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The production of regurgitation pellets in the great tit (*Parus major*)

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ABSTRACT

The nesting behaviour of the great tit (*Parus major*) from nest preparation to fledging was followed continuously by video-recording in two identical nest-boxes over two seasons during 2020 and 2021 in the same large rural garden. Unexpectedly, the production of a series of regurgitation pellets (RPs) by the nestlings was recorded during the 2020 season. No pellet regurgitation was recorded during the 2021 season. Recordings were also made of adult great tits producing small RPs as they roosted. I suggest that this unprecedented RP production by nestlings resulted from the consumption of suboptimal food items due to the poor breeding season of 2020.

INTRODUCTION

The capacity for flight allows birds to travel rapidly to explore food resources and to escape ground-based predators, but this ability comes at a cost. Weight must be minimised and so the digestive track is as short as possible. This compromises complete digestion and so many birds remove indigestible material regularly by expelling it from the upper gut in the form of a regurgitation pellet (RP) (Leprince *et al*., 1979). Many birds of prey produce RPs as they tend to swallow prey whole and their RPs consist almost entirely of fur, feather and bone. Many passerines are reported to produce RPs (Tucker, 1944; Davies, 1977a,b; Taylor & O'Halloran, 1997; Jehl, 2017), and RP production has been also reported in nestlings (da Paz Pereira & Melo, 2012). RPs are often produced when the bird preys on large quantities of insects, particularly when these insects have heavy chitinous exoskeletons. Chitin, a linear β1,4-linked polymer of N-acetyl-D-glucosamine, is digested by a range of chitinases and its complete digestion involves several different enzymes (Suginta *et al*., 2010). The chitin in the cuticle of insects is present as a matrix of proteins, lipids and, in some species, significant amounts of calcium (Kramer *et al*., 1995) and these affect the digestibility of the cuticle, meaning that digestion is seldom complete and chitin fragments are expelled as faecal pellets or regurgitation pellets. This observation has been used by many workers to investigate the diet of birds (Taylor & O'Halloran, 1997; Davies, 1977a,b).

The reasons why birds produce RPs differ substantially between bird groups. In the case of birds of prey, regurgitation is necessary to rid the bird of indigestible material such as fur and bone from their prey. In other cases, the reason is less clear. Members of the grebe family, for example, are known to eat their own feathers and produce feather-containing RPs (Simmons, 1956; Kop, 1972; Jehl, 2017). Many explanations for this well-documented habit have been offered but it seems most likely that the feather mass allows more complete digestion of their often poorly digestible food by delaying gut transit (Jehl, 2017).

Illness or intoxication is another cause of regurgitation. For example, the spotted fritillary butterfly *Melitea didyma* sequesters iridoid glycoside toxins from its plant food, which has been found to cause vomiting/regurgitation in a great tit (Tesařová *et al*., 2013). Plants such as mistletoe *Viscum album* (Mathiasen *et al*., 2008) and guelder rose *Viburnum opulus* (Hernández, 2009) rely on birds to spread their seeds through regurgitation and faecal discharge. These plants stimulate regurgitation while providing the birds with some nutrition.

The ability of birds to regurgitate is suggested to be a function of the musculature of the gizzard (Hart & Thompson, 1995) and this has been crudely quantified by the administration of emetics (Prŷs-Jones *et al.*, 1973). The spot-winged tit *Periparus melanolophus*, a relative of the great tit, was discovered to be able to regurgitate by this criterion (Prŷs-Jones *et al.*, 1973).

Here I report that great tits produce RPs at the roost, and I report for the first time that nestling great tits produce RPs. I hypothesise that, in the case of the nestlings, RP production results from the ingestion of suboptimal food items during a poor breeding season.

METHODS

A small wi-fi connected video camera (NCIP2WF, Green-feathers.co.uk, Bristol, England) was installed in a new 21 mm thick pinewood nest-box (31×13×13 cm internal dimensions, with an entrance hole 32 mm in diameter) in a large garden in Penicuik, Midlothian, Scotland (Lat. 55.8280, Long. -3.2449, elevation 224 m). The garden faces open agricultural land and deciduous woodland. This nest-box (hereafter referred to as the spruce nest-box) was firmly attached to an 8 m high Norway spruce tree (*Picea abies*), 2.5 m off the ground and 9 m from buildings, with the entrance hole facing northward to avoid direct sunlight, and the prevailing winds. A white-light LED lamp with a daylight detector illuminated the interior of the nest-box until the hours of darkness, when the white LED lamp was switched off and the camera switched automatically to infrared mode, to continually capture video. Some videos were recorded using an infrared camera (CMOSNC720M) in a similar setup, but without the LED lamp. Video was displayed and recorded on a Windows 10 PC using OBS Studio 25.0.8 video recording and streaming software (obsproject.com). A second identical nest-box with a video camera was placed 20 m away from the first in the same garden. This nest-box was attached to a holly tree (*Ilex aquifolium*) 2 m off the ground. In the 2021 season, this nest-box (hereafter referred to as the holly nest-box) was occupied by a pair of great tits which laid six eggs and successfully fledged all six chicks. Videos were annotated and edited using Moviemaker (Microsoft). Times and dates were automatically displayed on all video recordings. Still photographs outside the nest were taken with a Canon EOS 760D SLR camera with a Sigma DG 150-500 mm telephoto lens.

RPs were taken from the nesting material after the 2020 season nestlings had fledged. One RP which was recorded being produced by a nestling was retrieved from the nest periphery and was the known product of a nestling and not either parent. The composition of this RP was investigated by staining with three classic histochemical stains: Oil Red O (Sigma Aldrich, Dorset), which is specific for oils/droplets; Congo Red (benzidinediazo-bis-1-naphthylamine-4-sulfonic acid sodium salt) (Sigma-Aldrich), which stains chitin (the principal component of insect exoskeletons) and cellulose (plant cell walls); and Coomassie Blue R250 (Sigma Aldrich), which is specific for proteins. Congo Red and Coomassie Blue dyes were dissolved in a 50 mM phosphate buffer at pH 7.5, and Oil Red O was dissolved in isopropanol. The pH of other RPs was investigated by placing small RP fragments, moistened with deionised water, on litmus paper. The RP material available was very small and so a more thorough qualitative investigation was not possible. The pellets were investigated microscopically using a Leica inverted microscope with Hoffman modulation contrast optics. Images were recorded using a Canon EOS 1100D camera attached to the microscope.

RESULTS

Having built the nest alone, as is usual for the great tit (Perrins, 1979), the female roosted overnight on 11th May 2020 and by 19:37 BST on 12th May it was apparent that three eggs had been laid in the spruce nest-box, but they were obscured first by the female and then by the nest material. A further single egg was laid at approximately 20:00 on 14th May. After a 13-day incubation period (starting from when the last egg was laid), on 27th May, all four eggs hatched, the first at 10:55, the second at 11:50, the third at 14:53 and the fourth at 16:30. The standard time of incubation for great tits is 13-16 days (Perrins, 1979; Gosler, 1993). Both parents began feeding the hatchlings as soon as they were hatched (Fig.1 a,b). Nestlings usually fledge after 18-21 days (Gosler, 1993), but both parents continued to feed nestlings in the nest on day 22, when the surviving

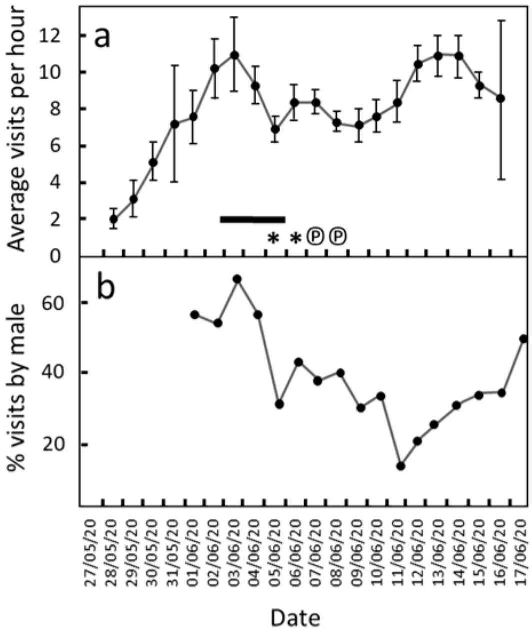

Fig. 1. Spruce nest-box: feeding rate and contribution of male to feeding. (a) Feeding rate. This reached a peak by 3rd June 2020 but then a three-day run of bad weather (black bar) reduced the rate, after which two nestlings died (*). New plant material brought into the nest is indicated by ℗. The rate then slowly increased until 14th June, after which the rate declined until fledging. Error bars indicate standard error. (b) Contribution of male to feeding. This exceeded that of the female until 5th June 2020. The male's relative contribution steadily declined, rising again on the day before fledging but only because the female's effort decreased.

pair fledged. A clutch of only four eggs is small compared with the average for great tits of 8.1 for this location (pers. obs.) and this clutch had a longer than average fledge time as is typical of suboptimal habitats such as gardens (MacKenzie, 2010). A total of 2,132 visits by both parents was recorded during the entire nestling period (Fig. 1a). Very few caterpillars were brought to the nestlings until day 13, as the temperatures were below average. The daily feeding visits built up over the first few days with the male effort being most important in the earlier days but gradually reduced (Fig. 1b).

Several episodes of pellet regurgitation were recorded, the first occurring when the nestlings were eight days old (Fig. 2a) and while all four were still alive in the nest. These regurgitation events (Fig. 3a-d; video 1 at https://youtu.be/LKZ0OMNLbF4) occurred day and night, although some events occurred unseen under the roosting female at night, the RPs being visible the following dawn. The RPs all had the same distinct appearance, varied in size from 1-5 mm, and often had an angular shape. These events were not obviously correlated with other small-scale events, such as being harassed by a fellow nestling or being jostled by the female while nest cleaning. The RPs were removed from the nest on the next visit by the parent or, if they were expelled at night, as soon as feeding visits resumed the next morning. Parents were also observed removing the

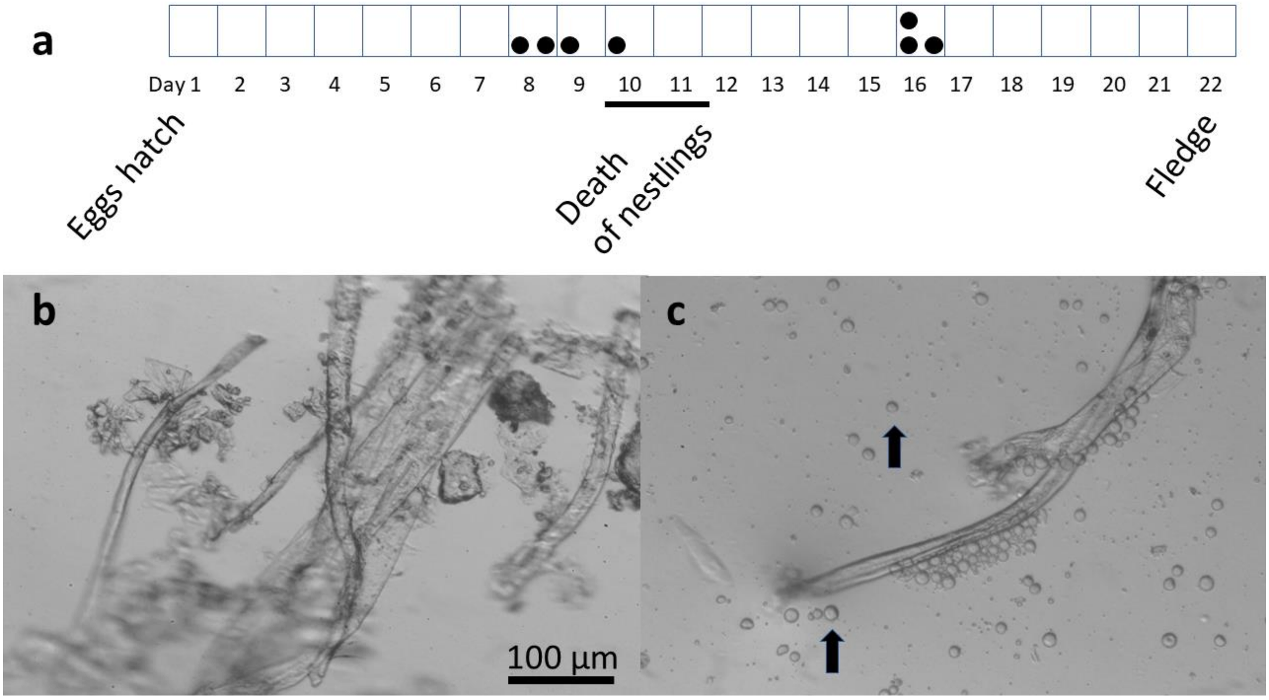

Fig. 2. Spruce nest-box: regurgitation pellet **(**RP) production and composition in the 2020 season. (a) RP production events (black spots) during the 22 days that the nestlings spent in the nest after hatching, in relation to other significant events. (b,c) Micrographs showing the nature of the RPs. Black arrows indicate putative lipid droplets that are both free and attached to fibrous material. The scalebar applies to both micrographs.

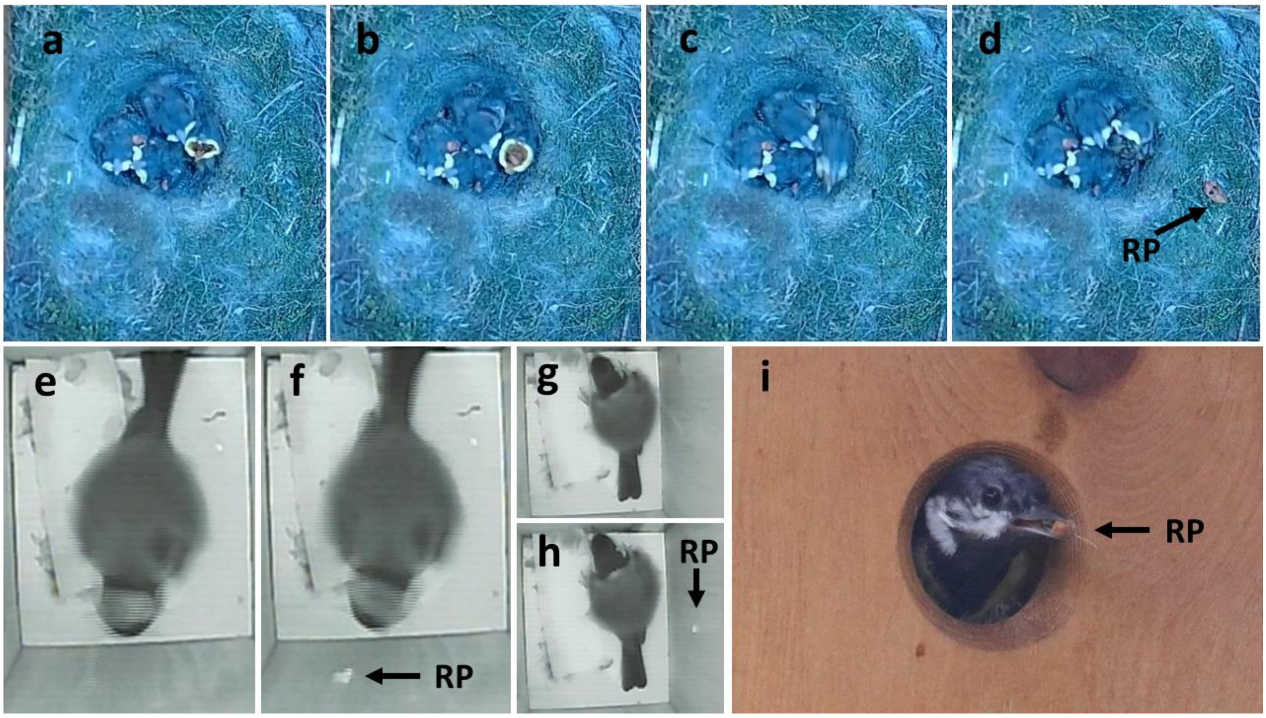

Fig. 3. Stills from videos showing regurgitation pellet **(**RP) production in nestlings (a-d) and adults (e,f and g,h). (a) The nestling on the right of the image begins to open and close its mouth. (b) A large RP appears in the gullet. (c) The nestling shakes its head vigorously. (d) A large RP is expelled and lands on the rim of the nest (arrow). The RP visible in (d) contains cuticular chitin (two black specks). (e) An adult shakes its head and (f) spits out an RP which adheres to the nest-box wall. (g,h) Similar events as in (e,f). (i) An adult female great tit removes from the nest the RP produced in the event shown in (a-d). Note that the red-orange colour of the RP is very different from the grey-white colour of the faecal sacs that were also removed from the nest-box by the parents.

RPs from the mouths of nestlings (video 2 at https://youtu.be/wCbXDkaCJtk). Two regurgitation events were also recorded in an adult great tit roosting in the spruce nest-box, one at 23:09:33 on 24th August 2020 and the other at 23:54:16 on 2nd Sept 2020 (Fig. 3e-f, g-h; video 3 at https://youtu.be/-ElSoAHK-r4).

A single RP was recovered from the periphery of the nest after fledglings had departed. This particular pellet had been seen to be moved to the peripheral position during the roost by the female and it had been overlooked in subsequent hygiene sweeps by the parent birds. When removed from the nest the pellet was pale orange (having been more distinctly orange on production), and of a chalky composition. The pellet was found to be largely insoluble in water and contained fibrous material and oily globules that were both free and attached to the fibres making up the bulk of the material when viewed with the microscope (Fig. 2b). Small fragments of the RP stained distinctly with Oil Red O, a lipid-specific stain, and Congo Red, which stains both chitin and cellulose. There was some staining with Coomassie Blue, a protein-specific dye. The fibrous material showed no indication of being organised into cells and did not appear to be of plant origin. Litmus paper indicated that the pH of the RP fragments was 5-6.

In the 2021 season nest building commenced on 5th April in the holly nest-box and the first of six eggs was laid on 11th May; all hatched on 29th May; and all six hatchlings fledged on 18th June. The parents were recorded making 4,134 visits to the holly nest-box and there were no RPs produced throughout the entire period. The 2021 female was a different individual from that of 2020, but the male could have been the same individual who raised the 2020 brood.

DISCUSSION

Perhaps most birds can produce RPs, but they only do so under certain circumstances. Many birds are believed to produce RPs when their food has a high content of indigestible material. Whereas there is a single report that great tit adults normally produce RPs (Gibbs, 1957) after eating seed, I now report that great tit nestlings also produce RPs and present video evidence in support of this observation. Nestlings were seen disgorging RPs with some difficulty (video 1, https://youtu.be/LKZ0OMNLbF4). Regurgitation in both the nestlings and the adults was accompanied by vigorous head-shaking movements, apparently in an instinctive attempt to use centrifugal force to dislodge the RPs. While only a very preliminary biochemical investigation of these RPs was possible because of the limited material available, it was found that the great tit RPs were acidic (pH 5-6), in agreement with the findings of Leprince *et al*. (1979) who studied the RPs of raptors. They contained protein, again in agreement with former studies (Leprince *et al*., 1979), lipid and chitin.

The parents reacted to the production of RPs by helping the nestlings remove them from their mouths. As parents also remove excess food from the mouths of nestlings if the nestlings cannot swallow the mass, this action pattern may explain RP removal when adults are not familiar with RP production by nestlings. Similarly, there is a well-documented instinct to remove objects from the nest in many passerines (Poláček *et al*., 2013), and so it is also possible that RPs are removed on this non-specific basis alone, without there having to be a specific familiarity with the regurgitation process or its products.

The first series of RP productions coincided with the death of two nestlings. It was not possible to identify which nestling produced the RP and so it is possible that the two events were unrelated. However, it remains a possibility that the nestlings died as they choked on RP pellets or that the RP pellets obstructed their gullets. Although the removal of the dead nestlings was video-recorded, the corpses could not be found after they had been removed from the nest-box by the mother, so the cause of death could not be investigated. Obstruction of the gullet is, however, a genuine threat to great tit nestlings: Cowie & Hinsley (1988) reported that they found dead nestling great tits with a large piece of peanut wedged in their gullets on three occasions.

On two consecutive days after the death of the second nestling the female adult brought fresh plant material into the nest. The first appeared to be a mass of grass, the second moss. The reason for this is unknown but aromatic plants brought into the completed nest of blue tits (*Cyanistes caeruleus*) were found to reduce the bacterial load on the nestlings but not on the parents (Mennerat *et al*., 2009), and so this may have been an instinctive act to sanitise the nest.

It is perhaps surprising to make a new observation in a bird species that has been so intensively studied, especially regarding such a striking behaviour. The recent availability of small cameras has greatly facilitated this study, but in addition it is possible that the production of RPs in great tit nestlings is uncommon and that it occurs only under specific conditions. Usually great tit parents time their reproduction to coincide with maximal caterpillar production, caterpillars being the favoured food for their nestlings (Barba *et al*., 1995, Naef-Daenzer *et al*., 2004). The 2020 breeding season in this study was sub-optimal in south-eastern Scotland, with cold weather delaying the appearance of caterpillars. The parent birds struggled to find enough caterpillars for their small brood of four, and instead were seen feeding the nestlings other insects such as small beetles, hoverflies, moths, green-veined white butterflies (*Pieris napi*) and spiders. These parents made a total of only 2,132 visits to the nest-box, compared with the 4,727 visits made in raising nine nestling great tits observed in a similar study (Betts, 1955), and 4,134 visits made by parents in the 2021 season with six nestlings in the same garden, again reflecting the poor breeding season reported here in 2020. It was only later in the 2020 season, after two chicks had died, that caterpillars dominated the visible food items brought to the nest-box whereas caterpillars were dominant from the beginning of the 2021 season. RP production may have resulted from the large amount of cuticular chitin present in the caterpillar-poor diet, which the chicks were unable to digest, and large pieces of insect exoskeleton were visible in some of the RPs. If this is the case, then RP pellets may also be produced in regions such as northern Finland where it has been shown that great tits do not have access to caterpillars early in the nestling season (Veistola *et al.*, 1995). It is also evident that, in the event of a second brood, the incidence of adult insects in the diet would be greater, as would the risk of dehydration, because caterpillars would be less abundant at this time.

An alternative possibility is that artificial food from surrounding bird tables could have been fed to the nestlings, causing their gullets to be obstructed. If this is the case, then this cannot be described as normal RP production but merely the spitting out of indigestible artificial food. It is possible too that the fatty material found in the RPs may originate from a "bird cake" or suet block from a local bird table. However, the fibrous appearance of the RPs under the microscope, the visible exoskeletal remains in the pellets, together with the observation that they were produced at night, long after feeding, all suggest that the pellets were genuine RPs. Finally, RPs were recorded from two adult birds, 5 h 20 min and 5 h 1 min after they returned to the roost and thus after their last opportunity to feed, again suggesting that these are genuine RPs produced to expel indigestible components of the diet.

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