Na channels may develop after a brief exposure to TTX. (iii) The depolarizations induced in the crayfish muscle fiber are long-lasting, whereas Na activation of spike electrogenesis is usually short-lived, being reversed by depolarizing Na inactivation (*10*). However, under abnormal ionic conditions even squid giant axons develop prolonged spikes which may reflect marked slowing of Na inactivation (*13*).

The increase in Na permeability which is indicated by an increase in membrane conductance as well as by the depolarizing electrogenesis is only transient. The muscle fiber repolarizes even in a Ca-free medium. The intracellular concentration of Ca in crayfish muscle fibers is rather high (about 20 mmole/liter) (*14*), much of it presumably in bound form. The disturbance of the Ca gradient across the membrane, caused by removal of Ca from the medium, probably initiates an outward movement of this cation. Thus, it seems likely that the repolarization which signals a decrease in Na permeability is linked with the replenishment of Ca in the membrane and its vicinity. This conclusion is supported by the finding that a fiber repolarized in a Ca-free medium undergoes a second depolarization on addition of EDTA, or merely on changing to a fresh Ca-free medium. The replenishment of Ca should be depressed more by larger quantities of EDTA. This expectation is also fulfilled (Fig. 3).

No adequate morphological studies are available for correlation with the physiological data. However, an alteration in the Na : Ca ratio of the bathing medium causes transient changes in the membrane resistance and in the thickness of the unit membrane of the amoeba (*15*).

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Gametophytes of Four Tropical Fern Genera Reproducing Independently of Their Sporophytes in the Southern Appalachians

Abstract. *Vegetative reproduction and dispersal by way of gemmae are known to occur in four types of fern gametophytes. Although they belong to basically tropical rain-forest genera, all four types have now been discovered growing naturally in the vicinity of Highlands, North Carolina, as clones on shady, damp rocks. Their sporophytes were rare or absent. The gametophytes are now identified as* Grammitis nimbata *(Jenm.) Proctor, Grammitidaceae;* Hymenophyllum tunbridgense *(L.) J. Sm., Hymenophyllaceae;* Vittaria lineata *(L.) J. Sm., Vittariaceae; and* Trichomanes, *probably representing several species, Hymenophyllaceae. Identification of the* Grammitis *was facilitated by the presence of some juvenile or dwarfed sporophytes; this constitutes the first report of this species, genus, and family for temperate North America. Growth and spread of fern gametophytes independently of, and distant from, the corresponding sporophytes is a novel phenomenon which should be investigated in other parts of the world.*

The gametophytes or sexual plants of most fern families and genera lack any method of reproducing themselves. New thalli may occasionally be formed from branches or by fragmentation due to senescence of older parts, but usually fertilization and the formation of a sporophytic plant prevent further growth of the original gametophyte. However, in a few primarily tropical fern genera, single gametophytes can form large, long-lived populations. These gametophytes have specialized vegetative structures of dispersal, gemmae, which are tiny bodies usually made up of two to ten cells. The gametophytes are thus able to spread by air and form new colonies, generally resembling patches of liverworts or algae. Such gemmaebearing gametophytes may occur independently of their sporophytes, in some cases separated from them by hundreds of miles (*1, 2*).

The clonal fern gametophytes that reproduce by gemmae fall into four distinct types: (i) an elongate-cordate type with "string-of-beads" type of gemmae; (ii) a branching ribbon-like type with marginal rhizoids and small, plate-like gemmae; (iii) a branching ribbon-like type with diffuse rhizoids and spindle-shaped gemmae; and (iv) a filamentous type with spindle-shaped gemmae. These are found respectively in the Grammitidaceae (*3*); the Hymenophyllaceae, genus *Hymenophyllum* (*4*); the Vittariaceae (*1*); and the Hymenophyllaceae, genus *Trichomanes* (*4*). The new concept of pteridophyte gametophytes existing and reproducing as self-dispersing clones, often far from their sporophytes, is of considerable botanical interest.

We now describe an unexpectedly rich display of this phenomenon in the vicinity of Highlands Biological Station, Macon County, North Carolina. The region was originally chosen for study of clonal gametophytes because of earlier reports that, in nearby Pickens County, South Carolina, there was a single colony of sporophytes of *Hymenophyllum tunbridgense* (L.) J. Sm. (*5*) unknown elsewhere in the United States, and its other occurrences being in the mild climates of Europe and the West Indies. All the basic types of gemma-reproducing gametophytes were encountered at Highlands, including the first record of a new family of ferns for temperate North America. Highlands is located in the southern Appalachian

mountains where the Blue Ridge escarpment runs nearly east to west and the ridge is cut by a number of narrow river valleys which form natural channels for warm, moist, southerly winds. The resultant high rainfall is reflected in luxuriant vegetation (*6*).

Gametophytes of the genus *Hymenophyllum* were found in all the river valleys examined, in some places 80 km (50 miles) away from the single site where the sporophyte is known in South Carolina. The gametophytic colonies of *Hymenophyllum* were often large, sometimes covering as much as a square meter of rock substrate. In a number of the colonies the gametophytes bore both archegonia and antheridia; nevertheless, sporophytes were encountered nowhere except in the original sporophyte locality. The gametophytes probably represent the same species as the sporophyte *Hymenophyllum tunbridgense* (L.) J. Sm. which grows in South Carolina.

The two vegetatively dispersed gametophytes which have been reported to grow in eastern United States in the absence of their sporophytic counterparts were also widely distributed in the Highlands area. One of these is the thallus known for over 30 years as the "Appalachian gametophyte" and more recently identified as probably the sexual stage of *Vittaria lineata* (L.) J. E. Sm. (*1*). The gametophyte grows as far north as Virginia and Ohio, but the sporophyte is known in the United States only in Florida and in one isolated station in Georgia. Identification of the other gametophyte, *Trichomanes*, reported from Illinois and Virginia (*2*, *7*), is more problematical, and, at least at Highlands, there may be several taxa involved. The identification is complicated because at least five species of *Trichomanes* in eastern United States probably have nearly identical gametophytes. Three of these, *T. punctatum* Poiret, *T. kraussii* Hook. and Grev., and *T. lineolatum* (v.d.B.) Hook., grow on rocks and mossy roots in southern Florida. The more temperate outliers of this primarily tropical genus are *T. petersii* A. Gray, which occurs from Louisiana and Florida north to the southern Appalachians (*5*), and *T. boschianum* Sturm, which is scattered in east-central United States (*8*). The *Trichomanes* in the Highlands area may be a combination of these species, but we found no means of distinguishing them with certainty.

The above-mentioned types of gametophytes may be expected to occur in a typical rain forest in tropical America on mossy tree trunks and rocks. In such areas, a fourth type is normally present, the elongate-cordate thallus type of the Grammitidaceae with its very distinctive gemmae. Although the possibility that this type of gametophyte might also exist at Highlands was entertained only as a remote one, it was indeed discovered there growing in large numbers behind a waterfall near the biological station. The identification was confirmed by the presence of about a dozen obviously juvenile or dwarfed sporophytes belonging to the species *Grammitis nimbata* (Jenm.) Proctor (*9*); the nearest locality for this species is Cuba, about 1280 km (800 miles) to the south. This is a remarkable new record for temperate North America. Possibly, this entire colony in North Carolina may have originated from the chance landing of a single spore from the south. The gametophyte has flourished locally by vegetative reproduction, but the sporophytes, judging from those growing at this single location, are unable to mature to their distinctive adult form. The tiny sporophytes, all less than 3 cm tall, entirely lacked reproductive structures.

On the basis of the foregoing observations, similar searches for clonal gemmiferous gametophytes might be made elsewhere, such as the temperate forest areas of Europe and eastern Asia. In the United States these gametophytes are usually found on damp, acidic rocks where the light intensity is too low for the growth of most bryophytes. Any gametophytes with gemma propagation may have the potential to exist continuously at long distances from their ancestral sporophyte populations.

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Ciguatoxin: Isolation and Chemical Nature

Abstract. *Ciguatoxin, the agent responsible for ciguatera, a disease produced in humans from ingestion of certain fishes, has been isolated from specimens of the moray eel,* Gymnothorax javanicus. *The toxin is apparently a lipid containing quaternary nitrogen, hydroxyl, and carbonyl functions.*

Ciguatera is a disease characterized by gastrointestinal and neurological symptoms and caused by ingestion of any one of a variety of tropical marine fishes. Although ciguatera was first reported as occurring in the Caribbean, most recent investigations on this disease have been made from occurrences in the Pacific (*1*). Unlike tetrodotoxin, which is probably produced by a single family of fishes (puffers), or unlike saxitoxin, which appears to originate in the phytoplankton and is found in plankton feeders such as clams and mussels, ciguatoxin, the agent which causes ciguatera, is found in a wide variety of fishes throughout the Pacific and Caribbean. Randall (*2*) and others have postulated that the toxin originates in a benthic alga and is transmitted through the food chain. Furthermore, the toxin-bearing fish may disappear from a given area and appear in an area previously considered free of poisonous fish. During the course of our work, toxicity of the red snapper, *Lutjanus bohar* (Forskål), has markedly decreased at Palmyra in the Line Islands and increased in the Marquesas Islands (*3*). In the Gilbert Islands the same species of fish is highly toxic on one reef and nontoxic on another reef a few hundred yards distant (*4*). Conditions in areas, such as the Line Islands, with no history of toxicity for over 100 years prior to the late 1930's, may suddenly change so that