house sparrow. In that species, pineal removal abolishes circadian rhythmicity (*10*) but denervation of the pineal does not (*11*); rhythmicity is restored to pinealectomized birds by the implantation of a donor's pineal into the anterior chamber of the eye (*12*) and the reconstituted rhythm has the phase of the donor bird (*13*); melatonin, administered at constant low levels has dramatic effects on circadian rhythmicity of intact birds (*16*). If, as is implied by these facts, the avian pineal is an oscillator with an hormonal output, then under appropriate conditions it should be possible to measure a continuing oscillation in vitro. The above demonstration of such an in vitro oscillation as well as that of others (*18, 19*) is, in conjunction with the earlier work, the strongest possible evidence that the avian pineal is a circadian clock and opens the way for an investigation of its molecular mechanism. Still to be unraveled are the functional relations between the pineal and other components of the circadian systems of birds. These relationships are known to be complex (*12, 20*) and may vary from one avian species to another (*21*).

Note added in proof: After submission of this manuscript a paper appeared by Binkley *et al.* (*22*) in which "a daily change during day 1 of organ culture in constant dark" was reported to occur in the NAT activity of chicken pineals in vitro. The authors further reported "equivocal persistance" of the rhythm for several days.

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16. The chicks were decapitated, and the heads were dipped into a warm (37°C) solution of disinfectant and then quickly rinsed in warm water. The skullcap was removed under a sterile hood. The pineal was removed, placed into a petri dish containing about 1 ml of Hanks balanced salt solution (*p*H 7.2), and at this time the adhering tissue was removed by dissection. Several small cuts were made in the gland, in order to increase the surface area and promote diffusion of gas and nutrients.
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4 August 1978

Microevolution and Clone Structure in *Spartina patens*

Abstract. *Analysis of the clone structures within a population of* Spartina patens *reveals considerable adaptive genetic divergence among adjacent dune, swale, and marsh sites. The dune subpopulation includes a small number of frequently encountered, spatially isolated clones that follow microtopography and have high reproductive output and colonizing potential. The marsh subpopulation consists of a large number of infrequent interdigitating clones with high vegetative biomass and competitive success. The swale subpopulation is generally intermediate for these traits.*

A general pattern has emerged that plant populations are geographically small in extent, with restricted interpopulation migration, and subject to intense selection pressure that varies in direction among populations (*1*). It is therefore no longer surprising to find microevolutionary divergence over distances of meters or even centimeters (*1*). In extending these generalizations to vegetatively reproducing perennial plant populations, a number of ambiguities confound the interpretation of evolutionary patterns. (i) Individual genotypes or clones may reach great age approaching a degree of immortality; (ii) replacement by seed occurs rarely, and selection will operate most strongly among perennating individuals; (iii) migration by vegetative spread may occur over long distances compared to breeding-population areas; and (iv) populations may comprise a small number of clones each with a large number of independent reproductive individuals.

In order to understand the genetic

Table 1. Means analysis on traits of *Spartina patens* plants grown in common environments.

Trait	Subpopulations		
	Marsh	Swale	Dune
Weight (g) per tiller (dry weight)	0.76 ± 0.06*	0.68 ± 0.04	0.58 ± 0.05
Leaf area (cm^2)	30.1 ± 1.9	36.8 ± 2.1	30.8 ± 1.5
Tillers per clone	30.5 ± 0.06	26.7 ± 2.1	35.3 ± 2.6
Seeds per clone	187 ± 33	288 ± 48	401 ± 96
Rhizome (g) per tiller (g) (dry weight)	0.75 ± 0.10	0.97 ± 0.07	1.05 ± 0.11
Index salt tolerance†	0.33 ± 0.05	0.40 ± 0.06	0.57 ± 0.05
Drought tolerance‡	0.41	0.50	0.83

*Mean and standard errors for traits based on a subset of individuals taken from the total sample. Further details of the statistical analyses and methods have been described (*5*). †Ratio of the increment in the length of the longest root over 48 hours in control solution to that in a solution of 1.75 percent synthetic sea salt. ‡Proportion of genotypes surviving 2 months in sand culture and watered every fourth day.

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structure and the evolutionary patterns of vegetatively reproducing perennial plants, it is important to identify individual clones in a population and the nature of the limits to their distribution. The size and pattern of spread of a given genotype may reflect chance and historical effects, or adaptation to specific microhabitats. Most previous attempts to elucidate clone structures in plants have been based on the identification of morphological variants (*2*). Less frequently, isozyme analysis has been used to identify clones by comparing zymogram patterns or by allelic analysis (*3*). In either case few attempts have been made to relate clone structure and distribution to microenvironmental factors (*4*).

I have used isozyme analysis to examine the clone structure of *Spartina patens* (Aiton) Mulh., a facultatively outbreeding, rhizomatous perennial grass, essentially confined to the coastal zone of eastern North America. In North Carolina this species displays a wide ecological amplitude, forming extensive colonies on sand dunes, swales (moist depressions between dunes), salt marshes, and adjacent habitats. A population of *S. patens* was examined along a 200-m transect across Core Banks, a barrier island on the coast of North Carolina. Individual tillers were collected at intervals of 1.5 m on the transect (105 tillers). In addition, three quadrats were established along the transect, one each in dune, swale, and salt marsh habitats. Ninety-nine tillers were collected from each quadrat at intervals of 1 m on a grid pattern established in each. These plants (402 in total) were cloned and grown in a greenhouse for 24 months prior to analysis. Crude protein extracts were prepared from whole tillers of cloned plants, and with the use of standard electrophoretic procedures (*5*) zymograms were obtained from six enzyme systems: acid phosphatase, esterase, glutamate dehydrogenase, leucine amino peptidase, malate dehydrogenase, and peroxidase.

Individual genotypes were equated with unique zymograms. Of 29 bands that could be scored, 14 were polymorphic. For three loci, allozymic variation could be directly inferred, while interpretation of the remaining more complex loci awaits formal genetic analysis. Band frequencies, levels of polymorphism, and average band differences between genotypes were sufficient to make detection of all unique genotypes highly probable (*5*). Among 346 plants surviving transplantation, 101 genotypes were identified: 15 genotypes among 90 dune plants, 31 genotypes among 91 swale plants, 46 genotypes among 75 marsh plants, and 47 genotypes among 90 transect plants. Of these genotypes, 32 occurred at more than one sampling site. Delimitation of these genotypes to specific sites was striking. Only three genotypes were common between dune and swale subpopulations, two between dune and marsh, six between swale and marsh, and 27 between transect and other sites. Clearly there is considerable genetic heterogeneity within and among these closely adjacent subpopulations of *S. patens*. By contrast, relatively little population heterogeneity or microevolution has been observed in vegetatively reproducing perennial plant populations from similar habitats (*3*).

Table 2. Variation in clone size within a population of *Spartina patens*.

Genotype frequency class	Number of unique genotypes			Mean maximum distance of clone spread (m)		
	Dune	Swale	Marsh	Dune	Swale	Marsh
1	6	14	32			
2 to 3	2	10	8	1.2	5.3	4.0
4 to 7	4	5	6	6.0	5.5	9.3
8 to 15	0	2	0		6.9	
16+	3	0	0	10.4		

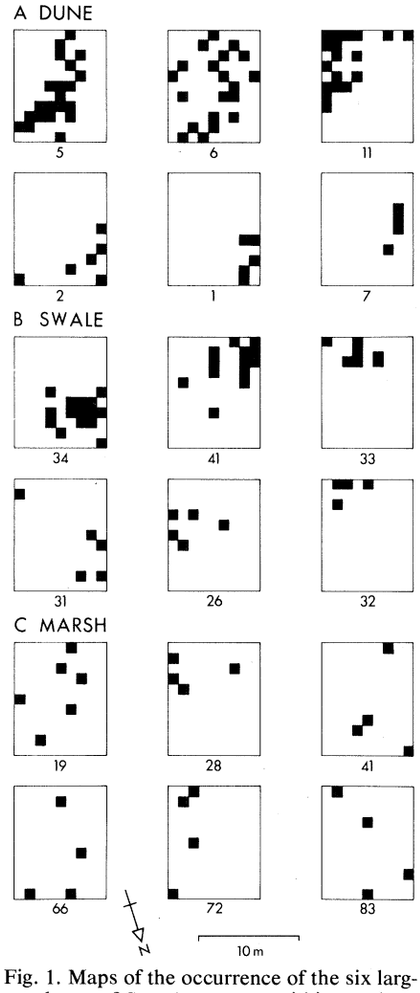

Fig. 1. Maps of the occurrence of the six largest clones of *Spartina patens* within quadrats established one each, respectively, in closely adjacent (A) dune, (B) swale, and (C) salt marsh subpopulations. Each square (1 by 1 m) represents an individual tiller collected at one point within the sampling grid. Numbers given below each map are arbitrary labels used to identify unique genotypes.

Subsets of these genotypes were grown in a common garden and in reciprocal transplant gardens. Biometric analysis of genetic components of morphometric and physiological characters provided additional evidence for considerable microevolution among subpopulations. The dune genotypes had significantly lower vegetative biomass; but they exhibited a higher reproductive output (tillers, seeds, and rhizomes) and a greater tolerance to salinity and drought than the swale or marsh genotypes (Table 1). Results of the reciprocal transplantation study showed that the genetic differences among subpopulations were adaptive, influencing survival and reproduction in each habitat (*5*).

In each subpopulation, resident clones were more fit than alien transplants, as assessed by differential survival and fecundity. Restriction of clones of *S. patens* to specific habitats thus reflects adaptation to specific sites. Selection in the dune has favored opportunistic genotypes with high seed, tiller and rhizome output, and colonizing ability in an environment that is comparatively unstable, being subjected to frequent oceanic overwash and rapid changes in sand accretion. In the more stable swale and marsh habitats, selection has favored genotypes with a higher vegetative biomass and lower reproductive output, promoting survival under conditions of high inter- and intraspecific competition, respectively. In all sites seedling establishment is rare, except after disturbances, and selection operates among clones through differential survival and fecundity.

Mapping of genotypes within sampling quadrats permits the investigation of microdistribution of clones (Fig. 1). The patchy distribution of clones within sub-

populations is most striking in the dunes and least evident in the marsh. Considering eight nearest neighbors per individual, the mean proportions of intraclonal contacts are, respectively, for dune, swale, and marsh: 0.29 ± 0.03, 0.18 ± 0.02, and 0.03 ± 0.01. These are significant departures from random expectations, which are, respectively, 0.18 ± 0.02, 0.06 ± 0.01 and < 0.01. The distribution of clones within the dune quadrat reflects local microtopography (*5*). A dune in the southeast third of the quadrat and another in the northwest third are dominated, respectively, by genotype 11, and genotypes 1 and 2. A shallow depression between dunes running northeast to southwest is dominated by genotypes 5 and 6. The swale and marsh quadrats were located in flat, more homogeneous sites. Patchy distributions of clones here could not be related to any microtopographic features or to any range of microenvironmental variables (*5*). Therefore, although environmental heterogeneity cannot be ruled out, chance or historical effects (or both) may play an important part.

The maps of genotype distribution (Fig. 1) also show significant differences in clone size among subpopulations. Large clones, in terms of proportional representation per unit area, predominate in the dune while many rare clones prevail in the marsh (Table 2). This is reflected in a greater genotypic diversity in the marsh than in the dune. Shannon indices of genotypic diversity calculated for dune, swale, and marsh were 2.11, 3.01, and 3.63, respectively. Alternatively, clone size may be viewed as the extent of vegetative spread, which was estimated as the maximum distance between two members of a clone (Table 2). Relatively little difference is seen among subpopulations in the spread of clones, although rare clones may spread further in the swale and in the marsh. In all subpopulations the potential spread of clones is more than 10 m. Some individuals in all populations will produce over 10 m of rhizomes within one season in a common garden, and individual tillers can be followed for more than 10 m in the field in both dune and swale habitats (*5*). The potential exists for individual clones to spread among subpopulations over several years. That this occurs rarely provides evidence that selection among perennating clones promotes local adaptation and restriction to narrow niches.

The foregoing analysis emphasizes the importance of small-scale sampling of clonal populations to resolve differences in the occurrence and pattern of the distribution of genotypes. It also points up the problems in determining genetic structure and estimating gene frequency in vegetatively spreading populations. Estimates of gene frequency and heterozygosity differ, depending on whether one considers individual reproductive units that make up a clone or genetically individual clones (*5*). Similarly, estimates of breeding population size necessitate the identification of genetic individuals within a population.

Thus, the dune subpopulation comprises a small number of common clones. The low genotypic diversity reflects a harsh environment with intense, largely density-dependent selection pressure sieving out a small number of genotypes capable of surviving. These genotypes allocate a greater proportion of available resources to reproduction, incorporating many opportunistic and colonizing characteristics. By contrast, the marsh and swale subpopulations consist of a larger number of interdigitating rare, but spreading, clones. Higher density-dependent regulation has selected genotypes with a greater proportion of biomass devoted to nonreproductive activity. These observations agree with theoretical expectations and observations made on a number of plant species (*6*). In all sites subpopulations are distinct. That interpopulation migration is restricted is supported by independent estimates of gene flow (*5*). As a result, interbreeding populations are small. Selection pressures in different subpopulations are intense. The striking degree of evolutionary divergence among subpopulations is enhanced by a flexible breeding system and a high level of genetic variation among and within populations.

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3 March 1978; revised 30 October 1978

Osmotic Shock Prevents Nuclear Exchange and Produces Whole-Genome Homozygotes in Conjugating *Tetrahymena*

Abstract. *Exposure of conjugating* Tetrahymena *to a hyperosmotic shock blocks the exchange of gametic nuclei and produces self-fertilized exconjugants that are homozygous for their whole genome. Cells are sensitive to this induction during a brief period after meiosis. The high efficiency of the treatment and the fertility of the progeny make this a useful method for the isolation of induced recessive mutations and enhances the value of* Tetrahymena *as an animal-cell model system in which genetic dissection is practical. The sharp peak of sensitivity is useful in the study of those cellular mechanisms responsible for the independent handling of several functionally distinct nuclei during conjugation.*

The single-celled ciliate *Tetrahymena thermophila* [formerly *T. pyriformis*, syngen 1 (*1*)] is a useful model system for the study of eukaryotic cell and molecular biology, since it can be cultured with ease and speed, crossed at will, and genetically manipulated (*2*). We and others (*3*) have been concerned with developing efficient methods to induce and isolate recessive mutants in order to increase its utility.

Tetrahymena (as a typical ciliate) maintains two functionally distinct nuclei within a common cytoplasm: the somatic (macro) nucleus and the germinal (micro) nucleus. Since the macronucleus is derived from the micronucleus during conjugation, procedures to isolate induced mutations must include a step in which the cells undergo some form of mating in between the step in which they receive mutagen treatment and the step in which phenotypes are selected. The isolation of cells expressing recessive mutations is further complicated by the diploid germinal nucleus; normal crosses of mutagenized cells yield heterozygotes. The problem does not arise in *Paramecium* because it spontaneously undergoes a type of self-fertilization called autogamy (*4*), a process not yet observed in *Tetrahymena*. In autogamy,

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