



Comparative Population Structure and Gene Flow of a Brood Parasite, The Great Spotted Cuckoo (*Clamator glandarius*), and Its Primary Host, the Magpie (*Pica pica*)

J. G. Martinez; J. J. Soler; M. Soler; A. P. Moller; T. Burke

Evolution, Vol. 53, No. 1 (Feb., 1999), 269-278.

Stable URL:

<http://links.jstor.org/sici?sici=0014-3820%28199902%2953%3A1%3C269%3ACPSAGF%3E2.0.CO%3B2-2>

Evolution is currently published by Society for the Study of Evolution.

Your use of the JSTOR archive indicates your acceptance of JSTOR's Terms and Conditions of Use, available at <http://www.jstor.org/about/terms.html>. JSTOR's Terms and Conditions of Use provides, in part, that unless you have obtained prior permission, you may not download an entire issue of a journal or multiple copies of articles, and you may use content in the JSTOR archive only for your personal, non-commercial use.

Please contact the publisher regarding any further use of this work. Publisher contact information may be obtained at <http://www.jstor.org/journals/ssevol.html>.

Each copy of any part of a JSTOR transmission must contain the same copyright notice that appears on the screen or printed page of such transmission.

JSTOR is an independent not-for-profit organization dedicated to creating and preserving a digital archive of scholarly journals. For more information regarding JSTOR, please contact support@jstor.org.

COMPARATIVE POPULATION STRUCTURE AND GENE FLOW OF A BROOD PARASITE, THE GREAT SPOTTED CUCKOO (*CLAMATOR GLANDARIUS*), AND ITS PRIMARY HOST, THE MAGPIE (*PICA PICA*)

J. G. MARTINEZ,^{1,2,3} J. J. SOLER,¹ M. SOLER,¹ A. P. MØLLER,⁴ AND T. BURKE²

¹Departamento de Biología Animal y Ecología, Facultad de Ciencias, Universidad de Granada, E-18071 Granada, Spain

²Department of Animal and Plant Sciences, The University of Sheffield, Sheffield S10 2TN, United Kingdom

⁴Laboratoire d'Ecologie, CNRS URA 258, Université Pierre et Marie Curie, Bât. A, 7ème étage, 7 quai St. Bernard, case 237, F-75252 Paris Cedex 5, France

Abstract.—The amount of gene flow is an important determinant of population structure and therefore of central importance for understanding coevolutionary processes. We used microsatellite markers to estimate population structure and gene flow rates of the great spotted cuckoo (*Clamator glandarius*) and its main host in Europe, the magpie (*Pica pica*), in a number of populations (seven and 15, respectively) across their distribution range in Europe. The genetic analysis shows that there exists a pattern of isolation by distance in both species, although the cuckoo data are only indicative due to a small sample size. Gene flow seems to be extensive between nearby populations, higher for magpies than cuckoos, and especially high for magpie populations within the area of distribution of the great spotted cuckoo. There is no correlation between genetic distances between magpie populations and genetic distances between cuckoo populations. We discuss the implications of extensive gene flow between magpie populations in sympatry with cuckoos for the population dynamics of hosts, in particular for the occurrence of egg rejection behavior in host populations and how the different rates of migration for both species can affect the dynamics of coevolutionary processes.

Key words.—Brood parasitism, coevolution, gene flow, host-parasite interactions, migration.

Received November 18, 1997. Accepted September 10, 1998.

The extent of gene flow between populations and the degree of genetic structure of a particular species are considered important factors in evolutionary processes in general (Slatkin 1987; Avise 1994) and coevolution in particular (Thompson 1994). Random genetic drift may lead to genetic differentiation and isolation of populations in the absence of gene flow, whereas high dispersal rate and gene flow between populations may act as a homogenizing force maintaining genetic cohesion and similarity among populations (Slatkin 1987).

In parasites, the lack of gene flow between populations might improve parasitic adaptations and lead to host-race formation (Price 1980). Price (1980) predicted that population structure of parasitic species would be that of a species subdivided into small populations with restricted gene flow between local demes. In the same way, gene flow between host populations may help spread adaptations against parasitism, and the ratio of the host/parasite migration rate (and therefore gene flow) is predicted to affect the probability of local adaptation for both species and the dynamics of the coevolutionary process (Gandon et al. 1996).

Avian brood parasitism is an interspecific interaction that has been proposed as a model system for the study of adaptation and coevolution (Rothstein 1990). Brood parasites depend on their hosts for reproduction, but simultaneously reduce the breeding success of hosts, thus generating strong selective pressures on the parasite to use the reproductive effort of hosts to their own advantage and on the host to counter parasitic adaptations and successfully avoid parasitism (Rothstein 1990).

The evolution of host defenses, especially the recognition and rejection of parasitic eggs, has been studied extensively

during recent years using different experimental and theoretical approaches (Rothstein 1975; Kelly 1987; Davies and Brooke 1989; Soler and Møller 1990; Moksnes et al. 1991; Briskie et al. 1992; Takasu et al. 1993). These studies indicate several aspects of the host-parasite relationship that are important to understanding the evolution of adaptations in both parties, such as the effect of parasitism on host reproductive success, population density of hosts and parasites, and parasitism rate. A few experimental studies have shown that responses against parasitism differ among populations of the same host species, depending on the duration of sympatry between host and parasite (Soler and Møller 1990; Briskie et al. 1992), and that populations not currently parasitized show rejection behavior against parasitic eggs (Davies and Brooke 1989; Briskie et al. 1992). The persistence of rejection behavior in unparasitized populations and the rapid increases of rejection rate in parasitized ones have sometimes been explained as the result of flow of rejecter genes from sympatric populations with a long history of parasitism to ones with a more recent relationship or to allopatric areas (Soler and Møller 1990, Briskie et al. 1992, Lotem and Rothstein 1995).

However, restricted gene flow must play an important role in the formation and maintenance of brood parasite host-races, like the ones described for the common cuckoo (*Cuculus canorus*) (Gibbs et al. 1996), and extensive gene flow between parasite populations will prevent the formation of host-races (Gibbs et al. 1997). Thus, the study of population structure and gene flow between populations of hosts and parasites is of great importance for understanding the dynamics of coevolution in brood-parasitic interactions.

However, no study has determined the genetic structure of brood parasite and host populations. Here we compare the population structure and gene flow rates of the magpie (*Pica*

³ Present address: Department of Animal and Plant Sciences, The University of Sheffield, Sheffield S10 2TN, United Kingdom; E-mail: j.g.martinez@sheffield.ac.uk.

pica) and its brood parasite, the great spotted cuckoo (*Clamator glandarius*), using microsatellite markers (Bruford and Wayne 1993; Jarne and Lagoda 1996). Magpies are the main European host of great spotted cuckoos (Cramp 1985; Soler 1990), and their interaction constitutes an example of co-evolutionary processes (Soler and Møller 1990, Soler et al. 1994). Gene flow has been used to explain some details of these interactions (for example Soler and Møller 1990, Lotem and Rothstein 1995), but this is the first study that measures the amount of gene flow and population structure between populations of magpies and great spotted cuckoos.

METHODS

Study Species

The host-parasite system of magpies and great spotted cuckoos and their interactions have been described elsewhere (e.g., Soler 1990, Soler et al. 1994). Briefly, magpies are members of the corvid family and are widespread in the Palearctic. They are territorial, sedentary, and long lived, with a well-known biology (Birkhead 1991). Great spotted cuckoos migrate from Africa to breed in Southern Europe. They are widespread in the Iberian Peninsula and reach the south of France at its northern limit (Cramp 1985). Cuckoos severely depress the breeding success of magpies, with parasitized magpie nests fledging few or no young (Soler et al. 1996).

Sampling and Study Areas

Samples were collected from several populations of the magpie's European range during 1993, 1994, and 1995. A number of magpie nests in each population were studied as part of a more extensive project. In every successful nest, a drop of blood (stored in ethanol) was taken from one magpie and/or cuckoo chick. A total of 15 populations were sampled (Fig. 1), nine within the limits of great spotted cuckoo distribution and six outside; we found parasitized nests only in seven of the nine populations within the cuckoo distribution area.

Microsatellite Isolation and Typing

DNA was isolated from blood using a standard phenol-chloroform extraction protocol (Bruford et al. 1992). Microsatellite loci were isolated in magpies and great spotted cuckoos following an enrichment procedure described by Armour et al. (1994); for details on microsatellite isolation and genotyping procedures see Martinez et al. (1998). We designed primers for three polymorphic loci of the magpie (Table 1) and for seven of the great spotted cuckoo (Martinez et al. 1998).

We genotyped a total of 173 magpies, six to 22 from each population, and a total of 27 cuckoos, all the chicks found in the seven populations where parasitized nests occurred, with the exception of the Guadix population from which we chose five random chicks. Table 1 summarizes information on magpie microsatellite loci, primer sequences, heterozygosity, and number of alleles; the information for the great spotted cuckoo loci can be found in Martinez et al. (1998). All loci were in Hardy-Weinberg (H-W) equilibrium, show-

ing nonsignificant deviation from H-W equilibrium (Markov chain exact test, $P > 0.05$) for all combinations of populations and loci in both species, except for the Frenuesse magpie population for loci Ppi3, which showed significant heterozygote deficiency (Rousset and Raymond 1995, U -test, $P = 0.03$). However, a global exact test (Rousset and Raymond 1995, Fisher test) for all populations and loci was nonsignificant, both for magpies ($\chi^2 = 86.8$, $df = 82$, $P = 0.34$) and cuckoos ($\chi^2 = 26.0$, $df = 26$, $P = 0.46$). We also tested the loci for linkage disequilibrium using a Fisher's exact test (Raymond and Rousset 1995) and found no evidence of linkage for all combinations of loci in both species (all tests nonsignificant, $P > 0.05$).

Statistical Methods

Calculations on allele frequencies, estimations and tests were carried out using the computer programs GENEPOP (ver. 3.1, Raymond and Rousset 1995) and FSTAT (ver. 1.2, Goudet 1995). Expected heterozygosity for each population/locus was calculated using Levene's correction.

We estimated population structure and gene flow from the genotypic data using different approaches. Genetic differentiation was quantified using Wright's F -statistics (Wright 1951) following the variance method developed by Weir and Cockerham (1984), which incorporates sample size and population numbers. The estimation of F -statistics is a standard practice, and the discussion of their properties has been based on the infinite allele model (Rousset 1996). However, microsatellites might evolve following a stepwise mutation process (Slatkin 1995). Although the exact nature of the mutation process is still not clear (Deka et al. 1995), whatever mutation model is appropriate for microsatellites, F_{ST} can be used in cases where the time scale of interest is sufficiently short for mutation to play a little role (Slatkin 1995). Population surveys in different species have shown a high efficiency of microsatellites in estimating population differentiation and genetic distances using F_{ST} -based approaches (Bowcock et al. 1993; Estoup et al. 1996; Perez-Lezaun et al. 1997). We assessed significant deviations from zero of F -statistics using permutation tests and obtained their standard deviations by jackknifing over populations (Goudet 1995; Chapuisat et al. 1997; see Table 3).

Gene flow was estimated as the product Nm , that is, the number of individuals or gametes that move from a particular population and is replaced from a source population in each generation (Slatkin 1985; Slatkin and Barton 1989), with N being population size and m the probability that each gamete in a population is an immigrant. There are several ways to estimate Nm (Slatkin and Barton 1989). Here we use two: the estimate of Nm from the F_{ST} -values (Wright 1951), $Nm = (1 - F_{ST})/4F_{ST}$, and the private alleles method (Slatkin 1985). These two measures are considered to provide reasonably accurate estimates of Nm under a variety of conditions, although F_{ST} tends to overestimate Nm for high rates of gene flow (Slatkin and Barton 1989).

We investigated the degree of isolation by distance by correlating $F_{ST}/(1 - F_{ST})$ estimates between pairs of populations and their geographical distances (Rousset 1997). The signif-

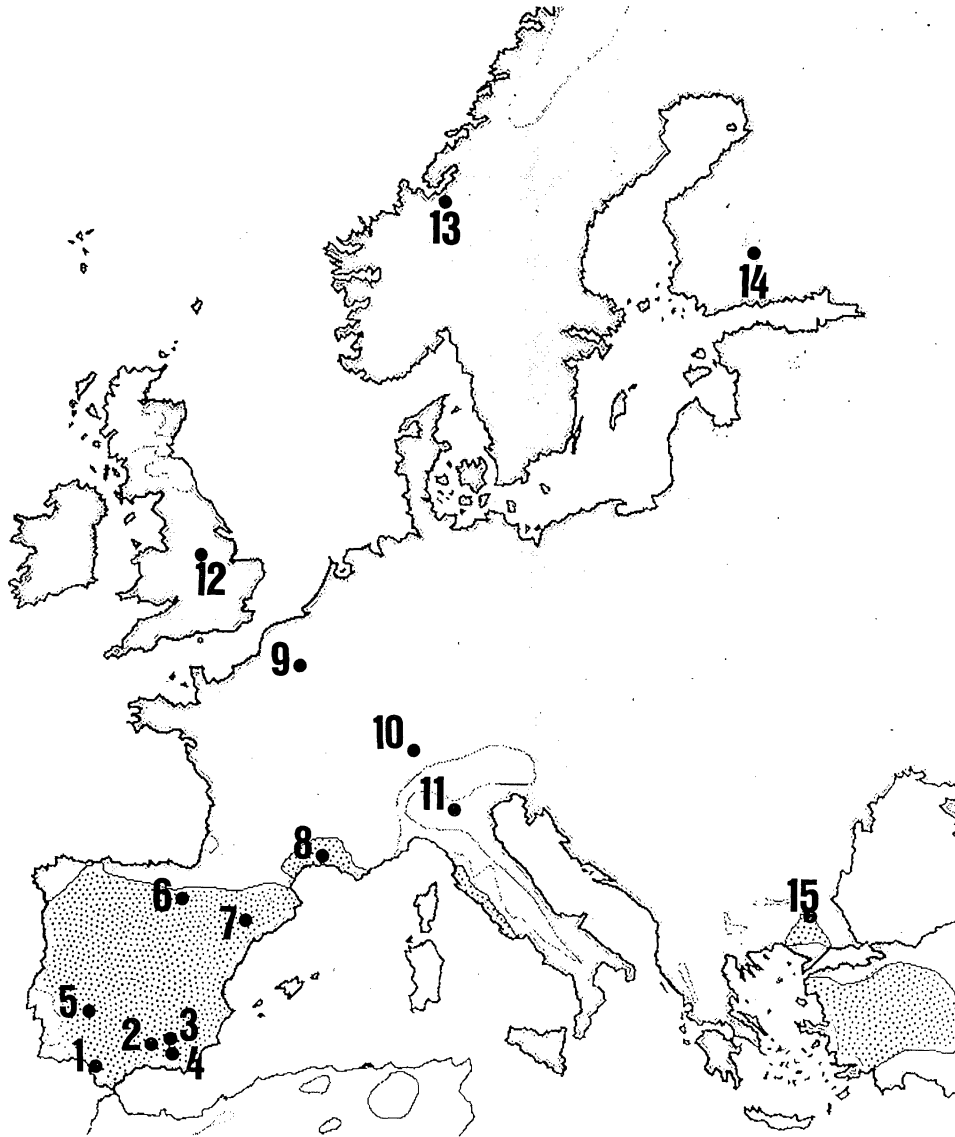


FIG. 1. Populations sampled (1–7 Spanish populations): Doñana (1), Santa Fe (2), Guadix (3), Laujar (4), Badajoz (5), Calahorra, Logroño (6), Torres del Segre, Lleida (7), La Camargue (France, 8), Frenuesse (France, 9), Bern (Switzerland, 10), Milan (Italy, 11), Sheffield (England, 12), Trondheim (Norway, 13), Jyvaskyla (Finland, 14), and Elhovo (Bulgaria, 15). The gray area corresponds to the distribution range of magpies and the dotted area to the distribution range of great spotted cuckoos. Magpies were sampled in all populations, whereas great spotted cuckoos were sampled in populations 1, 2, 3, 4, 5, 7, and 8. Adapted from Cramp (1985, 1994).

ificance of the correlation coefficient was assessed using Mantel tests (Mantel 1967) as implemented in GENETPOP.

We carried out analyses of population structure, gene flow, and isolation by distance for all host populations first, but

also for the subset of populations in sympatry with great spotted cuckoos and the ones in allopatry independently to check for possible effects of cuckoo presence on the population structure of magpies.

TABLE 1. Variability of the three microsatellite loci of magpies. *n*, total number of alleles; mean *n*, mean number of alleles per population (SD in parentheses); mean *H_o*, mean value of observed heterozygosity per population (SD in parentheses).

Locus	Primer sequence	<i>n</i>	Mean <i>n</i>	Mean <i>H_o</i>
Ppi	Forward: TTATCCACATCCACGCAGTC Reverse: GAAAGGGCTGCAATGATTTC	11	6.00 (2.04)	0.80 (0.10)
Ppi 2	Forward: CACAGACCATTGGAAGCAGA Reverse: GCTCCGATGGTGAATGAAGT	16	7.80 (2.75)	0.85 (0.11)
Ppi 3	Forward: CCAAACACAAGTACAGCTGCA Reverse: TTTTGCTGGGAGAGGACG	14	6.27 (1.66)	0.72 (0.19)

TABLE 2. Mean (SD) genetic variability measures for magpie populations and for populations in sympatry and allopatry with the great spotted cuckoo.

Population	Mean sample size per locus	Mean number of alleles per locus	Mean number of private alleles per locus	Mean frequency of private alleles per locus	Heterozygosity	
					Observed	Expected
Doñana	18.7	8.67 (2.08)	0	0	0.84 (0.11)	0.83 (0.06)
Santa Fe	6.0	4.67 (1.15)	0	0	0.78 (0.09)	0.77 (0.07)
Guadix	17.0	8.33 (2.15)	0	0	0.80 (0.07)	0.84 (0.05)
Laujar	6.0	6.00 (2.64)	0	0	0.77 (0.07)	0.85 (0.08)
Badajoz	22.0	10.00 (2.64)	0.33	0.008	0.92 (0.05)	0.86 (0.06)
Logroño	12.3	9.00 (1.00)	0	0	0.78 (0.05)	0.87 (0.05)
Lleida	6.3	6.33 (0.58)	0	0	0.74 (0.31)	0.87 (0.02)
La Camargue	13.3	8.33 (2.31)	0	0	0.82 (0.08)	0.86 (0.06)
Frenuesse	9.0	5.33 (0.58)	0	0	0.67 (0.39)	0.76 (0.06)
Bern	6.3	5.67 (0.58)	0	0	0.84 (0.02)	0.80 (0.07)
Milan	9.3	6.67 (2.08)	0	0	0.85 (0.13)	0.81 (0.03)
Sheffield	6.3	4.33 (2.08)	0.67	0.046	0.79 (0.18)	0.73 (0.17)
Trondheim	11.3	4.67 (0.58)	0	0	0.77 (0.14)	0.76 (0.05)
Jyväskylä	7.7	6.00 (1.73)	0	0	0.70 (0.06)	0.75 (0.10)
Elhovo	6.0	6.33 (2.31)	0.33	0.028	0.78 (0.19)	0.80 (0.14)
Sympatric populations	11.9	7.52 (2.39)	2.33	0.053	0.80 (0.13)	0.84 (0.07)
Allopatric populations	8.3	5.44 (1.46)	0.43	0.127	0.77 (0.18)	0.77 (0.08)

Finally, we examined the relationship between host and parasite population structure. We estimated genetic distances between pairs of populations of both magpies and great spotted cuckoos using the chord distance (Cavalli-Sforza and Edwards 1967) obtained with the computer program GENDIST (PHYLIP, Felsenstein 1993). We then calculated the correlation between the pairwise distances between cuckoo populations and the distances between magpie populations for the seven populations where we sampled both magpies and cuckoos. The significance of the correlation coefficient was also assessed using Mantel tests.

RESULTS

Genetic Variation

Magpie Genetic Variability

Table 2 shows several measures of genetic variability for the different magpie populations. There were no significant differences among populations in the mean number of alleles per locus (Kruskal-Wallis test, $H_{9,30} = 16.34$, $P = 0.06$), mean observed heterozygosity ($H_{9,30} = 6.62$, $P = 0.67$) and mean expected heterozygosity ($H_{9,30} = 7.67$, $P = 0.57$), despite sample sizes differing among sample units ($H_{9,30} = 27.15$, $P = 0.001$). There was a significant correlation between mean number of alleles per locus and sample size ($r_s = 0.68$, $P = 0.005$, $n = 15$). When comparing the subset of populations in sympatry with the ones in allopatry with the great spotted cuckoo sample size did not differ (Mann-Whitney U -test = 184.5, $P = 0.17$), but the mean number of alleles per locus and the mean expected heterozygosity were significantly higher in sympatric populations ($U = 118.5$, $P = 0.0035$ and $U = 116$, $P = 0.003$, respectively), although the observed heterozygosity showed no significant differences ($U = 214$, $P = 0.5$). Private alleles were present only in three

populations, Badajoz and Elhovo among the sympatric populations and Sheffield among the allopatric populations. Although the number of private alleles was higher in the sympatric populations, their frequency was smaller than the mean frequency of private alleles within the allopatric populations (Table 2).

Great Spotted Cuckoo Genetic Variability

Sample size and number of alleles per locus were significantly different among populations (Table 3; Kruskal-Wallis test, $H_{6,48} = 40.37$, $P < 0.0001$ and $H_{6,49} = 14.72$, $P = 0.02$, respectively), but there were no differences between populations in observed heterozygosity (Table 3; $H_{6,45} = 8.09$, $P = 0.23$) or expected heterozygosity ($H_{6,45} = 5.05$, $P = 0.53$). There was a significant correlation between mean number of alleles per locus and sample size ($r_s = 0.88$, $P = 0.0008$, $n = 7$). The mean frequency of private alleles was 0.20. Private alleles were present in all cuckoo populations (Table 3).

Population Structure

Magpie Population Structure

F_{ST} and F_{IT} were positive and significantly different from zero for each of the three loci and over all loci (Table 4). This confirms that populations sampled were genetically differentiated and that nonrandom mating occurs at the population level. F_{IS} was not significantly different from zero, which is in concordance with the lack of deviation from H-W equilibrium and suggests random mating within populations. In other words, the values of F -statistics confirm population subdivision.

We plotted $F_{ST}/(1 - F_{ST})$ estimates against geographical distances to investigate isolation by distance (Fig. 2). We detected population viscosity for the whole range of popu-

TABLE 3. Mean (SD) genetic variability measures for cuckoo populations.

Population	Mean sample size per locus	Mean number of alleles per locus	Mean number of private alleles per locus	Mean frequency of private alleles per locus	Heterozygosity	
					Observed	Expected
Doñana	4.7 (0.7)	4.2 (1.6)	0.14	0.01	85.7 (15.1)	74.9 (15.4)
Santa Fe	4.7 (0.7)	3.1 (1.3)	0.14	0.05	53.2 (21.7)	65.1 (24.3)
Gaudix	5.0 (0.0)	4.0 (1.6)	0.28	0.03	71.4 (27.9)	66.3 (24.7)
Laujar	3.8 (0.4)	3.4 (0.9)	0.43	0.07	73.8 (25.2)	68.2 (20.6)
Badajoz	2.0 (0.0)	2.1 (1.2)	0.14	0.04	75.0 (28.9)	79.2 (21.0)
Lleida	3.0 (0.0)	2.7 (0.9)	0.14	0.02	80.9 (17.8)	63.8 (13.8)
La Camargue	2.8 (0.4)	2.2 (0.5)	0.14	0.07	66.7 (27.2)	58.6 (16.1)

lations studied because genetic differentiation between pairs of populations was significantly positively correlated with distance (Mantel test, 500 permutations, $r_s = 0.58$, $n = 105$, $P < 0.001$). This means that although populations are genetically distinct, their degree of differentiation depends on their geographic location; whereas populations very far from each other are quite different in their genetic composition, those close to each other are more similar.

Populations in sympatry with cuckoos show a slight, non-significant pattern of isolation by distance (Mantel test, 500 permutations, $r_s = 0.39$, $n = 36$, $P = 0.082$; Fig. 3, all dots). The slight positive correlation is due to the inclusion in the analysis of the remote Bulgarian population because it disappears when that population is not considered (Mantel test, 500 permutations, $r_s = 0.015$, $n = 28$, $P = 0.44$; Fig. 3, solid dots). For the allopatric populations there also exists a slight, nonsignificant positive correlation (Mantel test, 500 permutations, $r_s = 0.44$, $n = 15$, $P = 0.074$; Fig. 4, all dots) that does not change if we exclude the more distant Scandinavian populations (Mantel test, 500 permutations, $r_s = 0.66$, $n = 6$, $P = 0.068$). We conclude that the degree of isolation by distance is weaker when considering smaller spatial scales, although it seems to be higher in allopatric than sympatric populations, as shown by F_{ST} -values being higher for allopatric than sympatric populations (Table 5).

TABLE 4. F -statistics (SD) for magpie and cuckoo populations and probability values for their deviation from zero (permutation test; Chapuisat et al. 1977).

Locus	F_{ST} (SD)	F_{IS} (SD)	F_{IT} (SD)
Magpie			
Ppi 1	0.088 (0.023)**	-0.027 (0.033)	0.064 (0.042)*
Ppi 2	0.056 (0.016)**	0.007 (0.032)	0.062 (0.026)**
Ppi 3	0.076 (0.027)**	0.053 (0.059)	0.125 (0.062)**
All loci	0.075 (0.009)**	0.012 (0.023)	0.086 (0.022)**
Great spotted cuckoo			
Cg11	0.204 (0.106)**	-0.200 (0.113)	0.043 (0.146)
Cg12	-0.033 (0.046)	-0.025 (0.106)	-0.063 (0.084)
Cg13	0.104 (0.081)**	-0.110 (0.106)	0.001 (0.092)
Cg14	0.025 (0.045)	0.022 (0.082)	0.049 (0.111)
Cg15	0.264 (0.134)**	-0.194 (0.088)	0.125 (0.193)
Cg16	0.255 (0.215)**	-0.200 (0.065)	0.098 (0.227)
Cg17	0.032 (0.024)	-0.028 (0.127)	0.004 (0.112)
All loci	0.104 (0.041)**	-0.080 (0.035)	0.031 (0.022)

* $P < 0.05$, ** $P < 0.01$.

Great Spotted Cuckoo Population Structure

F_{ST} was significantly different from zero when calculated over all loci in the great spotted cuckoo populations (Table 4), although it did not differ from zero for three of the seven loci when calculated for each locus independently. F_{IS} was not significantly different from zero, in concordance with the lack of deviation from H-W equilibrium. There was a strong pattern of isolation by distance in cuckoo populations, because genetic differentiation was correlated with distance (Mantel test, 500 permutations, $r_s = 0.67$, $n = 21$, $P = 0.006$, Fig. 5).

Gene Flow

Magpie Gene Flow

The two types of estimates of Nm are well above or very close to one (Table 6). As expected from theoretical models (Slatkin and Barton 1989), Nm is higher when calculated from F_{ST} -values than when calculated using private alleles. However, in both cases Nm seems to be greater in sympatric than allopatric populations, considering all populations or excluding Bulgarian and Scandinavian populations (Table 6). The more restricted gene flow between allopatric populations might be due to larger geographic distances between these compared to sympatric populations. To test whether genetic differentiation is significantly lower between sympatric pop-

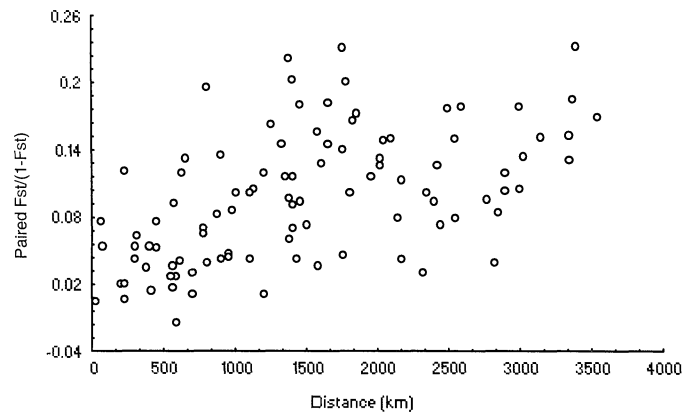


FIG. 2. Correlation between paired $F_{ST}/(1 - F_{ST})$ values and geographical distances between populations of magpies $r_s = 0.58$, $n = 105$, $P < 0.001$.

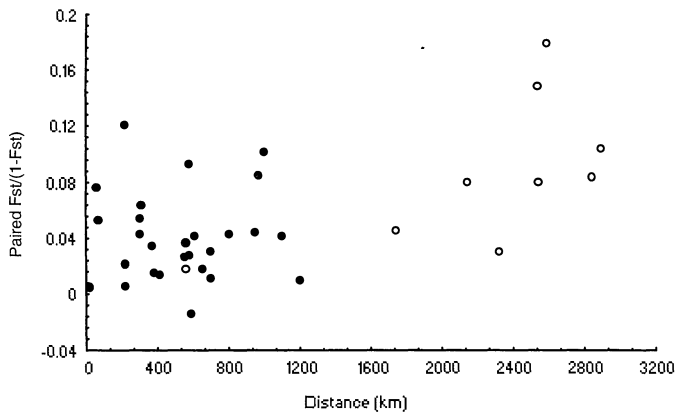


FIG. 3. Correlation between paired $F_{ST}/(1 - F_{ST})$ values and geographical distances between magpie populations within the distribution range of the great spotted cuckoo. Black dots are paired values excluding the Bulgarian population and white dots are paired values of the Bulgarian population against the rest. Correlations are as follow: $r_s = 0.39$, $n = 85$, $P = 0.082$ for the whole dataset and $r_s = 0.015$, $n = 28$, $P = 0.44$ excluding the Bulgarian population.

ulations independently of geographic distances, we have compared the residuals of the regression in Figure 2 for both sets of pairs of populations, sympatric and allopatric. Sympatric pairs of populations have significantly smaller residuals ($t = 5.61$, $df = 49$, $P < 0.00001$; Fig. 6), implying that for them the genetic differentiation is lower than expected from the geographic distances between them. The comparison is still significant after reducing the degrees of freedom to 14, that is, the number of population involved in the analysis minus 1 ($P = 0.0001$).

Great Spotted Cuckoo Gene Flow

The Nm -value for cuckoo populations calculated from the F_{ST} -value was 2.15, whereas that calculated by the private alleles method was 0.77. The low Nm -value when estimated

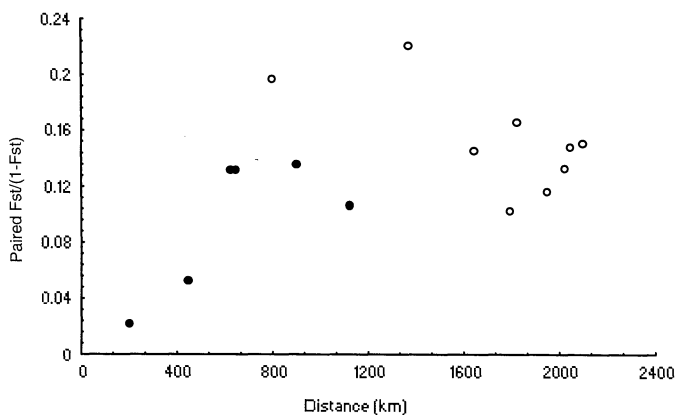


FIG. 4. Correlation between paired $F_{ST}/(1 - F_{ST})$ values and geographical distances between magpie populations outside the distribution range of the great spotted cuckoo. Black dots are paired values excluding the Scandinavian populations and white dots are paired values of the Scandinavian populations against each other and the rest. Correlations are as follow: $r_s = 0.44$, $n = 15$, $P = 0.074$ for the whole dataset and $r_s = 0.66$, $n = 6$, $P = 0.068$ excluding the Scandinavian populations.

TABLE 5. F_{ST} -values (SD) for magpie populations that are sympatric and allopatric with the great spotted cuckoo and their probability of deviation from zero (permutation test; Chapuisat et al. 1997).

	F_{ST} (SD)
All sympatric populations	0.040 (0.013)**
Sympatric populations excluding Bulgaria	0.034 (0.017)**
All allopatric populations	0.110 (0.016)**
Allopatric populations excluding Finland and Norway	0.079 (0.019)**

* $P < 0.05$, ** $P < 0.01$.

from the private alleles method is due to the presence of private alleles in all populations, which can be considered a consequence of the small sample size used in the cuckoo analysis. In any case, the estimates of Nm for cuckoos are clearly smaller than the values estimated for magpies, in particular for the subset of sympatric populations.

Therefore, we can conclude from the analysis of isolation by distance and Nm estimates that (1) gene flow is extensive in the magpie; (2) it is higher between magpie populations within the distribution limits of great spotted cuckoos than in populations outside these limits; and (3) gene flow between magpie populations is much higher than between cuckoo populations. Gene flow is also relevant between cuckoo populations, but the correlation of geographical distances and genetic differentiation suggests that cuckoos only disperse to nearby populations.

Correlation between Genetic Structure of Host and Parasite

The analyses of isolation by distance revealed a geographic component of genetic variation in both host and parasite. Accordingly, genetic distances (Cavalli-Sforza and Edwards' [1967] chord distance) were positively and significantly related to geographic distances in both species (Mantel tests, 500 permutations $r_s = 0.56$, $n = 105$, $P < 0.001$ for magpies, and $r_s = 0.71$, $n = 21$, $P = 0.01$ for cuckoos). However, there was no correlation between the pairwise estimates of genetic distances between magpie populations and the distances between great spotted cuckoo populations (Mantel

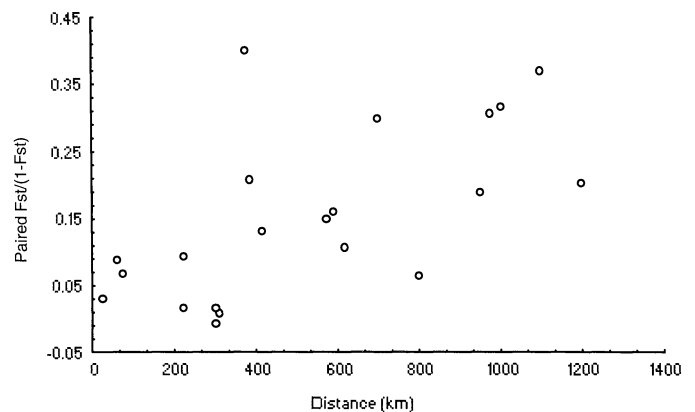


FIG. 5. Correlation between paired $F_{ST}/(1 - F_{ST})$ values and geographical distances between great spotted cuckoo populations ($r_s = 0.67$, $n = 21$, $P = 0.006$).

TABLE 6. Estimates of Nm derived from F_{ST} and the private alleles method for all populations and separately for populations in sympatry or allopatry with great spotted cuckoos.

	Nm from F_{ST}	Nm from private alleles
All populations	3.08	1.30
Sympatric populations	6.00	3.85
Sympatric populations*	7.10	5.89
Allopatric populations	2.02	0.92
Allopatric populations**	2.91	1.19

* Excluding Bulgarian population.

** Excluding Scandinavian populations.

test, 500 permutations, $r_s = -0.05$, $n = 21$, $P = 0.52$). The reason for this is the absence of geographic structure in the magpie populations in sympatry with cuckoos as shown before; accordingly, genetic distances were not related to geographic distances in the seven parasitized magpie populations considered here ($r_s = -0.06$, $n = 21$, $P = 0.78$).

DISCUSSION

Isolation by distance arises from the joint effects of gene flow, drift, and adaptation to local conditions (Wright 1943). Gene flow is an important determinant of genetic cohesion and similarity (Slatkin 1987), with patterns of isolation by distance reflecting limits to gene flow. For magpies, we found evidence of large-scale population structure and therefore evidence of limits to genetic exchange between distant populations, although gene flow appears to be extensive between nearby populations.

Cuckoos showed limited gene exchange between populations (a pattern of isolation by distance), but a rate of gene flow probably above one, which was smaller than that estimated for magpies. It can be argued that sample sizes for cuckoos are too small to be conclusive for a population genetic analysis, and caution must be exercised when interpreting these results. However, we found statistically significant population structure for cuckoos, implying that sample sizes are sufficiently large for meaningful analyses.

Despite geographic population structure, gene flow is relevant for both species and reflects dispersal from natal areas. There is no information on dispersal rates or distances of young great spotted cuckoos. Regarding magpies, direct estimates of dispersal are scarce and restricted to English populations; it is clear that a proportion of young stay in their natal area to breed (8.8% of young, Eden 1987; 13.3%, Birkhead and Goodburn 1989) and that dispersal distances from the natal territory to the first breeding attempt are very short, on average only a few hundred meters (see references in Birkhead 1991). Nevertheless, it is unclear what happens to those birds that do not return to their natal area, and long-distance dispersal cannot be ruled out. The reports by the Spanish Ornithological Society show that banded magpies are recaptured very anecdotally and always near the banding area. However, detailed studies on animal dispersal have revealed a bias toward reporting short-distance dispersers in traditional studies, and long-distance dispersal is strikingly more frequent than suspected (Koenig et al. 1996). In fact, there are a few records of long-distance movements for North

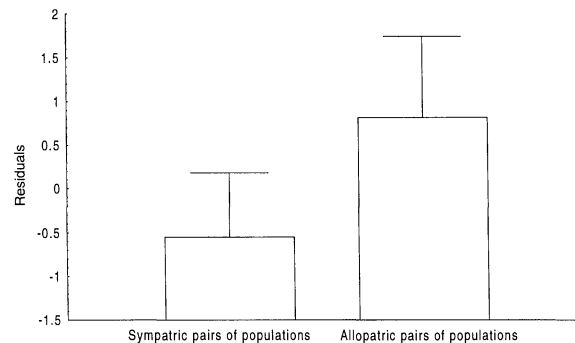


FIG. 6. Residuals of the regression between pairwise $F_{ST}/(1 - F_{ST})$ values and geographical distances (see Fig. 2) for the pairs of population in sympatry with the great spotted cuckoo ($n = 36$) and those in allopatry ($n = 15$). Bars are means and lines standard deviations; the comparison is statistically significant ($t = 5.61$, $df = 49$, $P < 0.00001$; see Results).

American magpies (Birkhead 1991), and a magpie banded as a chick in our Guadix population was found dead 250 km away from Guadix, in North Marocco (Cantos and Gomez-Manzanque 1997). Thus, even if English magpies usually disperse in small numbers over short distances, that does not imply that other populations must be similar. Natal philopatry has been shown to be an evolutionary labile trait that varies with ecological differences between populations of the same or closely related bird species (Weatherhead and Forbes 1994).

Population structure and gene flow in the magpie differed between populations that were sympatric and allopatric with the great spotted cuckoo (Tables 4, 5). F_{ST} -values differed between sympatric and allopatric populations of magpies with values being almost three times as large in allopatry as in sympatry (Table 4). Therefore, estimates of gene flow were three times as large for sympatric as compared to allopatric populations, when based on F_{ST} -statistics, and more than four times as large in sympatry as compared to allopatry, when based on private alleles (Table 5). These results suggest that there is extensive genetic exchange between magpie populations sympatric with the great spotted cuckoo, which results in reduced geographic patterns of genetic differentiation. Frequent extinctions and recolonizations of local populations can be an important source of gene flow (Slatkin 1987), and high prevalence of brood parasites may locally result in extinction of magpie hosts (see below). Alternatively, long-distance dispersal among parasitized magpie populations may result in a nongeographic population structure.

The findings on population structure and gene flow in magpie populations sympatric and allopatric with the great spotted cuckoo could be interpreted as being a consequence of the host-parasite interaction. Magpie dispersal would be favored in a heavily parasitized source population composed mainly of acceptors, that is, individuals that accept brood parasitic eggs (Rothstein 1990), if the recipient population is not parasitized, because offspring of acceptors will have a higher probability of successful breeding by dispersing than by remaining in the parasitized natal population. If the source population is composed of both acceptor and rejecter individuals (individuals able to identify and successfully reject

cuckoo eggs [Rothstein 1990]), but rejection entails reproductive costs larger than the cost of dispersal (e.g., due to rejection errors), then dispersing magpies would also be favored.

A different interpretation of the findings of our study is that extensive gene flow in parasitized host populations is a by-product of host-parasite interactions. High estimates of gene flow may result from extinction and recolonization of local populations of magpies being driven to extinction by heavy parasitism. Magpie populations within the distribution range of great spotted cuckoos are characterized by considerable variation in prevalence of the brood parasite and the degree of egg rejection (Soler et al. 1994; Soler et al., 1998): some populations recently parasitized show little resistance to the brood parasite and are the target of intense parasitism, and rejection behavior of cuckoo eggs has been demonstrated to increase rapidly in these populations (Soler et al. 1994). This intense parasitism may result in dramatic decreases in population size of the magpie, and sometimes even extinction of local populations (Soler et al. 1998), leaving a "gap" that can be filled by individuals from other populations.

Genetic exchange between host populations has important consequences for the dynamics of host-parasite interactions, especially when parasitized and unparasitized populations are connected by extensive gene flow. For example, the occurrence and spread of egg rejection behavior would be influenced in different ways. On one hand, when rejecters arise in a recently parasitized host population with a high prevalence of parasitism, the majority of dispersers to other populations will be the offspring of rejecters because most acceptors leave no offspring; that would result in a delay of local fixation of rejection alleles in that population as compared to predictions from models dealing with nondispersing hosts (e.g., Takasu et al. 1993). However, once egg rejection spreads in a parasitized population, the population will act as a source of rejecter alleles to nearby unparasitized host populations. This could result in a shortening of the duration of spreading of egg rejection in unparasitized host populations, because once they become parasitized by cuckoos some individuals would already be rejecters. On the other hand, we would expect to find acceptor individuals in parasitized populations immigrating from unparasitized populations where egg rejection is not common.

The interchange of rejecter and acceptor individuals between host populations may be an explanation for some intriguing observations such as the presence of acceptor individuals in host populations that have been parasitized for a long time (Rothstein 1990), the occurrence of rejection behavior in some unparasitized host populations (Davies and Brooke 1989; Briskie et al. 1992), and the fact that some recently parasitized host populations quickly evolve a high frequency of rejection behavior (Soler and Møller 1990; Lotem and Rothstein 1995).

The role of dispersal and gene flow has been completely neglected in most theoretical models of host-parasite interactions, but should be incorporated into these in the future. For example, May and Robinson (1985) argued that some populations of hosts of the brown-headed cowbird (*Molothrus ater*) are not replacing themselves due to losses from parasitism, and such populations may represent "sinks" that can

be maintained only by immigrants from areas exposed to less intense brood parasitism. However, their model assumed nondispersing hosts. Takasu et al. (1993) based their model on a host population in which newborn females were recruited into the adult population of nondispersing hosts, although they acknowledge that hosts and cuckoos may have different local populations and that host-parasite population dynamics may be dramatically affected by interactions at other localities. In general, for species engaged in a coevolutionary interaction, local populations may go extinct, but the interaction may persist at the regional level; alleles may appear through gene flow and disappear through genetic drift (Thompson 1994).

Metapopulation dynamics of hosts and parasites may dramatically affect the level of local adaptation of parasites to their hosts depending on the relative dispersal rates of hosts and parasites (Gandon et al. 1996), with migration being a source of polymorphism at the population level. The model by Gandon et al. (1996), which was conceived for general parasite-host associations, predicts that when hosts migrate more than parasites (present study) and parasite migration rates are not very high, the hosts are locally adapted.

This study has found a smaller migration rate for cuckoos than for magpies. Although the results must be interpreted with caution, as stated above, due to the small sample size for cuckoos, this conclusion is likely to hold true because everything else being equal, even with similar migration rates gene flow should be higher in magpies than great spotted cuckoos simply because of their larger population sizes.

Thus, from our data we would expect to find local adaptation in magpie populations. Because great spotted cuckoo populations are geographically structured, whereas magpie populations are not, it would be difficult for cuckoos to track magpie adaptations: genetic polymorphisms available through migration in magpie populations would make it difficult for cuckoos to evolve phenotypes that match local magpie phenotypes (e.g., egg appearance) and subsequently the formation of great spotted cuckoo "races." Nevertheless, because the relationship between Nm and F_{ST} only holds for models of drift and gene flow, we cannot disregard that strong selection pressures could promote local adaptation in cuckoos despite high levels of gene flow. At the same time, a high migration rate between magpie populations would provide an amount of genetic variation that would ease the appearance of magpie counteradaptations to new cuckoo adaptations. This would give an advantage to magpies in the coevolutionary arms race at a regional scale.

ACKNOWLEDGMENTS

We thank J. Neigel, M. Raymond, and two anonymous referees for their constructive comments that greatly improved the paper. We are indebted to P. Fitze, A. Jacot, J. Kilpimaa, J. P. Lenna, B. Milchev, J. Minguela, D. R. Opdahl, D. Parrott, H. Richner, and N. Saino for their help in data collection. Thanks to D. Dawson for her help in the cloning of magpie microsatellite loci. JGM was supported by an EC postdoctoral fellowship (HCM programm-ERBCHB1CT94 1288), JJS by an EC postdoctoral fellowship (HCM programm-ERBCHBCT92-0772), MS and APM by an EC proj-

ect (Science, ERB4002PL910307), and TB by a grant from the BBSRC.

LITERATURE CITED

- ARMOUR, J. A. L., R. NEUMANN, S. GOBERT, AND A. J. JEFFREYS. 1994. Isolation of human simple repeat loci by hybridization selection. *Hum. Mol. Genet.* 3:599–605.
- AVISE, J. C. 1994. Molecular markers, natural history, and evolution. Chapman and Hall, London.
- BIRKHEAD, T. R. 1991. The magpies. T. and A. D. Poyser, London.
- BIRKHEAD, T. R., AND S. F. GOODBURN. 1989. Magpie. Pp. 173–182 in I. Newton, ed. Lifetime reproduction in birds. Academic Press, London.
- BOWCOCK, A. M., A. RUIZ-LINARES, J. TOMFOHRDE, E. MINCH, J. R. KIDD, AND L. L. CAVALLI-SFORZA. 1994. High resolution of human evolutionary trees with polymorphic microsatellites. *Nature* 368:455–457.
- BRISKIE, J. V., S. G. SEALY, AND K. A. HOBSON. 1992. Behavioral defenses against avian brood parasitism in sympatric and allopatric host populations. *Evolution* 46:334–340.
- BRUFORD, M. W., AND R. K. WAYNE. 1993. Microsatellites and their application to population genetic studies. *Curr. Opin. Genet. Develop.* 3:939–943.
- BRUFORD, M. W., O. HANOTTE, J. F. Y. BROOKFIELD, AND T. BURKE. 1992. Single-locus and multilocus DNA fingerprinting. Pp. 225–268 in A. R. Hoelzel, ed. Molecular genetic analysis of populations. Oxford Univ. Press, Oxford, U.K.
- CANTOS, F. J., AND A. GOMEZ-MANZANEQUE. 1997. Informe sobre la campaña de anillamiento de aves en España. Año 1996. *Ecológia* 11:303–422.
- CAVALLI-SFORZA, L. L., AND A. W. F. EDWARDS. 1967. Phylogenetic analysis: models and estimation procedures. *Am. J. Hum. Genet.* 19:233–257.
- CHAPUISAT, M., J. GOUDET, AND L. KELLER. 1997. Microsatellites reveal high population viscosity and limited dispersal in the ant *Formica paralugubris*. *Evolution* 51:475–482.
- CRAMP, S., ED. 1985. The birds of the western Palearctic. Vol. IV. Oxford Univ. Press, Oxford, U.K.
- . 1994. The birds of the western Palearctic. Vol. VIII. Oxford Univ. Press, Oxford, U.K.
- DAVIES, N. B., AND M. DE L. BROOKE. 1989. An experimental study of coevolution between the cuckoo *Cuculus canorus* and its hosts. I. Host egg discrimination. *J. Anim. Ecol.* 58:207–224.
- DEKA, R., L. JIN, M. D. SHRIVER, L. M. YU, S. DECRIO, J. HUNDRIESER, C. H. BUNKER, R. E. FERRELL, AND R. CHAKRABORTY. 1995. Population genetics of dinucleotide (dC-dA)_n-(dG-dT)_n polymorphisms in world populations. *Am. J. Hum. Genet.* 56:461–474.
- EDEN, S. F. 1987. Natal philopatry of the magpie *Pica pica*. *Ibis* 129:477–490.
- ESTOUP, A., M. SOLIGNAC, J.-M. CORNUET, J. GOUDET, AND A. SCHOLL. 1996. Genetic differentiation of continental and island populations of *Bombus terrestris* (Hymenoptera: Apidae) in Europe. *Mol. Ecol.* 5:19–31.
- FELSENSTEIN, J. 1993. PHYLIP (Phylogeny Inference Package) version 3.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle, WA.
- GANDON, S., Y. CAPOWIEZ, Y. DUBOIS, Y. MICHALAKIS, AND I. OLIVIERI. 1996. Local adaptation and gene-for-gene coevolution in a metapopulation model. *Proc. R. Soc. Lond. B Biol. Sci.* 263:1003–1009.
- GIBBS, H. L., M. DE L. BROOKE, AND N. B. DAVIES. 1996. Analysis of genetic differentiation of host races of the common cuckoo *Cuculus canorus* using mitochondrial and microsatellite DNA variation. *Proc. R. Soc. Lond. B Biol. Sci.* 263:89–96.
- GIBBS, H. L., P. MILLER, G. ALDERSON, AND S. G. SEALY. 1997. Genetic analysis of brown-headed cowbirds *Molothrus ater* raised by different hosts: data from mtDNA and microsatellite DNA markers. *Mol. Ecol.* 6:189–193.
- GOUDET, J. 1995. Fstat version 1.2: a computer program to calculate *F*-statistics. *J. Hered.* 86:485–486.
- JARNE, P., AND P. J. L. LAGODA. 1996. Microsatellites, from molecules to populations and back. *Trends Ecol. Evol.* 11:424–429.
- KELLY, C. 1987. A model to explore the rate of spread of mimicry and rejection in hypothetical populations of cuckoos and their hosts. *J. Theor. Biol.* 125:283–299.
- KOENIG, W. D., D. V. VUREN, AND P. N. HOOGE. 1996. Detectability, philopatry, and the distribution of dispersal distances in vertebrates. *Trends Ecol. Evol.* 11:514–517.
- LOTEM, A., AND S. I. ROTHSTEIN. 1995. Cuckoo-host coevolution: from snapshots of an arms race to the documentation of microevolution. *Trends Ecol. Evol.* 10:436–437.
- MANTEL, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Res.* 27:209–220.
- MARTINEZ, J. G., T. BURKE, D. DAWSON, J. J. SOLER, M. SOLER, AND A. P. MØLLER. 1998. Microsatellite typing reveals mating patterns in the brood parasitic great spotted cuckoo (*Clamator glandarius*). *Mol. Ecol.* 7:289–297.
- MAY, R. M., AND S. K. ROBINSON. 1985. Population dynamics of avian brood parasitism. *Am. Nat.* 125:475–494.
- MOKSNES, A., E. RØSKAFT, AND A. T. BRAA. 1991. Rejection behavior by common cuckoo hosts towards artificial brood parasite eggs. *Auk* 108:348–354.
- PEREZ-LEZAUN, A., F. CALAFELL, E. MATEU, D. COMAS, R. RUIZ-PACHECO, AND J. BERTRANPETIT. 1997. Microsatellite variation and the differentiation of modern humans. *Hum. Genet.* 99:1–7.
- PRICE, P. W. 1980. Evolutionary biology of parasites. Princeton Univ. Press, Princeton, NJ.
- RAYMOND, M., AND F. ROUSSET. 1995. GENEPOP. Vers. 1.2. Population genetics software for exact tests and ecumenicism. *J. Hered.* 86:248–249.
- ROTHSTEIN, S. I. 1975. An experimental and teleonomic investigation of avian brood parasitism. *Condor* 77:250–271.
- . 1990. A model system for the study of coevolution: avian brood parasitism. *Ann. Rev. Ecol. Syst.* 21:481–508.
- ROUSSET, F. 1996. Equilibrium values of measures of population subdivision for stepwise mutation processes. *Genetics* 142:1357–1362.
- . 1997. Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. *Genetics* 145:1219–1228.
- ROUSSET, F., AND M. RAYMOND. 1995. Testing heterozygote excess and deficiency. *Genetics* 140:1413–1419.
- SLATKIN, M. 1985. Rare alleles as indicators of gene flow. *Evolution* 39:53–65.
- . 1987. Gene flow and the geographic structure of natural populations. *Science* 236:787–792.
- . 1995. A measure of population subdivision based on microsatellite allele frequencies. *Genetics* 139:457–462.
- SLATKIN, M., AND N. H. BARTON. 1989. A comparison of three indirect methods for estimating average levels of gene flow. *Evolution* 43:1349–1368.
- SOLER, M. 1990. Relationships between the Great Spotted Cuckoo and its corvid hosts in a recently colonized area. *Ornis Scand.* 21:212–223.
- SOLER, M., AND A. P. MØLLER. 1990. Duration of sympatry and coevolution between the great spotted cuckoo and its magpie host. *Nature* 343:748–750.
- SOLER, M., J. J. SOLER, J. G. MARTINEZ, AND A. P. MØLLER. 1994. Micro-evolutionary change in host response to a brood parasite. *Behav. Ecol. Sociobiol.* 35:295–301.
- SOLER, M., J. G. MARTINEZ, AND J. J. SOLER. 1996. Effects of brood parasitism by the great spotted cuckoo on the breeding success of the magpie host: an experimental study. *Ardeola* 43:87–96.
- SOLER, M., J. J. SOLER, J. G. MARTINEZ, T. PEREZ-CONTRERAS, AND A. P. MØLLER. 1998. Micro-evolutionary change and population dynamic of a brood parasite and its primary host: the intermittent arms race hypothesis. *Oecologia* 117:381–390.
- TAKASU, F., K. KAWASAKI, H. NAKAMURA, J. E. COHEN, AND N. SHIGESADA. 1993. Modelling the population-dynamics of a cuckoo-host association and the evolution of host defenses. *Am. Nat.* 142:819–839.

- THOMPSON, J. N. 1994. The coevolutionary process. Univ. of Chicago Press, Chicago.
- WEATHERHEAD, P. J., AND M. R. FORBES. 1994. Natal philopatry in passerine birds: genetic or ecological influences? *Behav. Ecol.* 5:426–433.
- WEIR, B. S., AND C. C. COCKERHAM. 1984. Estimating F -statistics for the analysis of population structure. *Evolution* 38:1358–1370.
- WRIGHT, S. 1943. Isolation by distance. *Genetics* 28:114–138.
- . 1951. The genetical structure of populations. *Ann. Eugen.* 15:323–354.

Corresponding Editor: J. Neigel