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Host-Race Formation in the Common Cuckoo

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The exploitation of a new host by a parasite may result in host-race formation or speciation. A brood parasitic bird, the common cuckoo, is divided into host races, each characterized by egg mimicry of different host species. Microsatellite DNA markers were used to examine cuckoo mating patterns and host usage in an area where a new host has been recently colonized. Female cuckoos show strong host preferences, but individual males mate with females that lay in the nests of different hosts. Female host specialization may lead to the evolution of sex-linked traits such as egg mimicry, even though gene flow through the male line prevents completion of the speciation process.

polygamous mating in a different cuckoo species (14). There are no demonstrable sex differences in the proportion of individuals that had multiple mates [within years, individuals being included only once, 7 out of 15 males and 3 out of 18 females had more than one mate (Fisher exact test, $P = 0.13$)].

To determine patterns of host use (specialist or generalist), we examined whether individual cuckoos that produced more than one offspring had offspring in the nests of more than one host species. Among males and females that were parents to two or more chicks, there were similar numbers of chicks (Fig. 2) ($n = 92$ chicks in males and 93 in females), but they were distributed differently. Seven of the 19 males but only 2 of the 24 females had offspring in nests of more than one host (Fisher exact test, $P < 0.05$). Thus, male and female cuckoos differ in host specificity, and males are more generalized in

Avian brood parasites lay their eggs in the nests of other bird species and raise none of their own young, often greatly reducing the reproductive success of their hosts. Parasitism typically results in the evolution of host discrimination and rejection of unlike eggs which, in turn, selects for improved egg mimicry by the parasite (1, 2). The common cuckoo, *Cuculus canorus*, is divided into different host races, or gentes, each characterized by egg mimicry (1, 2) but showing no differentiation in mitochondrial or nuclear DNA markers (3), which implies rapid host-race evolution. The first step in host-race formation is the exploitation of a new host, followed by adaptation to that host. In central Japan, cuckoos began to exploit the azure-winged magpie (*Cyanopica cyana*) 20 to 30 years ago as a result of expansion of the magpie's breeding range (4–6). Cuckoos also utilize two other hosts in the area: the great reed warbler (*Acrocephalus arundinaceus*) and the bull-headed shrike (*Lanius bucephalus*) (4–7). To investigate the process of host-race formation in this cuckoo

population, we have studied the mating system and patterns of individual host use (8).

To date, the mating system of the cuckoo has been impossible to determine directly because parents do not attend nests and are difficult to catch and mark. We used eight cuckoo-specific microsatellite DNA markers (9) to establish the parentage of 136 chicks collected from known host nests. From these data, we determined cuckoo mating patterns (monogamous, polygamous, or promiscuous) and individual differences in host use (specialist or generalist). We captured and genotyped 83 adult males and 79 adult females. Adult cuckoos were sexed on the basis of behavioral observations (4–6) or by use of a DNA-based sex identification test (10). Examination of variation at the different microsatellite loci enabled us to assign at least one sampled adult as the parent of 84% (114 out of 136) of nestlings sampled with a high degree of confidence ($P < 0.01$) (11). We assigned parentage only if parent or parents and offspring matched unambiguously at eight out of eight microsatellite loci (12).

To determine cuckoo mating patterns, we examined whether adults that produced more than one offspring also mated with more than one individual. The mating system is polygamous, with substantial numbers of both male and female cuckoos having multiple partners (Fig. 1). Previous studies implied that common cuckoos mate multiply (1, 5, 13), and a recent molecular study has shown

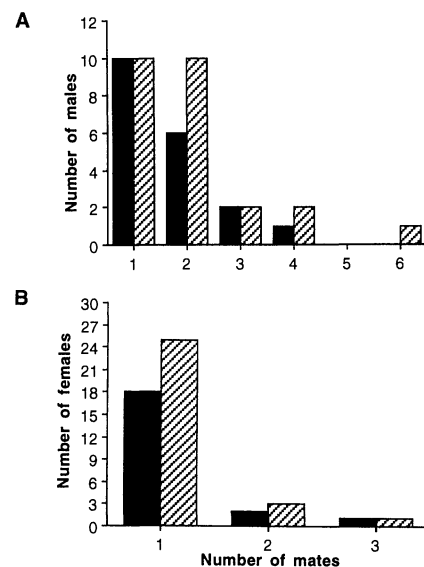


Fig. 1. Number of mates per individual male (A) and female (B) during 1991–1994. Only individuals producing more than one offspring are included. Individuals are tallied across all years combined (hatched bars) as well as separately for each season (black bars). In the latter case, if an individual had two or more offspring in more than 1 year, it was scored separately for each year. Total sample sizes are as follows: males within years, 19; males across years, 25; females within years, 21; females across years, 29.

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their host use than females.

Individuals that mated multiply had partners drawn from both their own and different host races, indicating that a high level of autosomal gene flow is occurring between host races [a conservative estimate of gene flow is about 9% between gentes through the male line alone (15)]. The high gene flow arises because there is movement through the study area (5) and because a few males gain many of the matings (as suggested by Fig. 2). For example, almost two-thirds of males (65%) and half of females (50%) sampled were not assigned any offspring.

Colonization by Japanese cuckoos of at least one host species (the azure-winged magpie) has occurred very recently relative to host colonization by European cuckoo gentes (4, 16). Unlike the Japanese population (6), different European gentes exhibit marked differences in egg characteristics that often strongly mimic those of their particular hosts (2) but show no detectable differentiation in mitochondrial or microsatellite DNA markers (3). Females from a particular gens lay eggs in the nests of hosts other than their own 5 to 10% of the time in Europe (17), which is similar to the levels documented here for Japan.

Because selection pressure on cuckoos to evolve mimetic eggs is imposed directly by the host, behavioral specialization on a particular host is required for the evolution of egg mimicry. An imprinting process by which females return to the same host in which they were raised can explain the similar degrees of host specificity observed in old

[European (17)] and recent [Japanese (4)] gentes as well as the rapid evolution of host specificity observed in Japanese cuckoos. Females in Japan exhibit behavioral host specialization, but this is not accompanied by much differentiation in egg characteristics. However, cuckoo egg types do vary somewhat among the different hosts, and nests of the recently parasitized azure-winged magpie contain eggs resembling the types of each of the other two gentes (6). This suggests that multiple females began to parasitize magpies as their breeding range expanded (6). Such behavioral host specialization by females may eventually lead to the evolution of female-specific sex-linked egg mimicry (18).

The use of hosts by cuckoos differs from many arthropod parasite systems in that exploitation of hosts affects the fecundity but not the survival of the parasite (19). Because male fecundity is generally increased by multiple mating but female fecundity is increased by survival of her offspring, host race exploitation creates a conflict between the sexes. We suggest that the resolution of this conflict in the common cuckoo has resulted in the evolution of egg mimicry (18), but high levels of male gene flow prevent completion of the speciation process. Among avian brood parasites this is not always the case (20, 21). In African parasitic finches, both male and female chicks learn the songs of their hosts, and song forms the basis of mate recognition (20). Some African cuckoos are also divided into species rather than host races (21). In both of these examples there has been a cessation of gene flow through the male and the female line, presumably attributable to behavioral factors and geographical separation of potential parasites.

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8. The Chikuma River study site in Nagano, central Japan, encompasses an area of approximately 7 km² and is the location of a long-term population study of cuckoos by Nakamura and co-workers (4–6). In each year, between 22 and 28 adult male and 21 to 26 adult female cuckoos were resident at this site. Nests of the three main host species (approximate numbers of magpie, warbler, and shrike nests found per year were 25, 180, and 40, respectively) were located in the reed beds and other areas surrounding the river and were regularly checked for cuckoo eggs. Using mist nets, we captured, measured, and collected blood samples for microsatellite DNA analysis from an estimated 75 to 90% of all resident male cuckoos and 62 to 73% of all resident females (4, 5). High rates of parasitism on host nests (13 to 57%) meant that we were able to collect blood samples from 16 to 56 cuckoo chicks in a given year. All blood samples (50 to 100 μ l, preserved

in lysis buffer) were taken from individual cuckoos throughout the entire period of study. To collect blood samples from nestling cuckoos, we removed cuckoo eggs from individual host nests and incubated them in the laboratory. We collected blood samples from nestling cuckoos after hatching and then returned the nestling to its original nest.

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11. We used eight highly variable, cuckoo-specific, microsatellite DNA markers (9) to determine the parentage of cuckoo chicks and hence to determine the mating patterns (monogamous, polygamous, or promiscuous) and individual differences in host use (specialist or generalist) of 162 adult cuckoos genotyped. The high level of variation at the different microsatellite loci (6 to 21 alleles per locus) enabled us to assign parentage through exclusion analyses with a high degree of confidence [the mean probability of exclusion, based on eight loci of a randomly chosen nonparental adult, is 0.992 (9)].
12. Of the 136 nestlings sampled, we were able to assign both parents to 84 nestlings, the male parent alone to 16, and the female parent alone to 14. Cases where more than one potential parent (probably because they were close relatives) matched a chick at all eight loci ($n = 8$) were excluded from the data set. None of the adults sampled matched 14 chicks. Fifty-four males and 39 females were assigned no offspring.
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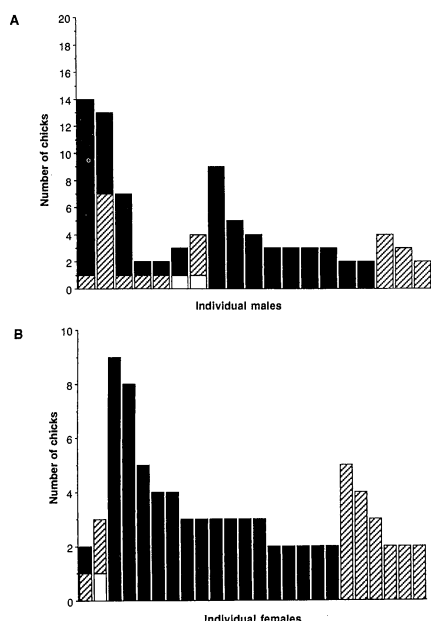


Fig. 2. Host use by individual males (A) and females (B) that produced more than one offspring during 1991–1994. An additional 10 males and 16 females were assigned a single nestling. Hosts are the great reed warbler (black bars), azure-winged magpie (hatched bars), and bull-headed shrike (white bars).