoptera are now thought to have occurred in at least Mesozoic times (*1–3*). Reports of fossil Lepidoptera of Tertiary age now include over 70 moths and 40 butterflies (*4*); the Cretaceous record consists of only a few adults (*3, 5*), larvae (*6*), and wing scales (*7*) of homoneurous Lepidoptera. Reports of the group in the Triassic include *Eoses triassica* (*8*) and material from South Africa (*9*) and Australia (*2*). Some investigators place these early fossils in Mecoptera (*1–3, 10*), and most agree that the Lepidoptera must have been present in the Triassic. Our report of fossil evidence of the vast moth family Noctuidae and of a heteroneurous lepidopteran in the Cretaceous is thus not unexpected. We are not aware of other descriptions of fossil lepidopteran eggs as such, but unidentified fossil "angiosperm seeds" (*11*) may prove to be lepidopteran (or other insect) eggs.

The egg was found in an organic-rich clay lens in lagoonal sediments of the (?)Magothy Formation on Gay Head, Martha's Vineyard. These sediments are dated as earliest Campanian (~ 75 million years old) on the basis of pollen analysis (*12*). The clay contains an array of vegetable remains including conifers (*12, 13*) and a diverse flora of angiosperm flowers, fruits, and seeds (*14*). The angiosperm fossils are less than 3.0 mm in diameter, requiring collection by sieving and examination with a dissecting microscope. The egg was found with plant material in this manner.

The ovoid to broadly ovoid, slightly flattened egg is approximately 500 to 600 μm in diameter and 600 μm high (Fig. 1, a and c). The surface is traversed by about 18 to 20 thick longitudinal ridges, at least one of which bifurcates in the lower hemisphere (extreme left, Fig. 1c). Each longitudinal ridge is connected to its neighbor by 15 to 20, smaller, latitudinal ridges. This surface pattern is stronger than that seen in comparable modern eggs (Fig. 1d). Inspection of the ridges at higher magnification gives no indication of aeropyles—conduits for gas exchange that are present in many modern insect