number of fungi producing detectable ethylene. Ethylene was determined in 2-ml samples of the atmosphere by gas chromatography (*8*) on an activated alumina column (0.65 m, 100 to 200 mesh) operated at 45°C with helium (50 ml/min) as carrier gas. Usually ethylene was identified by comparison of retention time with that of an authentic sample (*8*). Ethylene from selected fungi was verified by prior treatment with aqueous base and mercuric perchlorate and then by release with HCl (*9*).

The concentration of ethylene in 25 empty syringes sealed with short lengths of rubber tubing and incubated for 24 hours ranged from 0.05 to 0.16 parts per million (ppm). This extraneous source of ethylene presumably arose from the rubber tubing. Therefore, the fungi in sample syringes containing 0.16 ppm or less of ethylene were classified as those not producing ethylene. The air in the laboratory was monitored periodically for ethylene content. Ethylene, if present, was below detectable quantities.

Of 228 species of fungi examined (Table 1), approximately 25.6 percent produced ethylene, as judged by the production of gaseous compounds with retention times identical to that of authentic ethylene. The concentration of ethylene varied from 0.18 to over 500 ppm, and the fungi were classified into four groups on the basis of the concentration of ethylene produced: 3.9 percent, >1.0 ppm; 2.7 percent, 0.5 to 1.0 ppm; 19.0 percent, 0.17 to 0.5 ppm; and 74.4 percent, none. Ethylene was verified from 22 of these fungi; the concentration of ethylene (ppm) after incubation for 24 hours was: *Alternaria solani* (0.32), *Ascochyta imperfecti* (9.93), *Aspergillus candidus* (0.28), *Aspergillus clavatus* (514.0), *Aspergillus flavus* (12.8), *Aspergillus ustus* (0.86), *Aspergillus variecolor* (0.25), *Botrytis spectabilis* (0.25), *Cephalosporium gramineum* (12.40), *Chaetomium chlamaloides* (0.41), *Dematium pullulans* (0.61), *Hansenula subpelticulosa* (3.21), *Myrothecium roridum* (0.90), *Neurospora crassa* (0.90), *Penicillium corylophilum* (10.7), *P. luteum* (0.18), *P. patulum* (2.41), *Schizophyllum commune* (3.64), *Sclerotinia laxa* (0.48), *Scopulariopsis brevicaulis* (0.32), *Thamnidium elegans* (15.5), and *Thielavia alata* (0.25). The amount of ethylene produced by *Aspergillus clavatus* is noteworthy.

A survey of 20 unidentified streptomycetes routinely isolated from soil also showed the presence of ethylene in the atmosphere surrounding some of the cultures. Ethylene was verified in only one sample. Although we have not attempted to verify the production of ethylene by all of the fungi tested, we believe that ethylene is a common metabolic product of fungi and should be considered in studies of growth disturbances in healthy and diseased plants.

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Natural Hybridization between Two Sibling Species of Anolis Lizards: Chromosome Cytology

Abstract. *The Lesser Antillean lizards* Anolis trinitatis *and* Anolis aeneus *were both apparently introduced to Trinidad where they are hybridizing. The parental species have 36 and 34 chromosomes, respectively. Hybrids have 35 chromosomes. Meiosis in hybrids is abnormal. There is an accumulation of cells at metaphase I, and poor homolog pairing, characterized by a low frequency of chiasmata and numerous univalents.*

The lizard species *Anolis trinitatis* and *A. aeneus* were endemic to adjacent island banks in the Lesser Antilles. Both have apparently been accidentally introduced by man to the continental island of Trinidad, where there are several areas of contact (*1*). The distribution of the two forms is shown in Fig. 1.

The two species are quite distinct; *A. trinitatis* is bright solid green, and *A. aeneus* is gray and mottled. However, they differ in few other standard taxonomic characters, and museum systematists, working with darkened preserved specimens, have had difficulty telling them apart (*2*).

Hybridization between the two forms was suspected because individuals were found with intermediate color and pattern. Starch-gel electrophoresis of blood proteins confirmed our suspicions. The electrophoretic mobilities of the hemoglobins of the two parent species differ. Each species shows a single band; that of *A. aeneus* migrates toward the cathode at a rate slower than that of *A. trinitatis*. Suspected hybrids all had a double hemoglobin band corresponding to the two parental bands (*1*).

The two species also differ in karyotype. *Anolis trinitatis* has six pairs of metacentric macrochromosomes and 12 pairs of microchromosomes (diploid number, 2*n*, of 36), whereas *A. aeneus* has a similar macrochromosomal complement but only 11 pairs of microchromosomes (2*n* = 34). Neither species has clearly marked heteromorphic sex chromosomes (*3*).

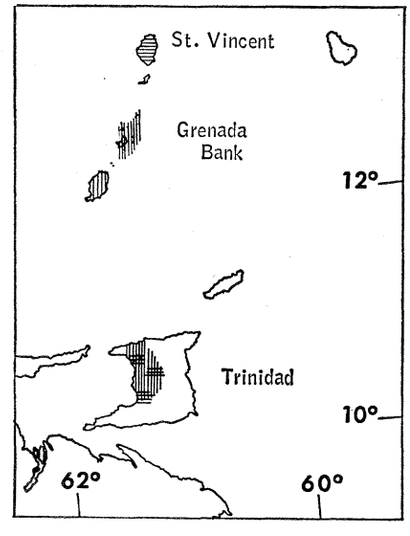

Fig. 1. The distribution of *Anolis trinitatis* (horizontal lines) and *A. aeneus* (vertical lines). On Trinidad, the range of *trinitatis* is disjunct and is encompassed by *A. aeneus*. Hybrids have been found in all areas of contact.

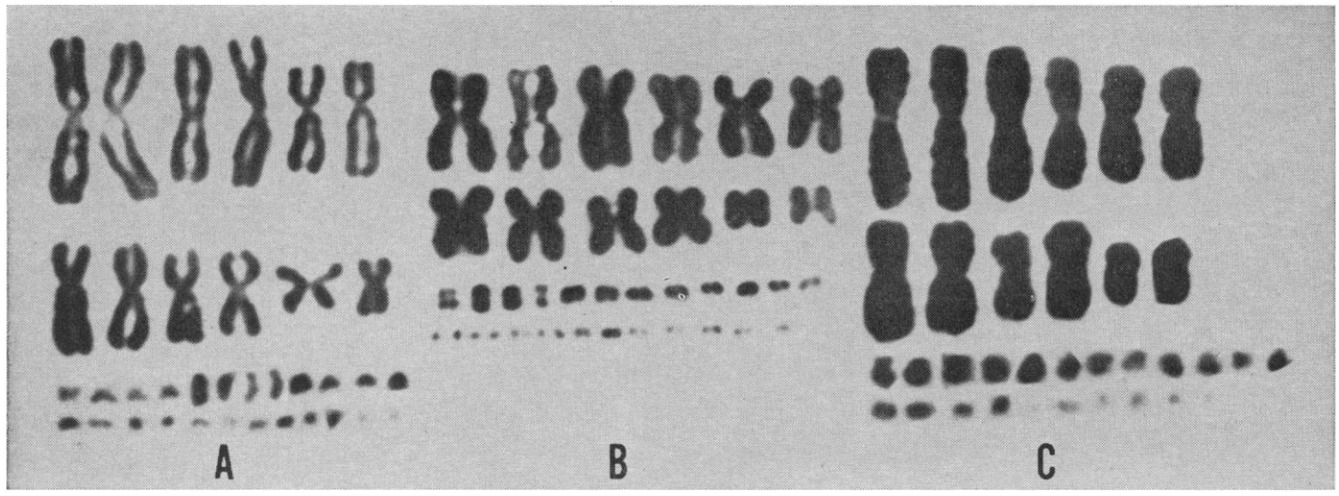

Fig. 2. Mitotic metaphase karyotypes of the two parental species and the hybrid. (A) *Anolis trinitatis* male; diploid number ($2n$) equals 36 with 12 macro- and 24 microchromosomes; leukocyte culture. (B) Hybrid male ($2n = 35$) with 12 macro- and 23 microchromosomes; leukocyte culture. (C) *Anolis aeneus* male ($2n = 34$) with 12 macro- and 22 microchromosomes; direct preparation of testis.

Recently, we have studied the chromosomes of three adult male hybrids, identified as such by their intermediate color and pattern. Meiosis was studied in testes minced in hypotonic solution, fixed in a mixture of methanol and acetic acid (3:1, by volume), and air-dried on slides. Mitoses were studied both in preparations of testes and in cultured leukocytes (*4*).

In mitotic metaphase, the hybrid has 35 chromosomes—12 metacentric macrochromosomes and 23 microchromosomes; this is the expected diploid number, intermediate between that of the two parental species (Fig. 2).

More instructive than mitosis has been an analysis of meiosis. Both parental species have six large bivalents and 11 (in *A. aeneus*) or 12 (in *A. trinitatis*) microbivalents at diakinesis (Fig. 3, A and B). Hybrid males differ significantly from the parental species in both qualitative and quantitative aspects of meiosis. There appears to be an accumulation of cells at metaphase I, implying an arrest at this stage. We examined slides prepared in a comparable manner at a magnification of × 125. Ten fields were chosen at random on each slide, and all cells at diakinesis or metaphase I were counted. In *A. aeneus* we found eight such cells, in *A. trinitatis* seven, and in the hybrid 136.

Furthermore, chromosome pairing appears poor in the hybrid. There is a variable number of apparent univalents, and the frequency of chiasmata is further reduced because the paired elements tend to have only a single terminal chiasma. In each cell there are from 6 to 12 macroelements (Fig. 3, C and D). A tabulation of 200 cells in diakinesis showed the following distribution of macroelements: 8 percent had six elements (fully paired), 17.5 percent had seven, 33 percent had eight, 19.5 percent had nine, 12.5 percent had ten, 5.5 percent had 11, and 4 percent had 12 elements (all univalents). Unlike reports for hybrid salamanders (*5*), no multivalent associations are formed.

Clearly, *A. aeneus* and *A. trinitatis* are good biological species which evolved in isolation and which had not had sufficient time to develop efficient behavioral or ecological isolating mech-

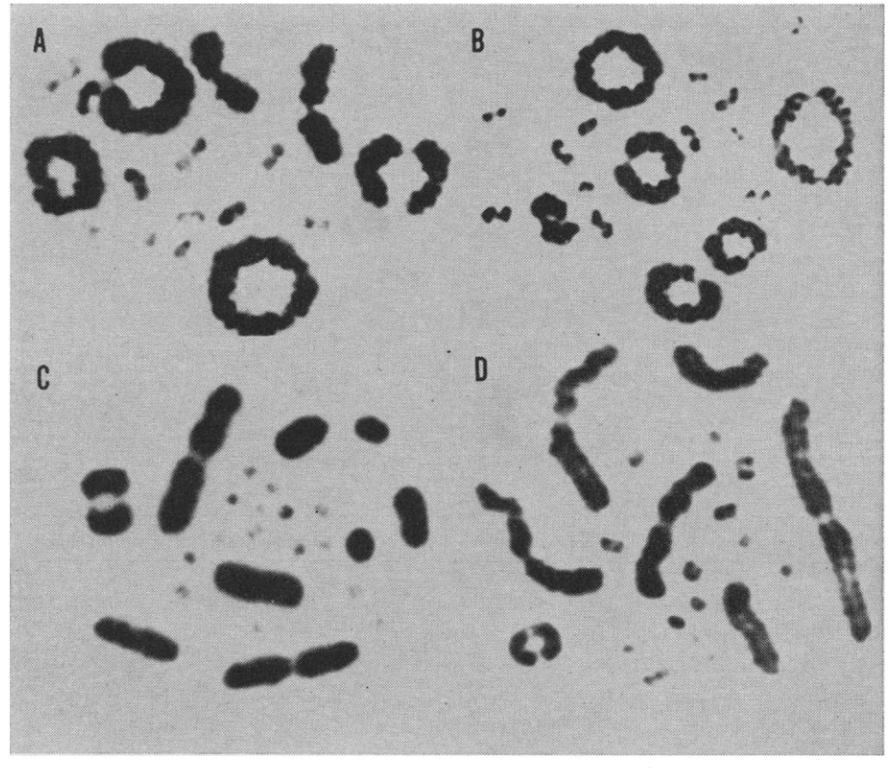

Fig. 3. Meiosis in the parental species and the hybrid. (A) *Anolis aeneus*. There are six large and 11 small bivalents ($n = 17$). (B) *Anolis trinitatis*. There are six large and 12 small bivalents ($n = 18$). (C and D) Two cells from a hybrid. There are three large bivalents and six large univalents in C and five large bivalents and two large univalents in D. Both cells have numerous microelements.

anisms to prevent wastage of gametes when the two species were artificially brought together. The two species do produce hybrids, but these hybrids obviously have reduced reproductive fitness, illustrated by difficulties in meiosis (*6*). We have here another instance of hybridization resulting from the interference of man. This appears to be the most common cause of hybridization in animals (*7*).

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Proton Magnetic Resonance of Transfer RNA

Abstract. *The temperature dependence of the areas under the proton magnetic resonance spectra of unfractionated yeast transfer RNA in 1.0 molar NaCl is a consequence of salt-induced aggregation and does not constitute a monitor of the melting of secondary molecular structure. Such melting can be observed by following the widths of the resonances in the various regions of the spectra. Peaks attributable to dihydrouracil and the methyl groups of the methylated bases are detected in the spectra of unfractionated transfer RNA and alanine transfer RNA.*

Studies of the unfractionated transfer RNA (tRNA) from yeast by proton magnetic resonance (PMR) at 60 Mhz have indicated that melting of the secondary molecular structure can be followed by measuring the areas under the two broad, but separate, PMR peaks due to the protons in the bases and those at the 1′-positions in the ribose moieties (*1*, *2*). The interpretation was based on similar experiments with other polynucleotides which showed that only the PMR peaks from disordered regions were narrow enough to be observed (*3*). We have studied the 100 Mhz PMR spectra of unfractionated yeast tRNA at various salt concentrations as a function of temperature and conclude that the previously observed area changes were due to disassociation of salt-induced aggregates rather than to melting of molecular secondary structure.

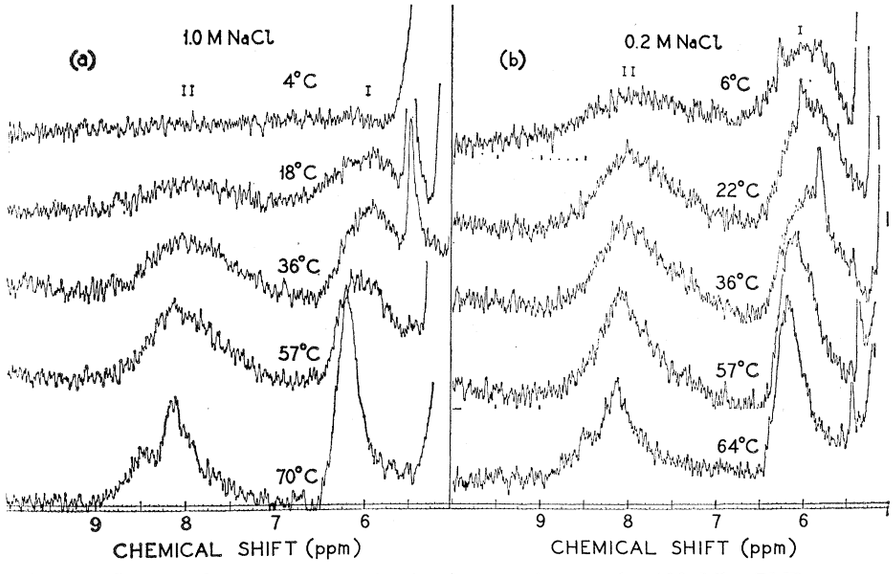

Fig. 1. Influence of temperature and salt concentration on the 100 Mhz PMR spectra of unfractionated tRNA from yeast, in D_2O, *p*D 6.8. The chemical shifts are measured in parts per million (ppm) from the external reference hexamethyldisiloxane present in a concentric capillary tube. The narrow peaks superimposed on region I are spinning sidebands from the intense HDO peak at 5 ppm.

In Fig. 1 we present the PMR spectra due to tRNA at 1.0*M* and 0.2*M* NaCl in D_2O, *p*D 6.8. These spectra were obtained on a Varian HA 100 spectrometer, operating in the internally locked, frequency-swept mode. The low-field peak (labeled peak II) centered on 8 ppm (parts per million from the external reference hexamethyldisiloxane) is due to the protons in the 2- and 8-positions of adenine, the 8-position of guanine, the 6-position of uracil and cytidine, and to most of the ring protons found in the unusual bases characteristic of tRNA. The higher field peak, labeled I, centered at 6 ppm, is due to all protons in the 1′-position of the ribose moieties and to the protons in the 5-position of uracil and cytidine. The drastic decrease in area with decreasing temperature for the 1.0*M* NaCl solution (Fig. 1a) is similar to that found by McDonald *et al.* (*1*), with the exception that the areas of peaks I and II behave identically (Fig. 2). Consideration of the average base composition of unfractionated tRNA from yeast (*4*) indicates that the area under peak I should be approximately 25 percent greater than that under peak II if all protons are mobile enough to be observed. This condition was satisfied for all the samples studied (within experimental error; Fig. 2). Therefore, we have no evidence for the involvement of the ribose protons in bonds stronger than those experienced by the base protons. The sugar protons appear to be liberated at the same temperature as the bases, as one would expect if the principal source of double-strand stabilization were base-base interaction.

However, the PMR behavior shown in Fig. 1b is obtained at all NaCl concentrations below 0.75*M*. No dramatic changes in area occur from 5° to 85°C, and the resonances are easily observable even at 3°C. The 0.5*M* NaCl solution behaves like the 1.0*M* solution, if it is brought up to 0.1*M* in $MgCl_2$.

The PMR spectra of both samples in Fig. 1 show an improved resolution above 55°C. Presumably at these temperatures disaggregation is complete even in the 1.0*M* NaCl samples, and