New record of intraspecific nest parasitism by the Silky Starling (*Sturnus sericeus*) [v1; ref status: not approved 2, <http://f1000r.es/V94LDx>]

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Abstract The Silky Starling (*Sturnus sericeus*) is endemic to East Asia and little is known about its breeding ecology. We found intraspecific nest parasitism (INP) by this species in a reproductive study conducted between March and June 2011. We found three nests were parasitized using the obvious morphological differences of eggs or partition of egg-laying. One egg appeared 3 days after the female in nest #26 had finished laying eggs. One egg was different in color from the other five eggs in the nest #27. The third instance was discovered in nest #37 after the fledglings had fledged. Our findings confirmed INP by the Silky Starling.

Introduction

Intraspecific nest parasitism (INP) occurs when a female lays her eggs in another female's nest¹. The number of bird species known to show INP has grown during the recent decades: Yom-Tov summarized INP from 53 species 30 years ago and up to 236 species in 2001^{1,2}. Gong and Lu (2003) added a further 14 species reported in China to the list. More and more species have been found to show INP, and the major reasons of this rapid increase include more detailed investigations of avian reproductive biology as well as the collection of reproductive data from more species³.

INP has been found and studied in four species of starlings: the Grey Starling (*Sturnus cineraceus*)⁴, the Spotless Starling (*S. unicolor*)⁵, the Rose-coloured Starling (*S. roseus*)⁶, and the European Starling (*S. vulgaris*)⁷. However, information on the Silky Starling is rare, mainly because they live just in China and the surrounding areas, and few studies have been conducted on its reproductive biology⁸. We provide the first description of INP by Silky Starlings and discuss possible reasons of INP in this species.

Methods

This study was conducted on the Pukou Campus (32° 10' N, 118° 41' E) of Nanjing University, China from March to June 2011. The campus is to the north of the Yangtze River. Elevation ranges from 2 to 50 m above sea level. Average annual temperature is 15.4 °C. Annual precipitation is 1,106 mm, ~ 60% of which occurs from June to September⁹.

We placed and numbered 15 artificial nests (16 x 16 x 35 cm) on the Pukou campus. Most nests were open to the east or south with a height range from 3 to 6 m. Distances between boxes were at least 15 m. They were mainly placed in the luxuriant trees just like metasequoia (*Metasequoia glyptostroboides*), weeping willow (*Salix babylonica*) and Phoenix Tree (*Firmiana platanifolia*).

We used 2 criteria to detect parasitic eggs in a nest⁴:

1) Partition of egg-laying period. The starling usually lays one egg per day, so if two or more eggs appear a day, or there is an average of >1 egg laid per day during the host egg-laying period, it indicates INP. Additionally, if extra eggs appear outside

of the egg-laying period, (e.g. the egg is laid after the host has completed its clutch), it also indicates INP.

2) Morphological differences of eggs. There are individual differences in egg morphological features, so the appearance of eggs which were of a different shape, size and color to other eggs in the clutch would also indicate INP.

The breeding season of the Silky Starling is from late-April to June. We checked nest boxes every two or three days to record the breeding status, and took notes and photos of any changes in the nests.

Results

We placed 15 nests on the Pukou campus on March 24th. We did not find any nest materials in these boxes until 2 weeks later. The first egg appeared on April 20th and hatched on May 9th. Seven boxes were occupied by Silky Starlings. They were nest numbers 26, 27, 30, 33, 37, 38 and 40. The probability of boxes used by Silky Starlings was 46.7%. We found the 26th, 27th, and 37th nests had parasitic eggs.

In nest box #26, we found three eggs on April 20th, and six eggs 4 days later. Three days later, we found a 7th egg. Birds

normally lay no more than one egg per day. On May 13th, 3 days after the six eggs hatched, the last egg hatched. As the incubation period is consistent within species this suggests that the host had completed its clutch before April 24th, but that on April 27th, another female laid the 7th egg. Unfortunately, the nest was destroyed by an unknown reason (probably predation) with only one dead hatchling left on May 17th. As a result, we confirmed that it was a parasitic egg (Fig. 1A-D).

In nest box #27, we found nest materials on April 28th. On May 6th, we found five eggs. On May 9th, there were six eggs in the nest. We numbered them with a pencil and found that egg # 5 was significantly paler than the others. On May 19th, we found the first hatchling in the nest, and 5 days later, there were five hatchlings. However, egg # 5 was still in the nest (Fig. 2A-B). On May 30th, this egg disappeared. As a consequence, we believe the most likely explanation is that the 5th egg came from another female; predation of the egg is unlikely to be an explanation as we would have observed broken egg fragments if this were the case. During our observation, we found that starlings often clean their nests; they take all excreta away after they have fed the hatchlings each time. This may suggest that the 5th egg was removed by the host when it was found that the 5th egg did not hatch.

Figure 1 Parasitized nest #26. (A). On April 27th, the seventh egg appeared in the nest, but we could not recognize which one was parasitic as they were all of similar size, shape and color; (B). On May 9th, we found six hatchlings and the last egg unhatched; (C). On May 13th, the last egg hatched (in red circle); (D). On May 17th, we found only a dead hatchling in the nest and the other hatchlings had disappeared.

Figure 2 Parasitized nest #27. (A). On May 9th, we found egg #5 was morphologically different from the others; (B). On May 24th, the hatchlings' color had become darker and had substantially grown, whilst the extra egg had still not hatched.

Figure 3 Parasitized nest #37. On 7 June, one parasitized egg was found after the nestlings had fledged.

In nest box #37, we found six eggs in the nest on May 6th. We did not find any morphological differences between the six eggs. On May 19th, the eggs hatched. It was not until June 7th that we found another egg in the nest after the nestlings had already fledged (Fig. 3). The egg appeared after the hatching season, so it was likely to be a parasitic egg.

Discussion

We had seven nests occupied by Silky Starlings, among them, three were parasitized by other Silky Starling females and so the parasitism rate was 43%. Two of the three parasitic eggs did not hatch, and so the hatching rate of parasitic eggs was 33%. The only hatchling died for unknown reasons. The fledging rate of parasitized eggs was therefore zero.

According to the criteria suggested by Yamguch and Saitou, we confirm INP exists in Silky Starlings⁴. That the extra eggs appeared after the female's clutch in nests #26 and #37 accords with the first INP criterion, and that the extra egg had a different color to the other eggs in nest #27 accords with the second criterion. With the above limited information, we can at least conclude female starlings can lay their parasitical eggs when they found the hosts had started hatching.

The parasitism rate seemed high in our study, although our samples were limited. Previous research on Spotless Starlings⁵ and Grey Starlings⁴, found intraspecific brood parasitism of Spotless Starlings was 19.1% in colony A and 25.3% in colony B, and parasitism of Grey Starlings occurred in 18.5% of 157 nests in 1992 and in 24.1% of 133 nests in 1993. The main reason behind the high parasitism rate in our study might be attributed to the lack of artificial or natural nests. This campus was built in 1993 and most of the trees were planted during this period. Therefore there are too few natural tree holes as nests for the Silky Starlings, while too many related neighboring species compete for the limited nests, such as Gray Starlings, Collared Owlet (*Glauucidium brodiei*), and even bumblebees (*Vespa manderinia*). Our other 8 artificial nests were just occupied by the above three species.

Our findings confirmed INP in Silky Starlings, but more studies should be done. The following questions could be addressed. Why have starlings evolved the strategy of INP? Could the hosts recognize the parasitical egg, or have the hosts evolved an anti-INP strategy? How about the reproductive success of these parasitical eggs? More artificial nests should be placed and longer monitoring should be done in future studies.

Author contributions

ZL was involved in designing the study, collecting and analyzing the data and writing the manuscript. RW and XJ helped in collecting the data and writing the manuscript. ZZ helped in collecting the data. All authors have read and approved the final submitted version of the manuscript.

Competing Interests

No competing interests were disclosed.

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This paper examines the occurrence of intraspecific brood parasitism (INP) in the Silky Starling, whereby females lay eggs in the nests of other species. INP is widespread in birds (over 200 species) but because it is difficult to detect without detailed field or genetic studies, it is likely to be more common than currently believed. Simply documenting the occurrence of INP in new species, along with some estimate of its frequency, is quite useful because it will eventually allow us to gain a solid understanding of the factors that favor the evolution of INP through comparative analysis.

The study of Silky Starlings by Zhongqui Li and colleagues uses two standard non-genetic techniques for detecting INP: the occurrence of multiple new eggs in 24 hours and morphological differences in eggs. These techniques have been criticized by some, who advocate that genetic techniques are the only proper way to detect INP. However, in my opinion, non-genetic methods can be very powerful when used carefully and properly. Thus, the power of these approaches depends entirely on how they are used—to yield convincing evidence for INP, nests need to be checked daily when parasitism rates are low (one parasitic egg per host nest), nests need to be checked at the same time of day each day, and it can also be helpful to know the time of day when eggs are normally laid. For example, if there is a 28 hour interval between nest checks, it would be possible to have two eggs laid by the same female, if the time of laying was just after the first check and just before the second nest check. In the present study, I am not convinced that the resolution of the data provide clear evidence for INP. I will discuss each of the cases.

Nest box 26. The sequence of events was as follows:

3 eggs on the first nest check

3 new eggs when the nest was checked 4 days later

1 new egg 3 days after that

These observations do not indicate that more than one egg per day was laid, nor do they necessarily indicate a very long pause between egg six and seven. Depending on the time of days when nests were checked and when Silky Starlings lay eggs, it is possible that there were no days

where there were skips in laying (i.e. egg 7 could have been laid 24 hours after egg six) or perhaps there was a skipped day. Even with the later, a skip of one day is not powerful evidence for INP because some birds will occasionally skip a day between laying consecutive eggs. In sum, the evidence for this nest for INP is very weak.

Nest box 27. The evidence from this nest was not laying sequence but egg color. From my own study of American coots, I often found that the last laid egg in a clutch was often paler in color. Sometimes the first laid egg can also be different. I think the pattern of last egg being different is quite widespread. I therefore believe that using egg color alone is very weak evidence for brood parasitism, especially because when dealing with the last egg laid in the clutch. Egg size is also not a very accurate indicator of INP because size often varies within clutches. Egg shape can be a pretty good indicator of INP but the validity of this method needs to be assessed for each species.

One thing that was not clear was whether the authors numbered the first five eggs before the sixth egg was laid or whether you numbered the eggs after all six were laid. In other words, are you certain that the pale egg was not the last egg? Even if you can show this, I would then want to know if the pale egg was the first egg. Regardless, egg color deviation alone is pretty weak evidence for INP.

Box 37. This seems fairly convincing as long as the egg was a new egg and not one that had been buried in the material. Based on the photograph, the egg looks pretty dirty. Are freshly laid eggs often dirty like this? As long as this example involves a new egg then I think this is fairly convincing evidence for INP.

To sum up, I do not find most of the evidence for INP very convincing. I think it is too early to be trying to publish this work—bad data are worse than not having data yet for a species. I encourage the authors to not be in such a rush to get these data out and to invest more time carefully collecting data that will be more convincing. Check nests daily, measure the eggs to assess shape, and determine when the birds lay eggs (video cameras can be useful for this). I think it would be worthwhile checking two recent studies that assess some of the methods for detecting INP, one by John Eadie (*Journal of avian Biology* 41: 163-176; 2010) the other by Hannu Poysa (*J. Avian Biol.* 40: 453-456; 2009). I think you should check these out. I also think my recent review on INP with John Eadie in *Annual Reviews in Ecology Evolution Systematics* 39: 343-363; 2008) would be a useful source for you to consult.

I think the data from this species will eventually be very useful, but the study is not yet at the stage where it can contribute to our understanding of INP.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

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'INP' is not a helpful initialism because the most reasonable alternative to intraspecific nest parasitism is interspecific, which also starts with an 'I'. In the past 'conspecific brood parasitism' or 'conspecific nest parasitism', hence CBP or CNP, has been used. Alternatively, just drop the initialism and use the whole phrase.

The writing is substandard in places, with respect to spelling (e.g., 'its' '), colloquialisms (e.g., 'more and more'), grammar (e.g. 'reasons of', or referring to the starling as 'they'); all of these examples are from the introduction.

The pattern of nest visits to nest #26, combined with the numbers of eggs found, does not establish that more than one egg was laid in a given day, nor that there was a day gap between two eggs. Seven eggs could have been laid consecutively between April 18th-24th. In this case a nest visit on the 20th after laying would have met with 3 eggs; a nest visit on the 24th before laying would have met with 6 eggs; and a nest visit on April 27th at any time would have met with 7 eggs. This is consistent with the report, so no parasitism can be concluded.

There is insufficient evidence of parasitism for nest #27. A paler egg, also failing to hatch, do not constitute conclusive evidence for parasitism.

The egg laid in nest #37 a month after one clutch was laid could have been laid by the same or another female. The gap method of determining parasitism is relevant during the laying and incubation period of a female, not a month later.

So it turns out that all three of the nests where parasitism is claimed are problematic. There is insufficient evidence here for nest parasitism.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

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