DO THERMOPHILOUS FUNGI OCCUR IN THE NESTS OF LUNDY HOUSE SPARROWS?

by

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ABSTRACT

The micro-fungi in the nest cups of the House Sparrow (*Passer domesticus*) collected from farm buildings on Lundy in 2020 were investigated in 2025 by two methods: 1, incubation of samples in damp chambers; 2, preparation of a dilution series on a selective agar medium to count colony forming units (CFUs). Incubation was at 20°C (for mesophilic fungi) and 40 °C (for thermophilous fungi). Taxa were identified and counted by microscopy. Six species of thermophile and nine species of mesophile were found, totalling 15, of which 13 were new records for Lundy. Populations of thermophilous fungi were lower than reported in most previous studies of bird's nests, possible due to the long storage of the nests (3 years).

INTRODUCTION

The population of House Sparrow, *Passer domesticus,* living and breeding on Lundy is the subject of long-term study and represents one of the longest running wild genetic pedigree systems on earth (for methods, see Dunning, Burke & Schroeder 2023). In recent years two days of field work on the fungi of Lundy have been successfully added to the programme of the postgraduate field course to give students experience of a completely different set of organisms, beyond sparrows. The week ends with a seminar session entitled 'Sparrows and Fungi', a unique idea but really just a serendipitous juxtaposition of presentations on the separate findings of their field work.

However, there is a real connection between House Sparrows and fungi, and indeed with many other birds; their cup nests provide an ideal place for some fungi to grow, especially because the temperature of the nest material is raised during the incubation of eggs and young chicks, often to 30- 40 °C.The heat, retained well by the nest construction, allows populations of heat loving micro fungi ('moulds') to develop in the nest material, which is also damp, the only similar environments being places like compost heaps and self- heating hay where temperature is raised by microbial activity, and where there are high populations of these fungi. Some have optimum growth temperatures of around 45°C (described as thermophilic, heat loving, by Cooney & Emerson 1964) although the term thermophilous, used by Apinis & Pugh in 1967, encompasses a wider range of fungi with optima around 35 °C and was employed in our study. In contrast mesophilic fungi found in soil and litter grow optimally around 20-25 °C. In their pioneering study Apinis & Pugh (1967) found a rich diversity of fungal thermophiles in nests of Passerine birds, with populations far

higher than in the surrounding environment and several thermophilous species were first found and described from nests. Subsequent studies have confirmed the high populations of thermophilous fungi in nests of many species of bird e.g. Satanarayana *et al* 1977; Chaturvedi & Sarethy 2022. For the most part the association remains benign, but scarcely studied, with the fungi benefitting from the warmth but also able to use nest material, especially plant materials, as a food source, but also including keratin from the feather linings.

No studies have been carried out on fungi in bird's nests on Lundy. An opportunity came during Covid lockdown when the then Warden Rosie Ellis and Jamie Dunning collected nests of House Sparrow, following the breeding period, and sent them by post to John Hedger who had the intention of studying the fungal population during lockdown. Procrastination led to the study being delayed until 2025, when the stored nests were eventually investigated and the results are presented in this short paper.

METHODS

Collection of House Sparrow nests on Lundy

Nesting boxes on Lundy are clustered into 'neighborhoods', with a median distance of 7.8 m (SE = 0.52) between boxes within each neighborhood. Each cluster is associated with farm infrastructure, and the majority are inside farm buildings (Dunning, Burke, and Schroeder 2023), with associated fitness cost/benefits to the breeding pair - that is, some nest sites are of higher quality than others through factors proximity to resources and their environment (Schroeder *et al*. 2012). A minority of House Sparrows on Lundy use either nesting boxes on the outside of farm buildings or, to a lesser extent, 'wild' nest sites, often in drystone walling on the periphery of existing neighborhoods. Plate 1 shows a typical nest.

Three nest cups were collected during annual nest box cleaning in December 2022, outside of the sparrow breeding period, from two neighbourhoods, both inside farm buildings and so all nest cups were drier and warmer than outdoor nest sites. House Sparrows typically have two to three broods of four or five eggs per breeding season (Westneat *et al* 2014), and so nests were generally incubated by either parent birds or thermoregulating chicks between April and July. Nest cups were stored in dry bags kept in the buildings within which they were collected, until sent off for mycological sampling.

Plate 1 Sparrow's nests with eggs © Jamie Dunning.

Ethical Note

Although all handling of birds on Lundy is subject to licenses from the British Trust for Ornithology and the UK Government Home Office, no specific laws prevent the cleaning of nest boxes, or the collection of passerine nests, outside of the breeding season

Mycological Studies

Samples were taken in January 2025 from the three nests, labelled 1-3, which had been stored dry in polythene bags since 2020. A sample of about 100g dry weight was removed with a knife from the inside of each nest cup. It consisted of woven grass (including *Festuca ovina* seed heads), moss, feathers, invertebrate frass and bird droppings. They were stored in a labelled sterile petri dishes. Two approaches were used to examine the fungal populations in the samples: -

1) *Estimation of relative abundance of fungal species by direct observation of nest material held in damp chambers.* 10 randomly selected small pieces (5mm maximum length) of nest material were incubated in damp chambers (petri dishes lined with damp filter paper). Ten dishes were set up for each nest sample. Five were sealed in plastic bags and placed in an incubator with temperature controlled at 40-43 °C (average 40 °C). Five were sealed in bags and kept at room temperature (range17-22 °C, average 20 °C). After one week and two weeks incubation the nest material samples were checked under a dissecting microscope and sporulating fungi identified, removing samples for mounting on slides under a compound microscope for final checking of identity. Each species was given a score of one (in one damp chamber) to five (in all five).

2) *Dilution plating on a selective agar medium to give quantitative estimates of the fungal populations.*10g samples from nests 1,2 & 3 were weighed out and separately added to 1 litre of tap water in a 5 litre plastic can. The top was replaced and the contents shaken vigorously for five minutes. This suspension was a dilution of 1:100. 1ml was then added to 9ml of water, giving a dilution of 1:1000. 1.0ml samples of the dilutions were withdrawn with a wide mouthed pipette and added to one of eight petri dishes. About 20ml of liquid (at around 50°C temperature) Rose-Bengal agar medium (Oxoid Ltd.), containing 0.1g/l Chloramphenicol to suppress bacterial growth, was poured into each dish and the contents swirled to disperse the suspension. After the agar had set, the petri dishes were sealed in plastic bags and incubated at 40-43 °C (average 40 °C), for development of thermophilous fungi, and at room temperature 17-22 °C (average 20 °C) for development of mesophilic fungi (four plates/each dilution). Fungal colonies were counted after one week of incubation and identified using dissecting and compound microscopes. Average numbers of colonies per plate vs dilution factor (1 x 102 and 1 x 103) were used to calculate populations as colony forming units (CFUs) derived from propagules like spores and hyphae, per g dry weight of the nest material.

RESULTS

The results are shown in Table 1, incubation at 20 °C (average) and Table 2-incubation at 40 °C (average). The data for the damp chambers shows presence/absence of a species in each of five dishes, so 5/5 = 100% record. The data for the dilution plating represents numbers of colony forming units (CFUs) per g. of material, calculated from the colony counts and dilution factor.

At 40 °C *Aspergillus fumigatus* had the highest colony count on plates from all three nests and it was also found on all the incubated material, as were two other fungi, *Myceliophthora thermophila* and *Absidia ramosa* (illustrated in Plate 2) . Three species, *Rhizomucor pusillus*, *Coprinopsis cinerea* and *Scytalidium thermophilum* were recorded on the incubated material but only one of these species, *Rhizomucor pusillus,* also grew on the plates. The plate-count totals gave a figure of 1-2,000 CFUs/ g/dry weight nest material for thermophilous fungi.

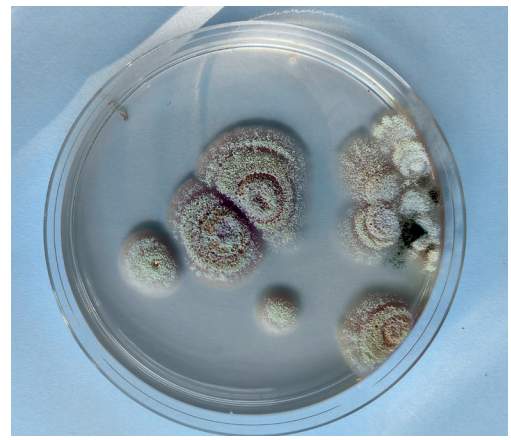


Plate 2 Fungal colonies growing on Rose Bengal/ Chloramphenicol selective medium. Incubated at 20°C. Dilution 1:1000 from Nest 2. The yellow/ white colonies are *Scopulariopsis brevicaulis*.

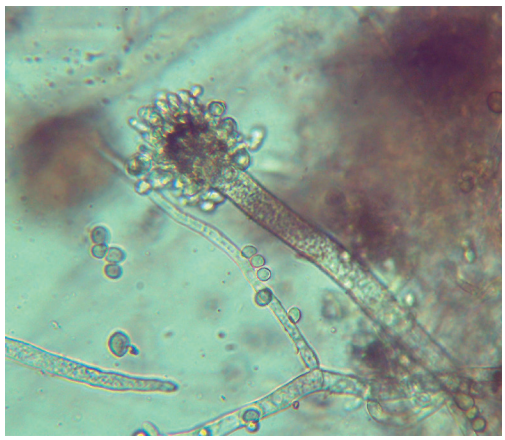
Table 1a Frequency of fungi in five Damp Chambers (40°C incubation).

	Nest 1	Nest 2	Nest 3	Total Score/15
Apergillus fumigatus	4	5	3	12
Absidia ramosa	4	2	0	6
Myceliophthora thermophila	4	1	5	10
Rhizomucor pusillus	4	0	1	5
Coprinopsis cinerea	1	0	0	1
Scytalidium thermophilum	1	1	0	2

Table 1b Numbers of fungi (CFUs) /g dw nest material (40°C incubation of agar plates).

	Nest 1	Nest 2	Nest 3
Aspergillus fumigatus	2000	1000	1500
Scopulariopsis brevicaulis	0	0	62
Absidia ramosa	0	250	250
Myceliophthora thermophila	0	0	62
Rhizomucor pusillus	0	250	0
Unknown	150	0	0
Aspergillus flavus	100	100	200

At 20 °C eight species were recorded by plating out and damp chambers, though the lists differed: *Aspergillus candidus, A.flavus* and *Scopulariopsis brevicaulis* were recorded by both methods; the two *Chaetomium* species, *C.elatum* and *C.globosum* were only found on the incubated nest material; *Absidia ramosa, Cladosporium herbarum, Penicillium brevicompactum*, *Stachybotrys dichroa*



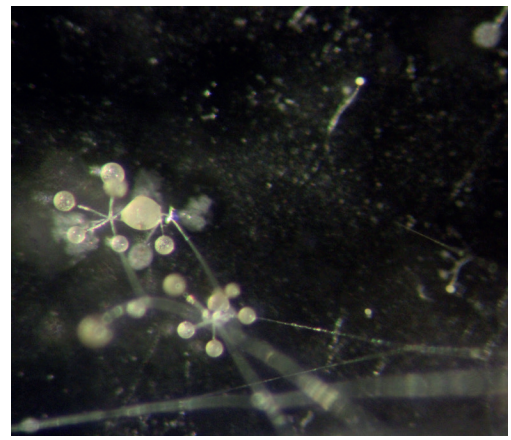


Plate 3 Photomicrographs of thermophilous microfungi
3a *Aspergillus fumigatus* conidial head (x 400). **3b** *Absidia ramosa* sporangia (x 20).

were only found by plating out. *A.flavus* and *A.candidus* were recorded from incubated material of all three nests but only by plating out from nest three; no colonies grew on plates from nests one and two. *Scopulariopsis brevicaulis* was easily the most abundant fungus on all the incubated nest material but colonies only occurred on plates from the nest two dilutions.Green/yellow colonies of *Trichoderma* completely dominated plates from dilutions of nest one material, but only a few grew on plates from nests two and three. The conidia were covered in small spines, placing the fungus in the *Trichoderma viride* species complex.

The total population of mesophilic fungi calculated from the plate-counts was 10-20,000 propagules/g/dry weight nest material.

Table 2a Frequency of fungi in five Damp Chambers (20°C incubation).

	Nest 1	Nest 2	Nest 3	Total Score/15
Apergillus fumigatus	4	5	3	12
Absidia ramosa	4	2	0	6
Myceliophthora thermophila	4	1	5	10
Rhizomucor pusillus	4	0	1	5
Coprinopsis cinerea	1	0	0	1
Scytalidium thermophilum	1	1	0	2

Table 2b Numbers of fungi (CFUs) /g dw nest material, (20°C incubation of agar plates).

	Nest 1	Nest 2	Nest 3
Aspergillus fumigatus	2000	1000	1500
Scopulariopsis brevicaulis	0	0	62
Absidia ramosa	0	250	250
Myceliophthora thermophila	0	0	62
Rhizomucor pusillus	0	250	0
Unknown	150	0	0
Aspergillus flavus	100	100	200

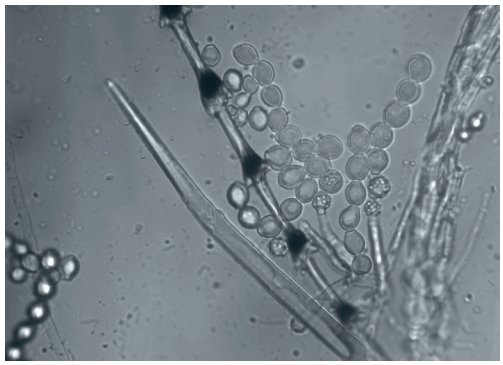


Plate 4 Mesophilic microfungi
4a Conidiophores and conidia of *Scopulariopsis brevicaulis* on nesting material x 400.
4b Perithecia of *Chaetomium elatum* on nesting material (x20).

DISCUSSION

The project set out to study the micro fungi within the nest cups of Lundy House Sparrows, with a prediction that thermophilous species would take advantage of nest cup warmth whilst breeding is taking place. Apinis & Pugh (1969) were the first mycologists to investigate the nests of passerine birds and found 27 species of thermophilous fungi in a survey in Nottinghamshire. Our own results were somewhat disappointing in comparison, detecting only six species of thermophilous fungi, with populations of only c.2,000 propagules per g of nest material as against up to 20,000 for mesophilic fungi. *Aspergillus fumigatus* was the most frequently found in all three nests. It is thermophilous, with an optimum growth temperature of 37 °C and is sometimes of concern because of its known pathogenicity to birds (Beernaert *et al* 2010).

Myceliophthora thermophila is thermophilic, as against thermophilous, with an optimum growth temperature of 45 °C and was also recorded for all three nests. It was also frequently found in passerine nests by Apinis & Pugh as its anamorph (asexual state), *Sporotrichum thermophile*. *Rhizomucor pusillus* and *Scytalidium thermophilum* (=*Mycothermus thermophilus*) are also thermophilic, with growth optima at 45 °C and were found as large populations in bird's nests by Apinis & Pugh (*S.thermophilum* as *Torula thermophila*). They did not feature much in the plating data in our study but were recorded in the damp chambers, so were probably at very low populations.

The other two *Aspergillus* species we found*, A.candidus* and *A.flavus* only appeared at 20 °C incubation but are near thermophilous, having growth optima around 30 °C . Both have been recorded from bird's nests e.g. Korniłowicz-Kowalska & Kitowski (2013) but are best known as food spoilage organisms in warm conditions, for example *A.flavus* on Peanuts where it releases potent toxins, Alfatoxins (Samson & van Reenen-Hoekstra 1988). *Absidia ramosa* (=*Lichtheimia ramosa*), is a 'pin mould', which is also near thermophilous and was recorded after incubation at both 40 and 20 oC on both nest material and by plating out. It is common in composts and other self-heating habitats.

Scopulariopsis brevicaulis (=*Microascus brevicaulis*), has been recorded from soils all over the world (Woudenberg *et al* 2017) and has a wide temperature/ growth range, with a few colonies appearing on the 40 °C plates (Figure 1). It often colonises keratin, e.g. hair, feathers,is

considered keratinophilic and can also be a dermatophyte on humans, infecting skin or nails. Its frequency on the 20 oC incubated nest material suggests a high population, confirmed by the dilution plating for nest 2 (20,000 propagules/ g) though it was inexplicably absent from nest 1 and 3 counts. It may well have been growing on the feathers lining the nest cups; perhaps nest 2 had feathers and the others not. Hubalek (1976) found keratinophilic fungi, including *S.brevicaulis,* to be active colonisers of feathers lining the cups of Tree Sparrow nests so its occurrence in the nests of Lundy House Sparrows is logical.

The record of *Coprinopsis cinerea* on material from nest 1 incubated at 40 °C in a damp chamber is interesting. This gill fungus normally grows in places rich in nitrogen such as manure heaps, where it forms white 'Inkcap' fruiting bodies .It was recorded from birds' nests by Apinis & Pugh in their 1967 study, as *Coprinus delicatulus.* We identified it by the presence of clamp connections (characteristic of basidiomycete fungi) on a white mycelium covering the nest material in one of the damp chambers. Further inspection found brown egg-shaped sclerotia characteristic of this species, which is thermophilous (Hedger 1974). One hypothesis is that its presence was linked to the Sparrow droppings in the nest cups.

The two *Chaetomium* species, *C.elatum* and *C.globosum* are well known as decomposers of plant remains and are widely recorded on hay and straw in storage (Ellis & Ellis 1988) so may have grown on the nest material such as grass stems before, and more probably, after the nests were taken down and stored in December 2022 *Stachybotrys dichroa* recorded on agar plates from nest three is most likely to have been a coloniser of the nest material before it was incorporated into the nest, persisting as spores which germinated on the plates. *Botrytis cinerea* colonies, found on plates from nest probably arose from its black sclerotia on the dead plant remains collected by the birds for nest material and is commonly seen on stems of dead plants in and around Millcombe, though only recorded officially in 2024!

A caveat must be added: all three nests had been stored for four years prior to the investigation of their mycoflora. They were 'dry', but at room temperature the storage may have altered the species composition compared to when they were' just used nests', with 'nest fungi' being replaced by mesophilic 'storage fungi' like the *Aspergillus* species, which can grow at low water contents. Nest one is a good example of a mycoflora affected by storage. *Trichoderma viride* made up most of the colonies on the dilution plates. This fungus is often recorded as a secondary invader of plant litter and wood, replacing other fungi by chemical and parasitic antagonism, but needs a higher water content (Watkinson *et al* 2016). So perhaps this nest was much damper than the other two, allowing *T.viride* to dominate.

CONCLUSIONS

This simple study has confirmed that thermophilous micro fungi can be found in the used nests of House Sparrows on Lundy. Numbers both in populations and species diversity were disappointingly low, although undoubtedly higher than in Lundy soils, but the nests had been in store for some years. Should the opportunity arise, fresher nests would give more accurate results. The study increased the number records of fungi on Lundy, since thirteen of the mesophilic and thermophilous species found are not on the current database (**https://www.lundy.org.uk/**).

It has also added another facet to the ongoing Lundy Sparrow Project, making the annual November seminar title 'Sparrows and Fungi' real rather than theatrical.

ACKNOWLEDGEMENTS

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