Conclusive evidence for conspecific brood parasitism in the blue tit *Cyanistes caeruleus*: a reply to Griffith et al.

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Previously we presented evidence for conspecific brood parasitism (CBP, the phenomenon of females depositing eggs into conspecific nests) in blue tits *Cyanistes caeruleus*. This evidence, from multiple nests, was based on the appearance of two eggs within a nest in a single day, sometimes for multiple consecutive days, one of which was always noticeably different in patterning compared to the rest of the eggs in the clutch (Vedder et al. 2007). However, based on the absence of molecular genetic evidence for CBP in a sample of 427 blue tit broods in Wytham Wood (Oxford, UK), despite the incidence of eggs with aberrant patterning, Griffith et al. (2009) suggested that our findings may have been misinterpreted and that the eggs we identified as parasitic and host may have been laid by the same female. Here we use molecular genetic data from two subsequent seasons, in conjunction with our previous criteria for identifying CBP, to provide unequivocal evidence of CBP in the blue tit and support for the validity of our previous criteria.

In the breeding seasons of 2006 and 2007, we checked all occupied nestboxes in our study population (De Vosbergen, Groningen, the Netherlands) on a daily basis, and identified and numbered all newly laid eggs. Shortly prior to hatching, all eggs were digitally photographed and placed into separate compartments in incubators, such that each hatchling could be assigned to the specific egg it hatched from. Hatchlings were uniquely marked, returned to their original nest, and DNA sampled within 1–6 days of hatching. These nestlings were later genotyped for six microsatellite loci together with their putative parents (see Magrath et al. 2009 for a more detailed description of field and lab procedures).

Examination of our daily laying records (n = 2156 eggs at 188 nests) revealed two clear cases (both in 2006), in different nests, where two eggs appeared in a single day (on the 4th and 10th day of egg laying, respectively). In both cases, a single new egg had been recorded on the day before and after the appearance of the two eggs. Additionally, in both cases, the digital images showed that one of the two eggs was distinctly different in patterning compared to the other eggs in the clutch. In contrast to previous studies on blue tits, we were able to compare the genotypes of the hatchlings from specific eggs with the genotypes of the two provisioning parents. For both nestlings from eggs of distinct appearance, there was a mismatch at four of the six loci with the feeding female. We found no maternal mismatches with any of the other nestlings in the broods. At the nest where the two eggs appeared on the 10th day of laying, the genetic mother of the parasitic egg was identified as a secondary female (of a polygynous male that sired all her eggs). The day after the host nest was parasitized, the parasitic female started laying a clutch of 10 eggs in a nestbox two territories away from the host nest, which contained a nest that was completed weeks before (i.e. possibly deserted by a previous pair). The parasitic egg was also sired by the polygynous male but no other nestlings in the host nest were sired by this male. The other parasitic egg could not be assigned to a female or male that successfully reared a brood in our nestboxes, however this does not exclude the possibility that the genetic mother bred successfully in a natural cavity or outside the study area.

The above scenario suggests a female in need of finding a nest to lay her eggs, but should, nevertheless, be classified as CBP because she increased her reproductive output without providing care for the nestling (Yom-Tov 1980). It also proves that females are capable of laying eggs in nests occupied by conspecifics and thus potentially unconstrained from using CBP as a strategy. Whether the behaviour has evolved as a conditional adaptive strategy, perhaps latently present in all females, or merely reflects an epiphenomenon of females preferring to lay their eggs in a nest instead of dumping them elsewhere, remains open to investigation.

The extremely low frequency of CBP that we report here may suggest it to be an epiphenomenon, but recently a study on an Austrian population of blue tits found a relatively high frequency of nestlings with maternal microsatellite mismatches (15.4% of nests) throughout their study area, after nest sites were limited experimentally in part of the area (Jacot et al. 2009). Hence, although uncommon, CBP in blue tits may be a more frequent phenomenon than previously assumed (Kempenaers et al. 1995, Griffith et al. 2009), and there is need for caution when generalising findings across populations. Since it has
been convincingly shown that aberrant egg patterning alone is an unreliable indicator for CBP (Griffith et al. 2009) and genetic mismatches alone cannot rule out nest take-overs with eggs from the first female becoming mixed with those of the new female, multiple criteria, as presented here, represent the most reliable evidence for the occurrence of CBP in blue tits. Nevertheless, we have shown in the current study that both cases that were identified as CBP using our previous criteria (Vedder et al. 2007) were corroborated by the molecular genetic evidence, suggesting that these two criteria are likely to be quite reliable.

References


