

THERMOPHILOUS FUNGI OF BIRDS' NESTS

by

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It is known that a number of fungi are associated with wild birds (cf. TIFFANY, GILMAN & MURPHY, 1955; BEER, 1963). PUGH (1965, 1966) found keratinophilic fungi on birds, as well as in birds' nests.

In 1912 NOACK reported occurrence of thermophilous fungi in nests, excreta and feathers of birds. WETMORE (1933) and FRITH (1959) mention self-heating of the nest material of Australian mound building Megapodes which incubate their eggs in mounds of plant debris undergoing microbial decomposition. Recently COONEY & EMERSON (1964) quote two species of thermophilous fungi isolated from the birds' nests: *Chaetomium thermophile* from a blackbird's nest and *Torula thermophila* from the straw of a chicken's nest.

A widespread occurrence of thermophilous microorganisms is reported from countries with a cool climate where they occur in soils (cf. MISHOUSTINE, 1950; APINIS 1963a, 1965b), vegetation (APINIS, 1963b, 1965ab), composts (LA TOUCHE, 1949; v. KLOPOTEK, 1962; FERGUS, 1964) and various agricultural produce (MIEHE, 1907; MISHOUSTINE, 1950) frequently causing damage by self-heating of hay, grain, straw and other stored materials. Birds' nests appear to be favourable sites too for the development of such organisms, because suitable organic substrata are present and the accumulated organic debris is warmed up in the summer as the temperature rises, as well as by birds while using the nests. The body temperature of birds varies from 40° to 43° C, which makes them well suited for warming up their nests during the incubation period of eggs which require an optimum temperature between 35° and 38° C (KENDEIGH, 1940; SPECTOR, 1956). However, exact information concerning temperature conditions of wild birds' nests is scarce or non-existent at present. According to NORDBERG (1936) birds' nests in Finland assume, at the beginning of incubation, a higher temperature of c. 34°—38° C which continues throughout the nestling period. The nest material is also warmed up for longer periods when the nest is used for roosting, as e.g., by the House Sparrow.

Thus the various organic materials built in the nests may become suitable substrata for the development of thermophiles. In 1965 a number of passerine birds' nests became available and it was decided to examine them for presence of thermophilous fungi.

MATERIALS AND METHODS

The 54 nests examined in this work were collected in the summer of 1965 in the Nottingham area. The identification of the nests was carried out by Mr. B. D. BELL who also collected a number of nests used in this study. According to the composition and the site (KEARTON, 1908) of the nests these may be grouped as follows:

a) The nests of Sandmartin, *Riparia riparia* (L.) FORSTER, from the sandstone holes, consisted of grass culms, leaves, and rhizomes, lined by some feathers.

b) The indoor nests of Swallow, *Hirundo rustica* L., consisted of hard mud casing which was strengthened by grass culms, rhizomes and straw, including a few small twigs, lined by grasses and feathers.

c) The nests of House Sparrow, *Passer domesticus* (L.) BRISSON, Starling, *Sturnus vulgaris* L. and Wren, *Troglodytes troglodytes* (L.) VIELLOT, belong to the more or less domed nests built either in trees or shrubs, as well as in holes or crevices. They consisted mainly of grass, leaves and straw lined by the Wren with mosses, and by the House Sparrow with feathers, hair, mosses and wool.

d) The medium cup-shaped nests of Blackbird, *Turdus merula* L., Mistle Thrush, *Turdus viscivorus* L., Song Thrush, *Turdus eritorum* TURTON and the five nests of Thrush (identified as *Turdus* species and probably belong either to Mistle Thrush or Song Thrush) consisted mainly of grass culms, leaves, rhizomes, straw, small twigs and other plant debris with a thick mud layer in the middle. The blackbirds' nests were lined by grasses, mosses, paper, wood chips, wool and other debris.

e) The small cup-shaped nests of Greenfinch, *Chloris chloris* (L.) CUVIER, Linnet, *Carduelis cannabina* (L.) BRISSON and of Hedge Sparrow, *Prunella medularis* (L.) VIELLOT are built usually in shrubs or hedges above the ground and consisted of grass culms, leaves, fine twigs of various plants, mosses and wool, lined by feathers, hair, mosses, wool and other fine materials.

f) A similar, small, cup-shaped nest built by Reed Bunting, *Emberiza schoeniclus* (L.) L., near the ground in a tussock of grass, consisted of grass culms, leaves and other plant material, lined by hair and other fine debris.

These notes on nest material and the state of its decomposition were made in the laboratory before the nest debris was prepared for incubation. No direct observations of the presence of thermophilous fungi on nest debris was attempted because in most nests the plant debris was affected by transportation to the laboratory.

For the incubation of the nest debris two sectors were cut from each nest, and each was placed in a sterile bottle. The various pieces of the debris of the first sector were plated directly with 2 % water agar in two Petri dishes, as well as in two further Petri dishes lined with thick filter paper and moistened by 10 ml sterile water (moist chambers). The nest material from the other sector was washed with sterile water and all the fine mineral and organic particles including spores were removed from the coarse organic debris. The various washed pieces were plated in soil extract agar, with an addition of streptomycin (0.04 mg/ml agar) to inhibit growth of bacteria, and in two moist chambers. Then the material of the washed and unwashed series was incubated at 38° and at 45° C for 8 days when the first examination and isolation of thermophilous fungi was carried out. After further examination of the thermophilous fungi growing upon the incubated nest debris, the relative frequency of occurrence of the respective fungal species was estimated for each nest surveyed as follows (cf. Table I):

- + - single fungal colony;
- 1 - present in one Petri dish on few pieces of debris plated;
- 2 - present in two Petri dishes;
- 3 - present in three Petri dishes on few pieces of debris plated;
- 4 - present in three Petri dishes, but colonizing at least one half of the debris plated;
- 5 - present abundantly in 4 Petri dishes.

RESULTS

Twenty seven species of thermophilous fungi were recorded on various plant debris of the nests surveyed (Table I) which represent 4 mucors, 7 ascomycetes, 1 agaric, and 15 deuteromycetes. Besides these fungi diverse thermophilous actinomycetes and bacteria appeared very frequently on the nest materials incubated at 38° and 45° C. The composition of species of the thermophilous fungus flora of birds' nests is reminiscent of that found in pasture soils and vegetation of the Trent Valley near Nottingham (APINIS, 1965a and 1965b), as well as in soils and vegetation of coastal grasslands (APINIS, 1963b and 1965a). Furthermore, a striking similarity in species populations also exist (cf. Table II) with the thermophilous fungus flora of mushroom composts (LA TOUCHE, 1949; FER-GUS, 1964), town refuse composts (v. KLOPOTEK, 1962) and self-heating hay (MIEHE, 1907). Whether some self-heating occurred in the nests of the birds examined is at present impossible to confirm or to deny, while accurate data on the temperature relations in the nests of birds are lacking. According to KENDEIGH (1940) the optimal temperature range for incubation of wild-birds' eggs was between 35° C and 38° C. The actual average wild birds' egg temperature measured by inserted thermocouples (HUGGINS, 1941) is 34° C. This and the relatively high body temperatures of birds, indicate

that the nests undergo some warming up while they are used by the birds (CADMAN, 1923; BALDWIN & KENDEIGH, 1932). On the other hand, nests are warmed up by air and sun during the warm season of the year and may even become overheated as has been stated by HUGGINS (l.c.). Therefore, it is reasonable to assume that the various organic debris of birds' nests undergoes changes in temperature which are favourable for the development of these thermophiles. This is reflected in the different temperature requirements of the thermophilous fungi recorded from the nests in which the optima and maxima for growth are above those for mesophiles (APINIS, 1963a, b). In thermophilic species, which require the highest temperature for growth, the optima are between 40° C and 50° C and maxima up to 60° C and they are unable to grow at a room temperature of 18° C, such as the common nest fungi *Allescheria terrestris*, *Chaetomium thermophile* and *Thermoidium sulphureum* (cf. Table II). The psychrotolerant thermophiles, with a wide temperature range, growing well at room temperature and at 48° C to 50° C, are also common in the birds' nests (e.g., *Aspergillus fumigatus* and *Sporotrichum thermophile*). Lower temperatures for growth are required by the microthermophilic species with an optimum between 25° C and 35° C and a maximum exceeding 40° C but not 45° C, such as *Coprinus delicatulus*, *Dactylomyces thermophilus*, *Dendrostilbella boydii* (conidial *Allescheria boydii*), *Emericella nidulans* and *Rhizopus arrhizus*, which never appeared on the organic debris of the various nests incubated at 45° C. The five transitional mesophilic species (cf. Table II) found infrequently on the nest debris incubated at 38° C possess the optimal temperature range of the mesophiles, i.e., between 18° C and 30° C, but are able to grow reasonably well at 38° C, e.g., *Aspergillus terreus*, *Endomyces lactis* and *Trichoderma koningi*.

The growth of various heterothropic thermophiles depends also upon the presence of a suitable food base. It is known that thermophilous fungi in their natural distribution are confined to the plant debris of the surface soil, the turf layer and the senescent or decaying vegetation (APINIS, 1963a, b). Similarly, the organic debris of the nests, such as grass culms, leaves, rhizomes and roots, is the main substratum upon which all the thermophilous fungi develop. On such substrata, the thermophilous actinomycetes and bacteria are abundantly present too, but both latter groups of microorganisms colonize other substrata as well, i.e., mud particles and the lining material of the nests (consisting of feathers, hair, mosses and wool, which are plentifully present in nests of Greenfinch, Hedge Sparrow, House Sparrow, Wren and other birds) upon which the thermophilous fungi are very scarce or absent. In general, there is little difference in species of fungi whether or not their substrata, i.e., the various plant debris, are incorporated in a layer of mud in the nests. However, in most nests with a mud layer, such as in the nests of Blackbird, Swallow and the Thrushes, the plant deb-

TABLE I.

Occurrence of thermophilous fungi in the nests of twelve passerine birds
 The figures indicate frequency variation, average frequency (in brackets) and presence in per cent of respective thermophilous recorded

Fungi recorded	a		b		c		d		e		f		
	Sandmartin 10 nests Freq. Pres.	Swallow 4 nests Freq. Pres.	House Sparrow 4 nests Freq. Pres.	Wren 1 nest Freq. Pres.	Starling 1 nest Freq. Pres.	Blackbird 15 nests Freq. Pres.	Mistle Thrush 1 nest Freq. Pres.	Song Thrush 3 nests Freq. Pres.	Thrush 5 nests Freq. Pres.	Greenfinch 1 nest Freq. Pres.	Hedge Sparrow 6 nests Freq. Pres.	Linnet 2 nests Freq. Pres.	Reed Bunting 1 nest Freq. Pres.
Phycomycetes													
<i>Absidia ramosa</i> (LINDT) LENDNER	1(1) 10			+(+)	1(1)	4-2(1) 20 2		+-2(1) 100	1-3(2) 60		1(1) 17	2(2) 50	
<i>Mucor pusillus</i> LINDT			+-1(+)			+-2(1) 33		+-2(1) 100	+-2(1) 60		1(1) 17	1(1) 17	
<i>Rhizopus oryzae</i> FISHER			+(+)								+(+)	1(1) 50	
<i>Rhizopus cobini</i> BERLESE & DE TONI			+(+)										
Ascomycetes													
<i>Allisleria terrestris</i> APINIS	+(+) 10	2(2) 25	3(2) 25 1		2	4-4(2) 77 1		+-1(1) 100	2-3(2) 80	1-3(3) 70			1
<i>Chaetomium thermophilum</i> LA TOUCHE		1-4(3) 100	2-3(3) 100 4		3	3-5(4) 86 3		2-5(4) 100	2-8(3) 100 3	1-5(4) 83		2-3(2) 100	2
<i>Dactylomyces thermophilus</i> SOPP						+-2(1) 13			3(3) 20				
<i>Enterocella nidulans</i> (BUDA) VUILL.			+(+)		+	1-2(1) 18			+(+)	1(1) 17		1(1) 50	+
<i>Endomyces lactis</i> (FRIES) WINDISCH													1
<i>Pseudocrotium</i> species	+(+) 25												
<i>Trichelia sepulchrum</i> EMMONS	1(1) 50		1(1) 25 3			+(+) 13 1			+-2(1) 60	1(1) 17		2(2) 50	2
Basidiomycetes													
<i>Coprinus delicatulus</i> APINIS	2(2) 20	2-3(2) 100	2-4(3) 100 3		2	1-2(1) 86 2		1-2(2) 100	+-2(2) 80 1	+-2(1) 83		3(3) 100	3
Deuteromycetes													
<i>Aspergillus fumigatus</i> FRESENIUS	+-1(1) 20	2-4(3) 100	1-3(3) 100 3		2	1-4(2) 100 3		3-5(4) 100	2-4(3) 100 3	2-4(3) 100		2-3(3) 100	4
<i>Aspergillus terreus</i> THOM								1(1) 33					
<i>Aspergillus</i> species						+(+)	7						
<i>Borytrichium</i> species	+(+) 25	+-1(1) 50	+-1(1) 50			+(+)	27		+(+)	20			
<i>Cephalosporium</i> species	1(1) 50	1-2(2) 50 1	1-2(2) 50		2	+-3(2) 79 1		1(1) 66	1-3(2) 80 1	+-1(1) 34		2(2) 100	
<i>Drepanostilbella boydii</i> SHEAR	1(1) 10	1(1) 25	1-2(2) 50			+-2(1) 33		+-1(1) 66					
<i>Hemicelia isolata</i> COONEY & EMERSON	1(1) 10	3-4(3) 100	3-5(4) 100		5	2-5(3) 93 2		2-4(3) 100	3-4(3) 80 2	2-5(3) 100		2-4(3) 100	
<i>Pactonyces variolus</i> BAINER	+(+) 25	+(+) 25	+-2(1) 50		+	1(1) 7			1(1) 20			1(1) 50	
<i>Penicillium diponti</i> GRIFFON & MAUBLANC s. EMERSON			+(+)		+	+(+)	20		+(+)	66			2
<i>Sporotrichum thermophilum</i> APINIS	2(2) 75	+-2(1) 75 1	+-2(1) 75 1		1	+-3(2) 59		+-1(1) 100	1-3(2) 80	2(2) 50		1-2(2) 100	3
<i>Stibelia thermophila</i> FERGUS			1-3(2) 13		1	+-3(2) 13							
<i>Thermotium sulphureum</i> MEHE	1-6(3) 100	3-4(3) 100 3	3-4(3) 100 3		2	+-4(3) 100 1		2-4(3) 100	+-5(2) 100 +	+-5(3) 100		3(3) 100	
<i>Thermomyces lanuginosus</i> ISIKLINSKI	+(+) 10	2-3(2) 100	2-3(2) 75 1		3	+-5(2) 86 2		1-3(2) 100	2-3(3) 40 2	2-3(3) 83		1(1) 100	+
<i>Tortula thermophila</i> COONEY & EMERSON	+(+) 10	+-1(1) 75	1(1) 25		3	+-2(1) 11		2(2) 33	+-1(1) 40	+-2(1) 100		1(1) 100	
<i>Trichoderma Roumigi</i> OUDEMANS			1(1) 25			+(+)	7						
Nos. of species recorded	8	15	19	12	14	22	11	15	17	15	15	15	10
Variation in nos. of species	0-2	9-11	9-15	12	14	8-13	11	11-14	10-12	9-10	9-15	9-15	10

ris enclosed in it appears in a more advanced state of decomposition than the outside debris.

Despite the similarity of plant debris in the various nests of passerine birds surveyed the presence of particular fungal species varies considerably, In this context three main groups of fungi may be recognized:

1) Fungi present in most of the nests, i.e., with presence as high as 50 % and over, such as the strictly thermophilic *Allescheria terrestris*, *Chaetomium thermophile*, *Humicola insolens*, *Thermoidium sulphureum*, *Thermomyces lanuginosus* and *Torula thermophila*, the psychrotolerant thermophiles *Aspergillus fumigatus* and *Sporotrichum thermophile*, as well as the microthermophilic *Cephalosporium* species and *Coprinus delicatulus*. Most species of this group also show a high frequency of occurrence (cf. Table I).

2) About one third of the thermophilous fungi recorded are less frequently present in various nests. They include the thermophilic *Mucor pusillus* and *Penicillium duponti*, the psychrotolerant *Aspidia ramosa* and *Paecilomyces varioti*, and the microthermophilic *Botryotrichum* species, *Dendrostilbella boydii* and *Emericella nidulans*.

3) The third group consists of those species with only a few records, such as the thermophilic *Stilbella thermophila* and the microthermophilic *Dactylomyces thermophilus*, *Rhizopus arrhizus* and *R. cohnii*. The five transitional mesophiles, of which just single records were made, belong here too (cf. Table II).

The site and the type of the nest, as well as other factors, may be reflected in the composition of the thermophilous flora of the nests of a particular bird species. In the material surveyed only single nests of Greenfinch, Mistle Thrush, Reed Bunting, Starling and Wren were available for examination, so general conclusions would be difficult to make due to the variations in their composition and in the sites of the nests. However, these nests (cf. Table I) revealed that the Greenfinch's nest had few thermophilous fungi (7 species), while the nest of Reed Bunting with 10 species was devoid of *Humicola insolens* and *Thermoidium sulphureum*, which were common in other nests, and that there was also an absence of mucors. The other birds' nests were available for examination at least in duplicate and the results given in Table I may be of general value. Here the number of fungi recorded from certain birds' nests vary between 8 (Sandmartins' nests) and 22 (Blackbirds' nests). In certain nests there was again a significant absence or very scarce occurrence of thermophilous mucors (the nests of Sandmartin and Swallow), as well as absence of *Thielavia sepedonium* (the nests of Sandmartin and Song Thrush). However, the most striking fact was an absence or a very low frequency of most thermophilous fungi, including actinomycetes, in Sandmartin's nests. The reason for this is somewhat obscure as the nest material is similar to that of the Swallow and other birds. There is a possibility that the nest

material in the sandstone holes is cool for most of the year and that this may prevent development of various thermophiles. However, according to Mr. R. S. BALTER (personal communication) all the nests of the Sandmartin were heavily infested by Collembola which

TABLE II
General occurrence of thermophilous fungi recorded from the nests

	Soil	Vegetation	Composts	Nests		Infections		
				Average relative frequency	presence %	Birds	Domestic animals	Man
a) Thermophilic species								
<i>Allescheria terrestris</i>	+	+	+	2	54			
<i>Chaetomium thermophile</i>	+	+	+	3	76			
<i>Humicola insolens</i>	+	+	+	3	76			
<i>Mucor pusillus</i>	+	+	+	1	31	(+)	+	+
<i>Penicillium duponti</i>	+	+	+	+	17			
<i>Stilbella thermophila</i>			+	2	6			
<i>Thermoidium sulphureum</i>	+	+	+	3	80			
<i>Thermomyces lanuginosus</i>	+	+	+	2	70		+	+
<i>Torula thermophila</i>			+	1	46			
b) Psychrotolerant thermophiles								
<i>Absidia ramosa</i>	+	+	+	1	26	(+)	+	+
<i>Aspergillus fumigatus</i>	+	+	+	3	85	+	+	+
<i>Paecilomyces varioti</i>	+	+	+	1	11		+	+
<i>Sporotrichum thermophile</i>	+	+	+	2	56			
c) Microthermophilic species								
<i>Botryotrichum species</i>			+	+	15			
<i>Cephalosporium species</i>			+	2	56			
<i>Coprinus delicatulus</i>		+	+	2	78			
<i>Dactylomyces thermophilus</i>		+	+	2	6			
<i>Dendrostilbella boydii</i>	+	+	+	1	20		+	+
<i>Emericella nidulans</i>	+	+	+	1	19	+	+	+
<i>Rhizopus arrhizus</i>	+	+	+	1	4	+	+	+
<i>Rhizopus cohnii</i>		+	+	+	2		+	
<i>Thielavia sepedonium</i>	+	+		1	24			
d) Transitional mesophilic species								
<i>Aspergillus terreus</i>	+	+	+	1	4	+	+	
<i>Aspergillus species</i>				1	2			
<i>Endomyces lactis</i>	+	+	+	1	4	+	+	+
<i>Pseudoeurotium species</i>				+	2			
<i>Trichoderma koningi</i>	+	+	+	1	4		+	

feed on various fungal spores and mycelia as well as on plant debris. In distinction to those nests with a limited occurrence of thermophiles, the organic debris in the nests of Blackbird, House Sparrow, Hedge Sparrow, Linnet, and the Thrushes is extremely rich in species of various thermophiles including actinomycetes, and a number of thermophilous fungi frequently occur on the plant debris (cf.

Table I). This indicates that the organic debris in such nests is warmed up by the birds (CADMAN, 1923; BALDWIN & KENDEIGH, 1932; NORDBERG, 1936) and by the sun during the warm season thus enabling the development of various thermophiles to the large extent that they are found on the nest debris. At present, there is no evidence that self-heating contributes to the warming up of the relatively small mass of debris of passerine nests.

According to AINSWORTH & AUSTWICK (1955) certain species of thermophilous fungi may be potential pathogens to animals including birds and man (cf. Table II). The number of species of such thermophilous nest pathogens varies. Usually the nests of Blackbird, Hedge Sparrow, House Sparrow, Linnet and the Thrushes appeared relatively rich in such potential pathogens, compared with the nests of some of the other birds examined. MACDONALD (1965), reporting on wild birds' mortality in Britain for 1962, states that the bacterial, mycotic and viral infections caused 19 % of deaths of which the cases with aspergillosis were 4 per cent.

The recent report by PARTRIDGE & WINNER (1965) of the widespread occurrence of *Cryptococcus neoformans* on birds', mainly pigeons', droppings in London is significant, because this known pathogen may occur in birds' nests too. However, this species, as well as certain other yeasts, was not recorded in the nests examined, probably due to the temperature of incubation used.

Summary

Twenty seven species of thermophilous fungi were found on plant debris in the nests of twelve passerine bird species in Nottinghamshire of which *Dactylomyces thermophilus*, *Humicola insolens*, *Penicillium duponti*, *Stibella thermophila* and *Torula thermophila* are new records for Britain.

Certain species, such as *Aspergillus fumigatus*, *Chaetomium thermophile*, *Coprinus delicatulus*, *Humicola insolens*, *Thermoidium sulphureum* and *Thermomyces lanuginosus* were present in most of the 54 nests surveyed, usually at a high frequency.

The species populations of thermophilous nest fungi are similar to those on plant debris on the soil surface and in the grassland vegetation. They are closely related to thermophilous floras known from various composts. The relatively high frequency of strictly thermophilic species found on organic debris of the various nests indicates that nests are warmed up by the birds while using the nests, and, probably, also by the sun and air during the summer months.

The number of thermophilous species and their frequency on the plant debris of the nests varies considerably in the twelve bird species. The number of species as well as their frequency was exceptionally low in Sandmartin's nests but the nests of Blackbird, Hedge Sparrow and of the Thrushes were rich in thermophilous fungi

All the 27 thermophilous fungi recorded from the birds' nests may be regarded a) as saprophytes with no known harmful relationships to birds and other animals, such as *Allescheria terrestris*, *Botryotrichum* species, *Chaetomium thermophile*, *Coprinus delicatulus*, *Humicola insolens*, *Penicillium duponti*, *Sporotrichum thermophile*, *Stilbella thermophila*, *Thermoidium sulphureum*, *Thielavia sepedonium* and *Torula thermophila*, or b) as saprophytes on the plant debris of the nests but with known potential pathogenicity to birds, other animals and man, e.g., *Absidia ramosa*, *Aspergillus fumigatus*, *A. terreus*, *Dendrostilbella boydii* (=conidial *Allescheria boydii*), *Emericella nidulans*, *Endomyces lactis*, *Mucor pusillus*, *Paezilomyces varioti*, *Rhizopus arrhizus*, *R. cohnii* and *Thermomyces lanuginosus*.

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