

## Parentage and host preference in the common cuckoo *Cuculus canorus*

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Microsatellite DNA markers were used to investigate parentage relationships in a population of common cuckoo *Cuculus canorus*. Thirty adults and 55 nestlings were genotyped at six loci from blood samples collected over a four-year period. To test whether each cuckoo female specialises in parasitising one single host species (Host Preference Hypothesis), the maternal relationships were used to record each female's host choice. The results supported the Host Preference Hypothesis since no female (N = 3) was recorded to have parasitised more than one of four congeneric host species breeding in the area. In contrast, the males (N = 4) did not show such specialisation since two of them sired offspring reared by different host species.

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The common cuckoo *Cuculus canorus* is an obligate brood parasite. Its eggs have been found in the nests of about 125 host species, but only 10–15 of these species are frequently parasitised (Wyllie 1981, Moksnes et al. 1990, Moksnes and Røskaft 1995, Davies and Brooke 1998, Røskaft and Moksnes 1998). Although the cuckoo as a species parasitises several hosts, individual females seem to specialise on a single host species (Wyllie 1981, Davies and Brooke 1998, Davies 2000, Honza et al. 2002, Vogl et al. 2002). This is in accordance with the Host Preference Hypothesis (Chance 1940, Baker 1942, Lack 1968) which predicts that each cuckoo female chooses to parasitise only one host species which eggs she mimics.

In this study we used data from microsatellite DNA markers to investigate the parentage relationships in a cuckoo population. Thirty adult cuckoos

and 55 offspring were genotyped using six microsatellite markers. The blood samples were collected during a four-year period from a cuckoo population in the Czech Republic. In our study area four main host species breed in close proximity in small-scaled habitats, allowing a single female cuckoo to watch up to four different host species from the same vantage point.

This unique setting allows us to test the prediction of the Host Preference Hypothesis, that an individual cuckoo female should not parasitise more than one host species (e.g. show a preference of 100% for her chosen species) even if several suitable nests of different host species are present (see Vogl et al. 2002). The results from the DNA parentage analyses were used to decide whether all known offspring of the same female were reared by the same host species.

## Methods

The field work was conducted over a four year period near Luzice in southern Moravia, Czech Republic (47° 40' N, 16° 48' E) from May to July in 1995–1998. Four host species were breeding sympatrically in the study area: reed warbler *Acrocephalus scirpaceus*, great reed warbler *A. arundinaceus*, marsh warbler *A. palustris* and sedge warbler *A. schoenobaenus*. Reed warblers and great reed warblers both breed in reed beds *Phragmites australis* close to the water, while sedge warblers and marsh warblers breed in grass and herb vegetation, respectively. The total number of nests detected (N = 1155) was distributed between species as follows: great reed warbler 14.0%, reed warbler 75.4%, marsh warbler 6.1% and sedge warbler 4.5%.

Blood samples were obtained from adult cuckoos caught in mist-nets and nestlings via alar venipuncture. For details about the field work see Øien et al. (1998), Stokke et al. (1999), Moksnes et al. (2000), Honza et al. (2001, 2002), Røskoft et al. (2002a, b) and Vogl et al. (2002).

## DNA analyses

Extraction was made by phenol/water/chloroform (MBI) followed by precipitation in microconcentration tubes such as Micron Centrifugal Filter Devices and Millipore MC Centrifugal Filter Units. Six microsatellite loci with cuckoo specific primers Ccμ 02, Ccμ 60, Ccμ 100, Ccμ 108, Ccμ 119 and Ccμ 137 were used (see Gibbs et al. 1998). The annealing temperatures at PCR amplification for each locus were 58°C, 60°C, 60°C, 55°C, 60°C and 58°C, respectively. For further description of the DNA analyses see Gibbs et al. (1998). Due to poor quality of DNA, 27 of the nestlings (~50%) included in the analysis were successfully genotyped at less than six loci. In the majority of these cases the genotype is missing at one locus only.

## Exclusion analyses

The program Parentage (Chakraborty et al. 1988) was used to conduct the parent exclusion analyses. Microsatellite data from 14 females, 16 males and 55 nestlings were obtained and these were all involved in the exclusion analyses. Parentage by a specific adult was excluded if he/she could not have contributed alleles found at all the six loci with a genotype in a given chick's DNA. A parental "match" was also excluded if a male and female contributed the same allele, but the chick was not homozygous at that locus. When genotypes of several adults matched a given chick's genotype, no adult was assigned as parent of this chick. In addition, if one female and one male matched a

given chick's genotype, but these two adults could not have been the parents of this chick, neither of them were assigned as parent for that given offspring. Finally, if more than one mother and/or more than one father was suggested as parents, but only one of the females and one of the males together matched as parents, the other adults were excluded. All parent assignments were included in the results.

Exclusion probabilities (mean =  $PE_m$  and combined =  $PE_c$ ) were calculated using the equations of Chakraborty et al. (1988); see also Alderson et al. 1999).

## Results

For a total of 26 chicks (47%) one or both parents were identified. Paternity and maternity were both determined for four offspring, whereas maternity only was determined for 11 and paternity only for another 11 offspring. Parentage was not assigned for 29 chicks (53%) of the genotyped individuals. Twenty-four of these did not match genotypically at one or more loci with any of the adults genotyped. Five chicks had multiple parental matches and could not unambiguously be assigned to any parent (see Methods). In addition, 50% of both the genotyped adult males and females (8 out of 16 and 7 out of 14, respectively) were not assigned to have sired any offspring. The mean exclusion probabilities at each locus for each sex ( $PE_m$ ) is given in Table 1 and show variable genotypes in four out of six loci tested ( $PE_m$  – values > 0.6). The combined exclusion probabilities for each single match,  $PE_c$ , ranges from 0.7594 to 1.000, but 65 out of the 78 calculations show values above 0.950, thus giving sufficient power to confidently assign parentage with these genetic markers.

No individual female parasitised more than one host species. Females 95-17 and 97-23 mothered six and two chicks, respectively, all reared by reed warblers. Female 96-01 mothered two chicks reared by great reed warblers (100%). The other four females were assigned to only one chick with each host. Two of the males, however, sired offspring reared by different host species. Male 97-22 sired four offspring, two of which were

Table 1. Mean exclusion probabilities,  $PE_m$  (Chakraborty et al. 1988) for each locus. N = number of offspring genotyped at given locus.

| Microsatellite | Male   | Female | Both   | N  |
|----------------|--------|--------|--------|----|
| Ccμ 02         | 0.6314 | 0.6220 | 0.6238 | 44 |
| Ccμ 60         | 0.6620 | 0.6668 | 0.6593 | 50 |
| Ccμ 119        | 0.7236 | 0.7629 | 0.7377 | 46 |
| Ccμ 137        | 0.6568 | 0.6519 | 0.6492 | 46 |
| Ccμ 100        | 0.2740 | 0.2038 | 0.2386 | 49 |
| Ccμ 108        | 0.0570 | 0.0915 | 0.0609 | 40 |

reared by great reed warblers (50%) and two reared by reed warblers (50%), while male 97-41 mated with female(s) which parasitised two different host species (one reed warbler and one great reed warbler). Males 95-18 and 96-03 sired three and two offspring, respectively, all found in reed warbler nests. The remaining four males were assigned to one chick only.

## Discussion

This is the first study where DNA markers have been used to test the preference of cuckoo females for closely related and sympatrically breeding host species. If a cuckoo female searches randomly for host nests within an area, we would expect the distribution of parasitic eggs for each host species to be similar to the distribution of observed host nests (see methods). However, the recorded preference for three individual females was 100% with regard to either great reed warblers or reed warblers. This confirms previous evidence that host nests are not randomly chosen.

The parentage data from this study indicate that individual females prefer to parasitise a particular host species. Radio tracking studies by Wyllie (1981), Dröscher (1988) and Nakamura and Miyazawa (1997) also suggested that individual cuckoo females show host specialisation. A radio telemetry study conducted simultaneously in our study area revealed, however, that the cuckoo females are not absolutely host specific (Honza et al. 2002, Vogl et al. 2002). Four out of six radio-tracked females, parasitised only one host species, while two distributed their eggs between two different host species' nests. This could simply reflect errors made by the cuckoo, or that nests of the preferred host species were not available. In a study by Marchetti et al. (1998) using microsatellite DNA markers it was showed that only two out of 24 females had offspring in the nest of more than one host species, which suggests a strong female host preference. In a study of cuckoos in Japan and Britain, microsatellite and mitochondrial DNA from cuckoo chicks and adults provided evidence for female-specific host races in both populations (Gibbs et al. 2000). On the other hand, a survey by Moksnes and Røskaft (1995) of European cuckoo eggs held in museum collections revealed that a large proportion of the parasitised host clutches contained cuckoo eggs of other morphs than their corresponding one. They also stressed that egg mimicry seemed less pronounced than what should be expected from strict host preference. A number of alternative hypotheses for the maintenance of gentes have been proposed to explain cuckoo female host choice, namely the Habitat Imprinting Hypothesis (Teuschl et al. 1998), the Nest Site Hypothesis (Wyllie 1981, Moksnes and Røskaft 1987, 1995) and the Natal Philopatry Hypothesis (Davies and Brooke 1991). These hypothe-

ses focus on the mechanisms involved in cuckoo host choice, but they are not mutually exclusive to each other. As yet there is no evidence for host imprinting (Brooke and Davies 1991), but there is correlative and experimental evidence for habitat imprinting as a mechanism to allow females to find the right hosts (Teuschl et al. 1998, Vogl et al. 2002).

Our genetic data and direct field observations (Honza et al. 2002, Vogl et al. 2002) suggest that each of the cuckoo females specialise on one of the four hosts in the study area. Despite this host preference, the cuckoo eggs in the area tend to show a general appearance between females. This was tested by Edvardsen et al. (2001) who found that the host preference documented in the area was not manifested in clearly differentiated and mimetic host egg morphs.

The fact that the males mated with females that laid eggs in the nests of different hosts gives further support to the view that host preference is maintained by the females. We found two males (out of four with multiple offspring) to be the father of chicks reared by two different species, which was also shown by Marchetti et al. (1998). These data are consistent with the Host Preference Hypothesis and may also indicate that egg morphology is a maternally inherited trait (Gibbs et al. 2000).

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