An investigation of a novel anomalous pink feather colouration in the Mute Swan *Cygnus olor* in Britain and Ireland

MERITA M. O'CONNELL[1]*, USNA KEATING[1], DEIRDRE MCELLIGOTT[1], PADDY O'REILLY[2], JULIANNA O'CALLAGHAN[2] & JOHN O'HALLORAN[1]

[1]School of Biological, Earth and Environmental Science, and Environmental Research Institute, University College Cork, North Mall Campus Cork, Ireland.
[2]Department of Microbiology, University College Cork, Ireland.
*Correspondence author. E-mail: merita.oconnell@gmail.com

Abstract

Pink feather colouration in normally white adult Mute Swans *Cygnus olor* is described here for the first time. Symmetrical salmon-pink colour was first evident on primary feather tips after moult (July–August), spread to secondary and tertiary remiges as the year progressed, darkened as winter approached, and sometimes developed to a brown colour. Affected feathers tended to become brittle, fragmented and lose their ability to repel water. Surveys made at nine sites in Britain and Ireland between May 2003 and January 2009 found 12–85% of swans with pink coloration. Highest prevalence occurred amongst flocks dependent on artificial food on eutrophic water bodies. Feather samples (white and pink), bill swabs and swabs of uropygial oil collected from swans in the field, and also pink fungus isolated from a bread sample, were cultured and subjected to high performance liquid chromatography (HPLC), to identify organisms and pigments respectively. Salmon-pink *Chrysonilia sitophila* fungus colonies developed on agars inoculated with samples from swans at Cork Lough and from the bread sample, but were absent from those inoculated with samples from swans at Lough Aderry and Rostellan Lake, where the birds feed mainly on aquatic vegetation. HPLC revealed that the dominant pigments in pink feathers were generally consistent with those found in *C. sitophila*, indicating that *C. sitophila* is the most likely agent responsible for the pink colour on swan plumage. Field experiments implied that *C. sitophila* was not transferred to the plumage through contact with water; we therefore suggest that *C. sitophila* is acquired through exposure to contaminated food via the bill and is preened onto the plumage. A layer of environmental contaminants and debris that coats the plumage of swans inhabiting eutrophic water bodies may provide a substrate for fungal growth.

Key words: Bread mould, carotenoids, *Chrysonilia sitophila, Cygnus olor*, Mute Swan, pigment, pink feathers, uropygial oil.

The principle pigments in birds' plumage are melanins, which produce black, grey and brown feathers. Carotenoids – naturally occurring, organic, fat-soluble pigments synthesised by plants, algae, fungi and bacteria, which produce reds, oranges and yellows (Pettingill 1985) – also have been reported in the plumage of several species including flamingos (*Phoenicopteridae* sp.), members of the pelican family (*Pelecanidae* sp.) and a number of gulls and terns (*Laridae* sp.) (Hudon & Brush 1990; McGraw & Hardy 2006). For instance, the pink hue of the Roseate Tern *Sterna dougallii* is obtained from the carotenoid astaxanthin, stored in the ovaries of their prey Sand Eels *Ammodytes* sp. (Hays *et al.* 2006), pink/red flamingo feathers originate from the application of pigmented uropygial oil (Grande *et al.* 2004), and Greater Hornbills *Buceros bucornis* similarly produce a yellow uropygial gland secretion that is preened on plumage areas that are used in threat displays (Reneerkens & Korsten 2004). Once formed, feathers lack any vascular supply beneath their outer surface, so cannot change colour or form except through fading, abrasion and staining, or through external treatment such as the application of pigmented uropygial oil.

Pigmented uropygial oil has not been documented in the Anatidae family and there are no published records of pink feather colouration from any swan species, although a rusty brown staining on the head and neck may occur when swans frequent iron-rich waters (Birkhead & Perrins 1986). Unusual pink feathers were noted (but the observation not published) in 2001 on Mute Swans at Cork Lough, a hyper-eutrophic, shallow lake in the suburbs of Cork city, in the south of Ireland (T. Kelly & C. Perrins, pers. comm.). Since then, anomalous pink feathering has been reported for swans elsewhere in Ireland, at sites in the United Kingdom and in Florida, United States of America.

This paper describes the prevalence of pink feathers on Mute Swans *Cygnus olor*, particularly in Ireland but also at sites in the UK. The factors that influence the distribution of the colouration on swans' plumage are assessed. For instance, we attempt not only to identify the agent responsible for producing the pink colour but to determine its source, as it may be obtained from the uropygial gland, the bill, or the environment. Whether the pigment is derived from microorganisms is also assessed. We hypothesise that spores of bread fungus float on the water surface and attach to feathers of swans when they are swimming and washing.

Methods

Study areas

Cork Lough in the western suburbs of Cork City, Ireland is a 6 ha hyper-eutrophic, shallow, freshwater lake regularly supporting over 70 Mute Swans (O'Halloran *et al.* 2002), including immature, non-breeding and post-breeding adults that frequently move to surrounding sites (Keane & O'Halloran 1992). Cork Lough wildfowl are highly dependent on bread and other artificial food supplied by the public, as aquatic vegetation is scarce (Irwin & O'Halloran 1997). Field observations, feather samples, bill swabs and uropygial oil samples were collected at Cork

Lough, and also at Rostellan Lake and Lough Aderry, which are located in more rural east County Cork, where swans are less dependent on bread. There is regular movement of swans between Cork Lough and these sites (O'Halloran *et al.* 1995).

Field observations were also made opportunistically at seven other sites in Ireland and the UK. These were located in west Cork, Wicklow, Dublin, Galway, Antrim, at Windsor in England and at the Cosmeston Lakes in Wales.

Field observations

During July 2003–September 2004 and July 2008–January 2009, Mute Swans were attracted with fresh bread at each site, to provide a clear view of their plumage. Flocks were scanned to quantify the number of fully white swans and those with pink colour on their feathers (Fig. 1). Cygnets were excluded from surveys as their dark plumage made it too difficult to ascertain whether or not they had pink feathering.

Focal bird observations were undertaken at Cork Lough, at 10-min intervals per swan, for birds with and without pink plumage. This involved an examination of the extent and distribution of pink feathers on the body of each bird. A total of 84 focal bird observations were undertaken: 45 for pink swans and 39 for non-pink swans. The

Figure 1. Symmetrical pink colour on Mute Swan feathers.

extent to which there was pink colour on the head/neck/breast area, the wing feathers, the tail feathers, the flank and the overall body (*i.e.* the whole plumage) was recorded on site, and later verified by photographs taken of the individuals.

Microbiology: examination of feathers

Swans with and without the pink feathers were caught at Cork Lough, Lough Aderry and Rostellan Lake under licence from the National Parks and Wildlife Service between July and December 2008. White, pink and brown feathers were clipped from different parts of the plumage (remiges, retrices, breast, flank and back), using surgical gloves and sterilised scissors, and then placed in sterile, zip-lock, labelled bags (Table 1). For swans with pink feathering, a sample of the pink, white and (if present) brown feathers were taken from each bird, brown feathers being associated with the late phase of the condition. Within two days of collection, pink and non-pink feathers were placed separately on selective media containing yeast extract glucose chlorophenicol (YGC) agar, tryptic soy agar (TSA) and sabouraud dextrose agar (SDA), to promote the growth of fungi, bacteria and yeasts that may occur on the feathers (Table 2). All of the agars were constituted in accordance with the manufacturer's instructions.

Agars with feathers were kept at constant temperature room (20°C) and monitored daily. Organisms producing coloured colonies which showed similarity to the pink colour of the pink feathers observed on swans were isolated using a sterile loop. The loop was then gently inoculated over the agar that best promoted its growth, incubated at 20°C for 1–2 weeks, and monitored daily.

To investigate whether the isolate could use different feather types as a substrate, a

Table 1. Number of Mute Swans feather-sampled at different sites.

Sample type	Cork Lough	Lough Aderry	Rostellan Lake
Pink feathers from swans with pink feathering	6	1	2
White feathers from swans with pink feathering	6	1	2
Brown feathers from swans with pink feathering	4	0	0
White feathers from swans without pink feathering	4	2	4
Bill swabs from swans with pink feathering	4	1	2
Bill swabs from swans without pink feathering	4	2	4
Uropygial oil from swans with pink feathering	4	1	2
Uropygial oil from swans without pink feathering	4	2	4

Table 2. Number of YGC agars inoculated with different sample types (*e.g.* pink feathers, white feathers, bill swabs, uropygial oil swabs) from swans with and without the pink feather colouration. YGC agars are only included in microbiological analysis of some sample types, as it became evident that no pink colonies were observed in any of the other agars following inoculation of feathers and bill swabs.

Sample type	Cork Lough	Lough Aderry	Rostellan Lake
No. of YGC agars inoculated with pink feathers from swans with pink feathering*	24	4	8
No. of YGC agars inoculated with white feathers from swans with pink feathering	12	2	4
No. of YGC agars inoculated with brown feathers from swans with pink feathering	8	0	0
No. of YGC agars inoculated with white feathers from swans without pink feathering*	16	8	8
No. of YGC agars inoculated with bill swabs from swans with pink feathering*	12	3	6
No. of YGC agars inoculated with bill swabs from swans without pink feathering*	12	6	8
No. of YGC agars inoculated with uropygial oil from swans with pink feathering	12	3	6
No. of YGC agars inoculated with uropygial oil from swans without pink feathering	12	6	12
No. of YGC agars inoculated which were attached to the experimental station	16	0	0

* = Sample types that were inoculated over same number of TSA and STA agars.

number of clean, white feathers were autoclaved for 15 min at 121°C. Loops of the cultured isolates were inoculated onto the agar that best promoted their growth and the autoclaved feathers were subsequently placed into these agars using sterilised forceps. Feathers and isolates were then monitored over a 6-month period.

Pink fungal colonies resembling the colour of pink-feathered swans were observed by chance on bread before its packaging had been opened. Isolates from this pink bread fungus were placed in YGC

agar with the autoclaved feathers and incubated at 20°C for six months and monitored regularly to ascertain whether this fungus could use the feather surface as a substrate.

Microbiology: examination of bill, uropygial oil and bread samples

Bill and uropygial oil swabs were collected in the field from swans at Cork Lough, Rostellan Lake and Lough Aderry (Table 1). Bill swabs were collected by rubbing a sterile cotton bud along the inside of the swan's bill. Uropygial oil swabs were obtained by rubbing the wick (a system of feathers that serve to elongate the uropygial gland) and the aperture of the uropygial gland with sterilised cotton buds. These cotton buds were then placed in sterile, labelled containers. Like the feather samples, swabs from the swans' bills and wicks were inoculated onto the selective media and incubated at 20°C for 1–2 weeks. Pink colonies that grew from these selective agars were again isolated, using the procedure described above.

Water monitoring

To test the possibility that the pink feather colouration was caused by staining from an agent in or on the water surface, and whether different feather types may be more prone to staining than others, an experimental station involving a system of floats was constructed at Cork Lough. Autoclaved, white, breast, wing and tail feathers, clipped from swans, were attached at a level which ensured the feathers were positioned at the water-air interface. Feathers were also positioned under the floats so that they remained fully submerged under water. Furthermore, as a control, feathers were positioned above the water surface. The feathers, deployed on the station in October 2008, were examined regularly to see whether they developed pink colour. After 6 weeks, feather clippings were taken using sterilised scissors, inoculated onto YGC, TSA and SDA agars, then incubated at 20°C and monitored daily to see whether they became contaminated by organisms that produced pink colonies (Table 2).

Microscopy

In the laboratory, white, pink and brown feathers sampled from swans with and without the pink feather colouration were examined and compared under a microscope. Some pink feathers were left in dry sealed bags at room temperature for 12 months. These feathers were subsequently examined under a microscope for any structural changes and colour degradation. Feather samples supplied from two Mute Swans in Orlando, Florida, USA, where similar pink feathers had been observed, were also examined under a microscope to see whether the same agent was responsible.

Analysis of pigments

Samples of white and pink feathers and samples of fungus isolated from feathers and bread were analysed using high performance liquid chromatography (HPLC). The feathers were washed in ethanol followed by hexane to remove surface lipids; 0.1 mg samples were then mixed with 300 l of 50% potassium hydroxide (w/v) and 2 ml of ethanolic pyrogallol (1%, w/v) in 10 ml

screw-top Pyrex tubes fitted with teflon lined screw caps. Tubes were kept at 70ºC for 30 min. When cool, 1ml of water and 4ml of hexane were added. Fresh pink fungus isolated from bill swabs, and an older (four month old) pink fungus isolated from bread, were treated similarly to the feathers.

The tubes were shaken vigorously and centrifuged at 2,500 rpm for 10 min. The upper hexane layer was removed and the extraction repeated with 2 ml of hexane. Combined hexane extracts were dried under nitrogen, re-dissolved in 200 µl cetonitile:methanol:dichloromethane (140: 40:20) and transferred to a HPLC vial (Buttriss & Diplock 1984).

A HPLC system consisting of an LC-10AD pump, SIL-10A autoinjector, SCL-10A system controller and SPD-6AV detector set at 450 nm (Shimadzu Corporation, Kyoto, Japan) was used to analyse the samples. The column system consisted of a Spherisorb ODS-2 C18 5µm PEEK guard column (150 × 4.6 mm) and a Spherisorb ODS-2 C18 5µm PEEK guard cartridge (Alltech Associates Applied Science Ltd), connected to a Vydac 201TP54 (250 × 4.6 mm) reversed phase C18 column (Grace Davison Discovery Sciences, IL, USA). Column temperature was maintained at 25°C using an Alltech column water jacket with a thermostatically controlled water bath (Lauda RM6 T; Lauda Dr. R. Wobser GmbH & Co. KG, *Lauda-Königshofen*, Germany). The mobile phase consisted of acetonitrile, methanol, dichloromethane (75:20:5) with 0.1% BHT and 0.05% triethylamine. The methanol contained 0.05M ammonium acetate. The flow rate was 1.5 ml/min. Chromatograms were recorded using Millenium 32 (version 3.05.01) Chromatography Manager Software (Waters Corp. MA, USA). The output was recorded as a series of peaks, the retention time characterising and hence differentiating each pigment that passed through the instrument, and the area under the peak indicating the quantity of the pigment present. The resulting chromatographs were compared to see if there were any pigments in common between the fungus and the pink feathers.

Data analysis

Kruskal-Wallis tests were used to determine any differences between sites in the percentage of pink colour recorded on different parts of the body for each swan. All statistical calculations were carried out using Minitab (Pennsylvania University, USA) and Microsoft Excel 2007 software.

Results

Field observations

Pink feather colouration was observed on the plumage of swans at all study sites in Ireland and the UK (Table 3). At Cork Lough, where Mute Swan numbers varied from 73–120, the mean proportion of birds with the pink feather colouration was 85%, suggesting that pink feather colouration is common and widespread on swans.

Focal examination of individual swans showed that the pink feathering occurred across the birds' plumage, but was particularly evident on the remiges (wing feathers) and retrices (tail feathers) (Table 4). The percentage of pink coverage recorded on different parts of the body (*i.e.* the

Table 3. Mean number of Mute Swans present (± s.d.) and the proportion of Mute Swans with pink feathers across a range of sites in Britain and Ireland. (Site information from O'Connell 2007.)

Location	Site type	Mean number of swans (± s.d.)	% Population with pink feathers
Cork Lough	Freshwater lake, hyper eutrophic	95 (±9.6)	85
East Cork	Freshwater lake, mildly eutrophic	40 (±12.6)	60
West Cork	Tidal estuarine, not eutrophic	44 (±14.2)	20
Galway	Tidal estuarine, moderately eutrophic	121 (±28.6)	76
Dublin	Tidal estuary, moderately eutrophic	75 (±0)	40
Wicklow	Tidal estuary, moderately eutrophic	58 (±0)	48
Antrim, N. Ireland	Freshwater lake, mildly eutrophic	12 (±0)	92
Windsor, England	Freshwater lake, mildly eutrophic	50 (±0)	30
Cosmeston Lakes, Wales	Freshwater lake, mildly eutrophic	65 (±0)	*c.* 55

head/neck/breast area, the wing feathers, the tail feathers, the flank and the overall body) differed significantly between sites (Kruskal-Wallis tests: $H_{head/neck/breast} = 35.14$, d.f. = 6, $P < 0.05$; $H_{remiges} = 68.11$, d.f. = 6, $P < 0.05$, df=6; $H_{retrices} = 52.45$, d.f = 6, $P < 0.05$; $H_{flank} = 38.48$, d.f = 6, $P < 0.05$; $H_{overall\ body} = 60.62$, d.f. = 5, $P < 0.05$) (Table 4).

Each year, the pink feathering appeared as a salmon-pink colour on the plumage between July and August, but darkened over time, and on some individuals progressed to a browner colour over the winter months. The feathers of these individuals appeared to become brittle and to lose their water repellence. In some cases, the plumage of these birds became completely water-logged, which was noted when the birds were caught by hand.

Inspection of feather samples

All YGC, TSA and SDA agars inoculated with pink and white feathers clipped from swans at Cork Lough displayed microorganism colonies after a week. Only salmon-pink fungus restricted to YGC agars produced a colour similar to the pink feather colouration and was identified as *Chrysonilia sitophila* on all 24 YGC agars inoculated with pink feathers sampled from swans at Cork Lough. This microorganism was also identified on all 28 YGC agars inoculated with white feathers sampled from swans with and without the pink feather colouration at

Table 4. Mean (± s.e.) % pink colouration on different parts of the body for swans inspected at a range of sites across Britain and Ireland.

Sites	No. of swans observed	Head/ neck/ breast	Remiges	Retrices	Flank	Full body view
Cork Lough	84	7.8 ± 11.7	30.6 ± 24.0	29.3 ± 23.6	6.9 ± 10.4	20.8 ± 17.2
East Cork	21	0.8 ± 1.5	2.1 ± 27.0	5.6 ± 9.8	0.2 ± 0.9	3.2 ± 5.3
West Cork	9	0	2.4 ± 40.0	0.5 ± 1.1	0	1.4 ± 2.5
Galway	30	6.9 ± 4.8	34.4 ± 25.0	33.1 ± 24.9	14.3 ± 18.3	23.2 ± 14.4
Limerick	5	0	15 ± 17.2	22.4 ± 30.7	0	*
Dublin	8	0	7.1 ± 10.8	16.9 ± 9.2	0	10.2 ± 9.6
Wicklow	5	1 ± 2.3	12.4 ± 16.1	15 ± 14.6	0.2 ± 0.4	11 ± 11.0
Total	162	2.3 ± 5.5	31.2 ± 28.0	24.9 ± 22.8	2.3 ± 5.2	18.4 ± 16.9

* = no information available.

Cork Lough. This fungus was easily distinguished by its initial pale pink floccose growth and subsequent salmon colour as spore formation occurred. Colonies covered entire Petri dishes, reaching the lid in tufts and shedding profuse salmon conidia at the rim (Pitt & Hocking 2009). Brown feathers associated with the advanced phase of the condition, and sampled from swans in Cork Lough in winter, did not yield *C. sitophila* after their inoculation to YGC agar. The fungus isolated from the bread sample was also identified as *C. sitophila*.

All YGC agars inoculated with pink and white feathers samples taken from three swans at Lough Aderry and six swans at Rostellan Lake displayed colonies of microorganisms after a period of one week. Unlike the YGC agars inoculated with feathers sampled from swans at Cork Lough, none of the 34 YGC agars inoculated with feathers sampled from swans at Lough Aderry or Rostellan Lake yielded colonies of *C. sitophila*, nor any other microorganism that appeared pink in colour.

Investigating the source of the colouration

Examination of uropygial swabs. Swabs of uropygial oil showed no pink colour in the oil. Moreover, none of the 24 YGC agars inoculated with uropygial oil swabs taken from pink and non-pink swans from Cork Lough displayed *C. sitophila* growth, or any other pink microorganism that might be associated with uropygial oil. Similarly, none

of the 27 YGC agars inoculated with uropygial oil swabs taken from swans at Lough Aderry and Rostellan Lake showed growth by *C. sitophila* or any other pink microorganism. These findings indicate that the uropygial gland was not the source of the pink feather colouration.

None of the feathers attached to the station and left floating in or on the surface of Cork Lough became pink over time. Clippings of these feathers inoculated onto 16 YGC agar yielded no *C. sitophila*, nor any other organism with a colour similar to that on the pink feathers.

Examination of bill swabs. All agars inoculated with bill swabs taken from Cork Lough Mute Swans developed colonies of microorganisms after one week. All 24 YGC agars inoculated with bill swabs sampled from swans with and without pink feathers at Cork Lough developed growths of *C. sitophila*. No other pink coloured microorganism was identified in these agars.

All agars inoculated with bill swabs taken from Mute Swans with and without pink feathers at Lough Aderry and Rostellan Lake also displayed colonies of microorganism after one week. None of the 23 YGC agars inoculated with these bill swabs yielded colonies of *C. sitophila*, nor any other microorganism that appeared pink in colour.

Detailed feather analysis. Differences between white feathers and feathers with pink colouring were evident under microscopic examination. Pink feathers sampled from swans in Florida appeared identical to those from swans at Cork Lough, indicating that the Florida swans were affected in the same way. The pink feathers tended to have large accumulations of an unknown material on them compared with clean, white feathers. These accumulations appeared to clog the barbules and to deactivate the ability of barbules to grip and interlock with adjacent barbules. It is likely that this unknown material is directly or indirectly responsible for the pink feather colour.

C. *sitophila* smeared onto clean, white feathers in the laboratory showed a pink colour after one week identical to that observed on the plumage of swans seen in the field. Feathers left for 12 months in the laboratory, with *C. sitophila* on their surface, showed no sign of physical degradation. The autoclaved feathers placed in YGC agar inoculated with *C. sitophila* showed no signs of physical degradation either, despite being left within an enclosed petri-dish at room temperature for a period of six months. *C. sitophila* left in petri dishes in a fridge for a period of four months showed considerable fading in comparison to fresher, more recently grown *C. sitophila*.

Pigment identification. HPLC extraction for four pink feathers taken from different swans at Cork Lough identified several carotenoids in the samples (Table 5). Carotenoids were identified by comparison of retention times with authentic carotenoid standards. The most common pigments in pink feathers were zeaxanthin, lutein, β-cryptoxanthin, astaxanthin, and β-carotene respectively (Table 5). Some white feathers also had low background levels of carotenoids (Table 5). These carotenoids were also present in the fungus samples with some differences between 'fresh' (< 1 week

Table 5: High performance liquid chromatography (HPLC) extractions on pink feathers, non-pink feathers, old mould (> 4 months old, isolated from bread) and fresh mould (< 1 week old, isolated from bill swabs).

Area (mm^2)*	Old mould	Fresh mould	Pink feathers	White feathers
Astaxanthin	52,882	40,940	16,734	727
Lutein	102,586	107,27	109,145	636
Zeaxanthin	0	10,363	463,036	0
Canthaxanthin	191,290	46,411	3,413	0
β-Cryptoxanthin	33,343	136,558	34,356	4,228
Lycopene	19,535	69,796	1,425	0
α-Carotene	1,191	60,919	1,035	0
β-Carotene	6,559	70,705	11,239	1,980

* = The area of the peak, as registered by the detector.

old) and 'old' (4 months old) samples. The dominant pigments in pink feathers were broadly consistent with those found in the fungus. The fresh fungus carotenoid profile matched the pink feathers closest (Table 5). As all carotenoids identified in the pink feather samples were also identified in the fungus samples, it is very likely that *C. sitophila* is the agent responsible for the pink colouration on the plumage of swans.

Discussion

Since 2001, up to 85% of Mute Swans at Cork Lough have had pink colour on their feathers. This colouration is common on swans at both estuarine and freshwater sites in parts of Ireland and the UK, and also occurs in the USA. High prevalence was associated with large swan flocks dependent on artificial feeding by the public (supplementary food being important at urban sites with little natural vegetation; Keane & O'Halloran 1992), on moderately to highly eutrophic water bodies.

The salmon-pink colour was first evident on primary feather tips and retrices immediately after moult (July–August), spreading to secondary and tertiary remiges, flank and breast feathers. The colour darkened as winter approaches and affected plumage may become brown. Affected feathers tend to become brittle and fragmented and appear to lose their water repellence. In severe cases, the pennae (external feathers) absorbed and retained large quantities of water and algae began to colonise the plumage. In such cases, water can actually be squeezed from these feathers.

The hypothesis that swans with pink feathers produce pink coloured uropygial oil was rejected since uropygial oil samples from these swans contained no pink pigment nor gave rise to pink coloured colonies of microorganisms after inoculation onto selective agars. Staining agents in the water inhabited by swans were also rejected as a potential source, because autoclaved feathers positioned above, on and below the water surface of Cork Lough showed no pink colouration after six weeks.

The fungus *C. sitophila* was present on all pink feather samples obtained at Cork Lough and HPLC extraction indicated that carotenoids present in *C. sitophila* are also present in pink feathers. Pink feathers were less prevalent among swans at Lough Aderry and Rostellan Lake, where swans are less reliant on bread than those at Cork Lough, suggesting that the presence of *C. sitophila* in the bills of swans from Cork Lough could be associated with their ingesting large quantities of bread. Pink *C. sitophila* fungus was cultured from bread left in a cupboard for some time and has been commonly reported on bread, pastries, nuts and maize (Pitt & Hocking 2009). Large amounts of "waste" bread are provided regularly to Cork Lough swans and exposure to *C. sitophila* contaminated bread likely explains the prevalence of *C. sitophila* in bill swabs taken for swans at the site, from where it could be applied rapidly to feathers during preening. Alternatively, swans may inadvertently preen bread material without fungus onto the plumage, forming a substrate for *C. sitophila* establishment from contact with the bills or feathers of conspecifics affected by the condition, or through airborne spores landing on the substrate.

Swans regularly move between Cork Lough, Lough Aderry and Rostellan Lake, potentially explaining the presence of pink feathers on some swans at Lough Aderry and Rostellan Lake. Swans initially contracting the colour at Cork Lough could transport the fungus elsewhere, as the colour did not disappear from feathers kept in the laboratory for up to one year. The fungus was not active on feathers from swans at Lough Aderry of Rostellan Lake; pink feathers from these swans yielded no *C. sitophila* after their inoculation to selective agars. *C. sitophila* maintained at constant room temperature for four months showed considerable fading compared to *C. sitophila* less than one week old and did not grow on fresh agars, indicating pigment persistence after the death of the organisms. This would explain why pink feathers from swans in Lough Aderry and Rostellan Lake did not yield *C. sitophila* after inoculation onto YGC agar.

Assuming that *C. sitophila* is the agent responsible for the pink colouration, it may be more prevalent on certain feathers, depending on their location on the swan. The condition appeared to be confined to pennae (outer feathers), confirmed by inspecting birds in the hand, perhaps because of more regular wetting of these feathers by the eutrophic waters upon which the swans occur.

Fungi have previously been recorded on birds' plumage. Beer & Kear (1975) reported that *Cladosporium herbarum* may act as a pathogen on the plumage of captive flamingos, and mentioned that plumage on the flamingos' backs may become "dingy

and frayed" and "wet and miserable looking". They also suggested that a bird washing itself in water contaminated with food and faeces would result in material adhering to the feather surface. This enriched feather surface would provide a substrate on which initial fungal growth is possible. There is some similarity between the fungus *C. sitophila*, identified on the plumage of swans in the current study, and the fungus *C. herbarum* described on the plumage of flamingos by Beer & Kear (1975). The symptoms of feather degradation are similar in both cases and *C. sitophila* resembles *C. herbarum* in that it is common on organic debris and produces many airborne spores. The spores of *C. sitophila* are also heavily pigmented and do not seem to succumb to light either. Furthermore, *C. sitophila* is opportunistic and can also grow on weakly nutritive substrates.

Microscopic examination of pink feathers revealed heavier debris accumulation compared to healthy white feathers. This debris clogged barbules and reduced their ability to grip and interlock with adjacent barbules. Breakage is likely the result from general environmental contact and friction between feathers, such as during preening, resulting in water penetration and retention within damaged regions of plumage and enabling algal growth. Subsequent algal decomposition in winter could explain later plumage browning. The likelihood of this waterlogged environment being unsuitable for *C. sitophila* is supported by absence of the fungus from brown feathers sampled at Cork Lough and inoculated over YGC agar.

In conclusion, it would appear that *C. sitophila* is cause of the pink colouration in Mute Swans. *C. sitophila* does not appear to cause direct feather degradation, but appears to be associated with a contaminant film (potentially food and faeces suspended in eutrophic waters) that adhere to the feather surface, as a medium for growth. Preening of contaminated feathers could spread *C. sitophila* to other parts of the plumage, potentially explaining the presence of *C. sitophila* in all samples taken from bills of swans at Cork Lough and the symmetrical distribution of the colour on the plumage. More detailed studies are required to determine whether pink-feathered swans suffer health or behavioural effects that could affect fecundity and survival rates compared to white swans, and hence affect management recommendations for swans at the sites concerned.

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Photograph: Mute Swan displaying at Cork Lough, by Mark Carmody.

Photograph: Cork Lough landscape, by Mark Carmody.

