

extent help to retain the dense, highly undersaturated waters over the (bottom) shelf and reduce circulation (13).

Of the 108 samples studied by me and others, 46 with calcareous faunas are from depths less than 550 m; only nine are from more than 550 m and below the solution boundary. Two of McKnight's samples with high calcareous percentages can be disregarded because one (IBM 028, 576 m) contained only one specimen and the other (IBM 011, 740 m), only one calcareous species. Of the remainder, three are from the northeast of Pennell Bank, three are from McMurdo Sound to the west of Ross Island, and one is from 192 km east of Ross Island—all but the last from areas of steep slope. These atypical calcareous faunas possibly result from displacement of bottom sediments and calcareous faunas from shallower waters, a common phenomenon in Antarctic waters (11). Preservation of calcareous specimens not in chemical equilibrium with the surrounding water may also reflect rapid burial because of either high rates of sedimentation or burrowing by benthonic animals.

The distribution of the contrasting faunas has been explained by others, working with relatively few samples. McKnight (1) and, with modifications, Pflum (2) proposed a rather complicated mechanism involving a hypothetical current, with high arenaceous and low calcareous foraminiferal percentages, passing over the stations dominated by arenaceous Foraminifera; this bottom current swept almost all planktonic Foraminifera out of the areas dominated by the arenaceous forms. My work shows, however, that the calcareous faunas occur in shallow areas that are probably more exposed to current action. The distribution of calcareous and noncalcareous faunas in Ross Sea bottom sediments is more readily explained by invocation of a calcium carbonate solution boundary.

JAMES P. KENNETT

*New Zealand Oceanographic Institute,  
Department of Scientific and  
Industrial Research,  
Wellington, New Zealand*

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## Ambrosia Fungi: Extent of Specificity to Ambrosia Beetles

*Abstract. Experiments with developing ambrosia beetles and fungi indicate that beetles may feed on more than one species growing in their tunnels. The yeast-like ambrosia propagules in mycangia of beetles arise from conidia and ascospores picked up by callow adults during their stay in tunnels.*

Despite earlier assumptions (1-3), each species of ambrosia beetle does not necessarily feed on a particular fungus throughout its life cycle; nor is it dependent on only one species of fungus. In nature, larvae feed usually on a primary ambrosia fungus while adults also eat one or more auxiliary ambrosia fungi. The primary fungus is brought into tunnels by females (rarely by males) at the time of burrowing (2, 3); it grows luxuriantly in tunnels and spreads from there to any cradles present. In some species the larvae are reared individually in cradles which they enlarge; other species do not construct such cradles. The mycelium ramifies into xylem, phloem, and ray elements, but usually sporulates only in the tunnels and larval cradles. By the time the first adult beetles emerge, auxiliary fungi may be growing throughout the entire tunnel system; one may sometimes grow beside the primary ambrosia fungus during the early stages of excavation of tunnels.

Most primary and auxiliary ambrosia fungi produce abundant conidia on short conidiophores of determinate length, and thus are well adapted to the limited space available in main tunnels and larval cells. Periodic removal of conidia by beetles ordinarily does not damage the growing point but rather appears to promote further sporulation.

Although larvae of several ambrosia beetles (4) were observed in the field feeding on the primary ambrosia fungus in their cells, young adults of the same brood may also feed on auxiliary fungi in the main tunnels. Such observations were confirmed in the laboratory in some instances by allowing

callow adults access to auxiliary fungi. When eggs and young larvae were incubated on wood chips colonized by only auxiliary fungi (in some instances by fungi other than ambrosia) the larvae ate the fungi and matured (Table 1).

Adults usually contain in the gut partially digested conidia and fragments of mycelium of auxiliary and primary ambrosia fungi. Most of the auxiliary fungi so far isolated belong to either the yeast genera (*Hansenula*, *Saccharomyces*, *Oidium*, and *Torula*) or the filamentous hemiascomycete genera (*Ascoidea*, *Cephaloscypha*, *Dipodascus*, *Endomycolopsis*, and *Endomyces*). In some instances *Graphium* and *Leptographium* also serve as auxiliary fungi. The primary ambrosia fungus of one beetle may sometimes be an auxiliary fungus for other species of ambrosia beetles.

A large population of *Trypodendron scabricollis* just beginning a brood, with its primary ambrosia fungus already established in tunnels, was obtained from the field during early summer of 1962 (2). Thirty females (two groups of 15) were aseptically removed from naturally infested logs and identified with differently colored paints. One group was transferred into tunnels bored with an electric drill in wood that had been inoculated, after sterilization, with conidia of *Endomycolopsis fasciculata*, a common auxiliary ambrosia fungus of many beetles. The second group was transferred to similar tunnels inoculated with conidia of *Ceratocystis minor*, a nonambrosia fungus commonly associated with bark beetles. A naturally infested log having 15 active burrows of approximately the same age and presumably lined with a palisade of primary and auxiliary fungi

Table 1. Beetles, adults or pupae, were reared from eggs or young larvae on a diet of various fungi growing as pure cultures in artificial tunnels or on wood chips. Symbols: +, successful development; 0, no pupae or adults developed; NT, not tested.

Fungus diet	Ambrosia beetles					
	<i>M. fasciatum</i>	<i>T. scabricollis</i>	<i>X. saxeseni</i>	<i>P. compositus</i>	<i>X. politus</i>	<i>G. materiarius</i>
<i>Cephalosporium acremonium</i> Cda.*	0	0	+	0	0	0
<i>Ceratocystis bicolor</i> David. & Wells*	0	+	0	0	0	+
<i>C. minor</i> (Hedg.) Hunt*	0	+	0	0	0	+
<i>Monilia brunnea</i> Verall†	+	NT	+	+	+	NT
<i>Endomycolopsis fasciculata</i> Batra†	+	+	+	+	+	+
<i>Tuberculariella ambrosiae</i> Funk†	+	+	+	0	+	+
<i>T. sulphureus</i> Batra†	+	0	+	0	+	0
(?) <i>Scopulariopsis brevicaulis</i> (Sacc.) Bain†	NT	NT	NT	0	+	+
<i>Dipodascus aggregatus</i> Fr.-Gros.‡	0	+	0	0	0	+
<i>D. uninucleatus</i> Biggs‡	NT	0	0	0	0	0
<i>Cephalosporium fragrans</i> Hanawa‡	NT	+	NT	0	0	+

\* Occasionally auxiliary fungi of *Xyleborus saxeseni*, *Trypodendron lineatus*, and *Trypodendron* sp., respectively. † Primary ambrosia of *Monarthrum fasciatum* and *M. mali*, *Gnathotrichus materiarius*, *Trypodendron scabricollis*, *Xyleborus saxeseni*, and *Xyleterinus politus*, respectively. ‡ Nonambrosia contaminants commonly associated with bark beetles but occasionally encountered inside tunnels of ambrosia beetles (particularly of *T. scabricollis* and *G. materiarius*) as auxiliary fungi.

was incubated under similar conditions as a control.

The beetles transferred to the artificial tunnels inoculated with auxiliary fungus or nonambrosia fungus resumed normal activity. After 4 to 6 weeks, adults of the next brood were obtained from all three sets of tunnels, which pack showed that larvae could live exclusively on *E. fasciculata* or on *C. minor*. The same experiment yielded similar results in 1963 (5).

Relatively fewer adults came from tunnels inoculated with auxiliary or nonambrosia fungi than from tunnels naturally colonized with primary ambrosia fungus; however, all adults appeared normal. The mycangia (specialized pouches containing fungal inoculum) of the adults reared in the artificial tunnels contained inoculum of the auxiliary or nonambrosial fungi on which they had fed as larvae.

Experiments similar to those described for *T. scabricollis* were also conducted with the ambrosia beetle *Xyleborus saxeseni*. Eggs, young larvae, and the accompanying females were aseptically removed from their parent tunnel systems and placed on wood inoculated with *Cephalosporium acremonium*, a nonambrosia fungus. Wood naturally colonized with the primary ambrosia fungus (perhaps a species of *Tuberculariella*) and containing eggs, larvae, and adults was maintained as a control. In both cases adults regularly developed from the eggs and larvae.

Although adults often feed on auxiliary or even nonambrosia fungi, their

mycangia contain predominantly the primary ambrosia fungus. Secondary microflora, in addition to the primary fungus, were often isolated from the mycangia of hibernating beetles as well as from beetles of summer broods of many species.

Most ambrosia fungi superficially resemble each other in having monilioid chains of cells called "ambrosia propagules" or "ambrosia cells"; perhaps it was this similarity that led Webb (6) to believe that all represented modified cells of *Leptographium*, particularly *L. lundbergii*. Although it is true that a few species of a fungus genus may be associated with closely related beetles, the superficial resemblance of ambrosia cells characteristically present in mycangia and in tunnels must not be taken to indicate relationship among all these fungi. Morphologically similar ambrosia propagules from mycangia and tunnels of various beetles, when plated, yield a varied mycoflora consisting of *Ambrosiomyces*, *Ambrosiella*, *Ascoidea*, *Ceratocystis*, *Dipodascus*, *Diplodia*, *Endomycolopsis*, *Monacrosporium*, and *Tuberculariella* (1-7); most of these are dimorphic, and their filamentous phase can be suppressed by manipulation of nutritional or environmental conditions in the laboratory (8).

The mycangia of most of the 48 species of ambrosia beetles examined by me contained ambrosia propagules, although some such fungi do not form such structures either in culture or in tunnels; thus was suspected that the beetles in some way convert conidia and ascospores into ambrosia propa-

gules. Glandular secretions containing proteinaceous and lipid materials often accumulate in mycangia, as do occasionally a few cells of host-tree and dirt particles. Mite eggs, nymphs, and what appeared to be their fecal pellets were observed in precoxal mycangia of *Monarthrum fasciatum* and in prothoracic pleural mycangia of *Trypodendron scabricollis*. The nutrients in mycangia probably are used by the fungi as a source of nutrition, so that many propagules may be tightly packed in limited space. The following experiment provides evidence for this assumption.

Female adults of *Trypodendron scabricollis* and *Monarthrum fasciatum*, excised aseptically from their pupal skins, were transferred to cradles and tunnels containing only the conidia-bearing, nonambrosia fungi *Aspergillus* sp., *Trichoderma* sp., and *Ceratocystis ulmi*. Similar pupae with their pupal skins still intact and callow adults, presumably smeared with primary ambrosia fungus, were also transferred to these nonambrosia fungi as controls. As in nature, the callow adults moved back and forth in their cradles for 2 to 5 days. It was at this time that the inoculum of each of the fungi growing around them was forced into the mycangia. Mycangia of such adults were dissected at regular intervals and the inoculum was examined microscopically and plated. The conidia of each fungus progressively lost their identity in the mycangia, being transformed into ambrosia propagules. Except for minor differences in the width of cells, propagules of the three unrelated fungi appeared similar. Contents of mycangia, when plated on routine laboratory media, yielded conidia-forming cultures of the respective fungi.

Airborne contaminants, together with ambrosia fungi, are regularly released in tunnels during excavation in nature. How the foreign fungi are suppressed in the presence of adult beetles is not known, but in their absence contaminants spread throughout the tunnels within 24 hours.

Mycangia seem to occur universally among the wood-boring, fungus-feeding Scolytidae and Platypodidae (3, 9). Some genera of the family Scolytidae (*Ips*, *Dendroctonus*, *Scolytus*) that ordinarily feed on bark also carry fungus inoculum in pockets similar to mycangia; they occasionally consume fungi growing in their emergence tunnels in addition to bark. Some of the bark

beetles are consistently associated with specific fungi and feed on them regularly but not exclusively. Thus, young adults of *Ips avulsus*, *I. calligraphus*, *I. grandicollis*, and *I. pini* were observed feeding on conidia and perithecia of *Ceratocystis ips* in North America; their intestinal tracts contained perithecial fragments and viable ascospores and conidia. *Ips grandicollis*, *I. pini*, and *I. oregonensis* were also observed regularly feeding in nature on sporodochia of *Tuberculariella ips*, which is invariably found in pupal cells and is morphologically very closely related to—perhaps congeneric with—*T. ambrosiae*, the primary ambrosia fungus of *Platypus wilsoni* (7) and *Trypodendron* spp. *Ips pini* and *I. oregonensis* are often smeared outside with the ascospores of *C. ips*, which bud in the intersegmental folds of the beetles, the resulting yeast-like growth resembling the ambrosia propagules in mycangia of ambrosia beetles.

*Trichosporium tingens* and *T. tingens* var. *macrosporum*, symbiotically associated respectively with the bark beetles *Tomicus minor* and *Ips acuminatus* in Sweden, are quite similar to some ambrosia fungi, particularly *Monilia ferruginea*, the primary ambrosia fungus of *Trypodendron lineatum* and other *Trypodendron* spp. in Canada, the United States, and Sweden. *Trichosporium* spp., as well as bark, serve as foods for these beetles, and hibernating *Tomicus minor* carries inoculum of *T. tingens*, *Ceratocystis cana*, and some yeasts "in a primitive manner in its median suture and in the lateral folds of the elytra" (3). Inoculum of *T. tingens* var. *macrosporum*, on the other hand, is contained in specialized pockets in the oral cavity of *Ips acuminatus*; such structures correspond to oral mycangia of some *Xyleborus* spp., which are true ambrosia beetles (3).

I have isolated 51 fungi from inoculum surviving as yeast cells or as ambrosia propagules on various parts of many Platypodidae and Scolytidae from India (unpublished). Such propagules are usually indistinguishable from each other, yet when plated they yield diverse fungus flora and many do not sporulate.

I therefore postulate that fungi that may have been fortuitously carried at one time into the tunnels of bark- and wood-inhabiting beetles today live symbiotically with ambrosia beetles in a truly mutualistic relation. Initially the fungi established themselves beneath

the bark, ramifying into the frass-packed tunnels. The fungi were occasionally consumed along with bark, as with *Ips*, *Tomicus*, and *Dendroctonus* spp. During the course of evolution some fungi perhaps invaded wood and made it possible for the beetles to penetrate weakened xylem while feeding on fungi. Eventually some beetles abandoned the eating of bark and wood, became wholly adapted to mycetophagy, and developed mutualism with ambrosia fungi.

LEKH R. BATRA

Department of Botany,  
University of Kansas, Lawrence

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5. Only 6 of 15 beetles in tunnels with *Endomycopsis fasciculata* and 4 of 15 with *Ceratocystis minor* maintained brood for a period long enough to yield adults in 1962; these were individuals that had not yet laid eggs in tunnels from which they were initially removed. Most of the mothers that did not initiate or maintain a brood to maturity had left eggs and larvae in the tunnels from which they were originally transferred; presumably they were already considerably worn out. In 1963, when only young beetles were used, mortality in their new tunnels was much lower than in 1962.
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## Chromosome Aberrations: Increased Incidence in Bone Marrow of Continuously Irradiated Rats

Abstract. *The number of chromosomal aberrations in the bone marrow of continuously irradiated rats temporarily decreases after exposure, and then increases as the total dose and time after irradiation increase. This increase in aberrations is greater than that expected as a result of age alone.*

Chromosomal abnormalities which may be a rough index of mutations (1-3) and, in accordance with the somatic mutation theory, can be related to aging (4), appear to be a suitable criterion for determining late radiation injury. While radiation-induced aberrations slowly decrease in tissues whose cells rarely divide (2-5), most aberrations in tissues with continually dividing cells, such as the bone marrow, are eliminated soon after irradiation (6). Nevertheless, one type of aberration, the polyploid forms, persists in bone marrow or in peripheral leucocytes long after irradiation (7).

To determine the hematologic changes in rats during continuous irradiation, male albino rats (Wistar, 7 to 12 months) were irradiated with various dose rates of  $Co^{60}$   $\gamma$ -rays for 23 to 23.5 hours per day. Abnormal and normal cells in anaphase or telophase were scored at 5 days, 6 months, and 18 months after cessation of the irradiation. Unfortunately, there were not

enough rats to form groups irradiated under the same conditions of dose and time (Table 1, groups II, III, and IV). Later, the incidence of aberrations during continuous irradiation (Table 1, group I) and the frequency of spontaneous aberrations, in relation to age, in nonirradiated rats were studied. Bone marrow was taken from the femur which had been fixed in acetic alcohol, and squash preparations were stained by the Feulgen method. Bridges and acentric fragments (4) in anaphase and telophase mitotic figures were scored and expressed as the percentage of total anaphase and telophase figures found in 10,000 marrow elements.

The percentage of chromosomal aberrations decreases within 5 days after irradiation (group II) and is about one-fourth that found in rats during exposure to an approximately equal total dose of irradiation (group I). Within 6 months after exposure (group III) the percentage of aberrations is more than twice that 5 days after exposure