

the papain Fc-fragment contained the class-specific antigenic sites for γ A (four-chain), γ G, and γ H immunoglobulins (8), we attempted to localize the two-chain γ A class-specific determinant on a papain fragment. However, two-chain γ A proteins from Adj. PC-6C and MOPC 47A have not yielded to papain hydrolysis in our laboratory. Thus, lacking Fc- and Fab-fragments from the two-chain γ A proteins, we were unable to localize the site of their class specificity.

The heavy chains from the two-chain γ A's and those from the four-chain γ A's were compared by reduction, alkylation, separation of light and heavy chains, and tryptic peptide mapping (6). The maps of heavy chains isolated from two four-chain γ A molecules (from Adj. PC-6A and MOPC 209B) and from two two-chain γ A molecules (from Adj. PC-6C and MOPC 47A) are shown in Fig. 2. There are about 20 ninhydrin-positive peptides common to all four chains and all other mouse γ A heavy chains studied in this laboratory. The difference in heavy chains between the four-chain γ A and the two-chain γ A is a set of five peptides indicated in Fig. 2. These five peptides were reproducible features of all the tryptic peptide maps prepared from heavy chains from Adj. PC-6A and MOPC 209B proteins and were not seen in either of the heavy-chain maps of the two-chain γ A's shown or in maps of heavy chains isolated from the two-chain γ A's of MOPC 88 and MOPC 116. The presence of these peptide spots was associated with the antigenic specificities of four-chain γ A's. Further, these specificities have been localized on the papain Fc-fragment of the heavy chain (9). Absence of these peptide spots results in a loss of these specificities and the appearance of a new set of specificities shared by all two-chain γ A's (class specificity of two-chain γ A's). It would appear that the heavy chains of the two-chain γ A's lack a structural unit which may be a prerequisite for larger forms of γ A molecules. However, covalently bound carbohydrate or polypeptide and tertiary configuration have not been conclusively eliminated as the source of these peptide and antigenic differences.

The existence of an intermediate in the assembly of four-chain immunoglobulin molecules has been proposed for γ G and γ H immunoglobulins (10). Probably there is such an intermediate

in the synthesis and assembly of γ A immunoglobulins, and the two-chain molecules described in this paper are the tangible evidence for their existence. Some error in the protein assembly process of these few plasma-cell tumors seems to have prevented the completion of the molecule beyond a two-chain stage—normally a very transient and probably totally intracellular stage.

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Production of Ethylene by Fungi

Abstract. *Ethylene was detected by gas chromatography, and verified by chemical means, as a metabolic product of 22 species of fungi. Because 58 of 228 species of fungi produced a gaseous compound with retention time identical to that of authentic ethylene, we believe that this compound is a common metabolic product of fungi.*

Ethylene, a natural regulator of plant growth, is ubiquitous among higher plants (1) and is also produced by subcellular particles from rat tissues (2). Among fungi, however, the production of ethylene has been convincingly demonstrated by only three species, *Penicillium digitatum* (3), *Blastomyces dermatitidis* (4), and *Agaricus campestris bisporus* (5). The production of ethylene by baker's yeast (6) and *Fusarium oxysporum* f. *lycopersicum* (7) has been questioned (1). We know of no comprehensive survey of ethylene production by fungi; we now present evidence that ethylene is produced by many fungi.

Fungi were cultured in 500-ml erlenmeyer flasks containing 90 ml of medium (Staley's corn steep liquor, 40 g; technical glucose, 40 g; CaCO_3 , 3.5 g; NaNO_3 , 3.0 g; K_2HPO_4 , 0.5 g; MgSO_4 , 0.25 g; and deionized water, 1000 ml). After inoculation, the flasks were placed on a reciprocating shaker (99 to 100 cycle/min with 7.6-cm strokes) at 28°C for 6 to 8 days. The cultures were filtered through Whatman No. 1 filter paper, and the mycelium was used directly. Usually, only submerged pellets were used, but in some cases it was

necessary to use mycelium adhering to the walls of the flask. Large pellets and compacted mycelium were sliced into sections (1 to 2 mm³). Fungi which grew as single cells were centrifuged at 2000g for 5 minutes and decanted to remove culture filtrate. The fungi, approximately 4 ml (0.1 to 0.2 g, dry weight) were incubated in 10-ml glass syringes for 24 hours. Although ethylene was detected from many fungi after 5 hours, the longer period increased the

Table 1. Summary of fungi tested for ethylene production.

Major groups	Genera tested (No.)	Species tested (No.)
<i>Phycomycetes</i>		
Mucorales	15	31
<i>Ascomycetes</i>		
Endomycetales	2	2
Eurotiales	7	116
Sphaeriales	5	9
Hypocreales	2	5
Pezizales	2	3
Helotiales	2	2
<i>Basidiomycetes</i>		
Agaricales	6	6
<i>Fungi imperfecti</i>		
Sphaeropsidales	2	2
Melanconiales	2	2
Moniliales	35	49
Mycelia-Sterilia	1	1

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 varicolor* (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, $2n$, of 36), whereas *A. aeneus* has a similar macrochromosomal complement but only 11 pairs of microchromosomes ($2n = 34$). Neither species has clearly marked heteromorphic sex chromosomes (3).

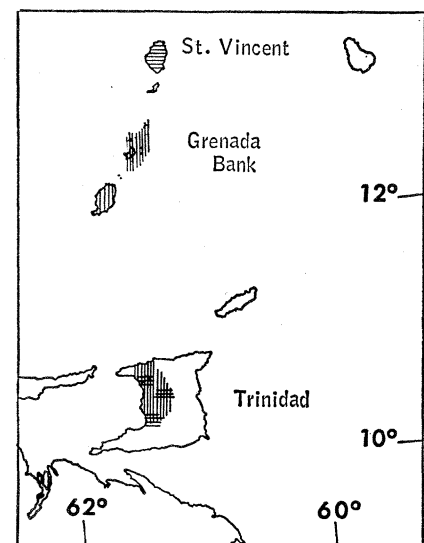


Fig. 1. The distribution of *Anolis trinitatis* (horizontal lines) and *A. aeneus* (vertical lines). On Trinidad, the range of *trinitatis* is disjunct and is encompassed by *A. aeneus*. Hybrids have been found in all areas of contact.