CUCKOO PARASITISM AND PRODUCTIVITY IN DIFFERENT MAGPIE SUBPOPULATIONS PREDICT FREQUENCIES OF THE 457bp ALLELE: A MOSAIC OF COEVOLUTION AT A SMALL GEOGRAPHIC SCALE

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The level of defense against great spotted cuckoo (*Clamator glandarius*) parasitism in different European populations of magpie (*Pica pica*) depends on selection pressures due to parasitism and gene flow between populations, which suggests the existence of coevolutionary hot spots within a European metapopulation. A mosaic of coevolution is theoretically possible at small geographical scales and with strong gene flow, because, among other reasons, plots may differ in productivity (i.e., reproductive success of hosts in the absence of parasitism) and defensive genotypes theoretically should be more common in plots of high productivity. Here, we tested this prediction by exploring the relationship between parasitism rate, level of defense against parasitism (estimated as both rejection rate and the frequency of the 457bp microsatellite allele associated with foreign egg rejection in magpies), and some variables related to the productivity (average laying date, clutch size, and number of hatchlings per nest) of magpies breeding in different subpopulations. We found that both estimates of defensive ability (egg rejection rate and frequency of the 457bp allele) covaried significantly with between-plot differences in probability of parasitism, laying date, and number of hatchlings per nest. Moreover, the parasitism rate was larger in more productive plots. These results confirm the existence of a mosaic of coevolution at a very local geographical scale, and the association between laying date

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and number of hatchlings with variables related to defensive ability and the selection pressure arising from parasitism supports the prediction of coevolutionary gradients in relation to host productivity.

KEY WORDS Avian interspecific brood parasitism, coevolution, egg rejection, genetic marker, host defenses, *Pica pica*, productivity gradients.

The ability to recognize and reject eggs of odd appearance is considered to be one of the main defenses of avian hosts against brood parasitism (Payne 1977; Rothstein 1990). It has been demonstrated that rejection is a direct consequence of the selection pressure exerted by brood parasites (see, e.g., Davies and Brooke 1989; Rothstein and Robinson 1998; Davies 2000) which, in turn, selects for counter-defenses in brood parasites, such as egg mimicry by parasite (Payne 1977; Brooke and Davies 1988; Rothstein 1990; Moksnes and Røskaft 1995). In general, both defensive (rejecter) and nondefensive (accepter) phenotypes co-occur within a host population independently of the intensity of selection and the extent of coevolutionary interactions between hosts and parasites, because host populations are not isolated from each other (metapopulation structure), and because selection pressures vary within the host metapopulation (e.g., Nuismer et al. 1999; Soler et al. 2001; Thompson 2005; Røskaft et al. 2006). Thus, the presence of adaptive and nonadaptive phenotypes within host populations exploited by brood parasites is hypothesized to be the consequence of geographically structured coevolutionary interactions in a complex landscape (Thompson et al. 2002).

A geographic mosaic of coevolution is theoretically possible even within a nongenetically structured population (e.g., Thompson 2005) because the selection pressure exerted by the parasite may not be constant across subpopulations, giving rise to subpopulations that vary in the frequency of adaptive phenotypes/genotypes. We have previously shown that magpie (Pica pica) populations in sympatry with the brood parasitic great spotted cuckoo (Clamator glandarius) do not show a pattern of isolation by distance (Martínez et al. 1999), and that variation in selection pressures (i.e., parasitism rate) predicted the frequencies of adaptive phenotypes after correcting for gene flow and geographic distances (Soler et al. 2001). Moreover, genetic, but not geographic, distances covaried with rate of parasitism, suggesting the existence of hot spots occurring relatively independently of geographical location (Soler et al. 2001). However, these patterns arose from a study at large geographic scale, in a number of European magpie populations in sympatry with the great spotted cuckoo. Because coevolutionary gradients, at least theoretically, may occur within a smaller geographic range (Nuismer et al. 2000; Thompson 2002), Thompson (2005) emphasized the importance of studying coevolution at different geographic scales. Thus, the first aim of this article is to explore the existence of a mosaic of coevolution (i.e., association between frequencies of adaptive phenotypes and parasitism selection pressure independent of geographic distances) at a local scale, in a single magpie population in southern Spain, Guadix, which comprises several geographically isolated but nearby subpopulations.

Habitat quality, as indicated by host productivity in terms of reproductive success in the absence of parasitism, could also be an important factor affecting coevolutionary relationships because it might affect both the distribution and abundance of hosts and parasites within their geographic range. If parasites select abundant host populations, they would be most influential on the population dynamics of hosts in these areas, rather than in areas scarcely used by hosts (Hochberg and Van Baalen 1998). In this scenario, host productivity should be related to coevolutionary gradients and, thus, selection pressure for the host's investment in defenses should increase with host productivity (Hochberg and Van Baalen 1998). Here, we test this hypothesis by exploring the association between plot quality and magpie productivity and the distribution of defensive phenotypes within the subpopulations of magpies in Guadix. The quality of magpie plots was estimated as the average laying date of first reproductive attempts that, at least in this species, reflects territory and individual quality (e.g., Birkhead 1991; Soler et al. 1995; Sorci et al. 1997; De Neve and Soler 2002). Moreover, we also used clutch size and number of hatchlings per nest as variables reflecting aspects of magpie productivity (see, e.g., Högstedt 1980; Goodburn 1987, for correlations between these variables and fledging success in magpies). The frequency of defensive individuals in different subpopulations was estimated not only by means of model-egg recognition experiments, but also by using information from a microsatellite locus (Ase64) (Richardson et al. 2000), one of whose alleles (457bp) strongly associates with the ability of magpies to reject foreign eggs (Martín-Gálvez et al. 2006). Rejection is more likely in those pairs in which we found the 457bp allele in the genotype of one of their chicks, suggesting that the frequency of this allele in different subpopulations can be used as an indicator of the genetic ability associated with the expression of the defensive behavior. Finally, we use the frequency of brood parasitism in different subpopulations as a variable related to the level of parasitism selection pressure.

Methods study area

The magpie population of Guadix (37°18′N, 3°11′W, southern Spain) is situated in a high-altitude plateau (approximately 1000 m a.s.l.). The vegetation is sparse, including cultivated cereals



Figure 1. Map showing the relative location and area of magpie subpopulations in Guadix. Letters (FE, FA, LA, CA, CH, HU, and CC) refer to subpopulations.

(especially barley) and many groves of almond (*Prunus dulcis*) and oak (*Quercus rotundifolia*) trees in which magpies prefer to build their nests (Soler 1990). The Guadix magpie population is composed of several nearby subpopulations, which are separated by arable land with few or no potential nest sites for the magpie (Soler et al. 1994). The patchiness of the study area allows us to distinguish several geographically isolated subpopulations of breeding magpies, of which here we use data from seven (Fig. 1). Previous studies have found differences in ecological conditions among these subpopulations, including synchrony, density, or different rates of brood parasitism (see, e.g., Soler et al. 1994, 1998a,b Martínez et al. 1996), which validate the use of these subpopulations as distinct units for this study.

FIELD PROCEDURES

Fieldwork was conducted during the breeding seasons of 2000 and 2001. At the beginning of spring, we searched intensely for magpie nests that we visited regularly to determine their laying date (Table 2). When a nest contained at least one magpie egg, a model egg made with plaster of Paris and painted to resemble those of great spotted cuckoos was introduced in the nest without removing any magpie egg. During the laying period we visited the nests at least three times per week to determine clutch size and natural parasitism (presence of great spotted cuckoo eggs or traces of parasitism such as broken eggs, see below). Similarly, 15 days after the start of incubation we visited the nests every second day to determine the number of nestlings that successfully hatched. Hatching success was estimated in unparasitized nests with no eggs broken by the great spotted cuckoo (see, e.g., Soler et al. 1997). We did not estimate fledging success because nestlings were used in some other experiments (i.e., cross-fostering, see De Neve et al. 2004a). Mean values of clutch size and number of hatchlings per nest for each study plot were used as an index

 Table 1. Parasitism rate, rejection rate, and frequency of the 457bp

 allele per subpopulation. Numbers in parentheses refer to number of

 magpie nests.

Zone	Parasitism rate (%)		Rejection rate (%)		Frequency of 457bp allele
Year	2000	2001	2000	2001	(%)*
FE	19.57 (46)	_	47.83 (23)	_	21.43 (42)
FA	19.05 (21)	-	50.00 (10)	-	16.67 (18)
LA	0.00 (12)	18.18 (22)	28.57 (7)	28.57 (21)	4.17 (24)
CA	36.23 (69)	34.78 (69)	57.14 (56)	27.87 (61)	15.00 (60)
CH	8.43 (83)	41.94 (62)	51.35 (37)	41.07 (56)	17.74 (62)
HU	78.13 (32)	69.57 (23)	47.83 (23)	47.83 (23)	22.73 (22)
CC	-	55.56 (18)	-	31.25 (16)	8.33 (12)

*Frequencies of 457bp allele were calculated from pooled data of both years because nests selected for genetic analyses from the year 2001 (N = 21) were in magpie territories not studied in 2000 (N = 101).

of magpie productivity (see Table 2). Egg-rejection experiments were performed independently of whether nests were parasitized by great spotted cuckoos to estimate the foreign egg recognition ability of magpies in different subpopulations. We classified magpie pairs as accepters if the experimental model egg remained in the nest after six days; otherwise, if the model disappeared from the nest or the magpie pair abandoned its clutch after experimental parasitism, it was considered a rejecter (Table 1). Because magpies may abandon the nest for reasons other than our experimental parasitism, we consider nest deserting to be a response to a model egg only if abandonment was detected two days after experimental parasitism. Thus, although the response to foreign eggs is not a fixed trait, and may depend on several factors such as egg mimicry (see, e.g., Davies et al. 1996), we refer to rejecter and accepter phenotypes in a probabilistic sense by assuming that those pairs that reject model eggs are more prone to reject natural parasitic eggs than those classified as accepters.

We used rate of parasitism for each study plot (Table 1) as a measure of selection pressure due to parasitism, because in a previous study in the same population both parasitism rate and cuckoo density were highly correlated (Soler et al. 1994, 1998b). We considered a nest as parasitized if it contained at least one cuckoo egg or showed traces of parasitism. Great spotted cuckoos typically cause breakage or denting of some magpie eggs during laying, easily detected by observers even if the cuckoo egg was rejected before visiting the nest (e.g., Soler and Martínez 2000). Nonetheless, following Rothstein (1971), our values of parasitism rate should be considered to be underestimates.

Due to logistic problems with capturing adult magpies, we sampled a randomly selected chick from each nest for genetic analysis. We took a blood sample from the brachial vein of the chick when it was 18 days old. Blood samples were stored in 1 mL of 100% ethanol. Moreover, nests selected for genetic analyses in 2001 were only from magpie territories not studied in 2000.

We had samples from 101 nests in 2000 and 21 nests in 2001. In addition, we only used data from first breeding attempts because magpies rejecting in the first breeding attempt may change their behavior to acceptance in replacement clutches due to the retaliatory behavior of great spotted cuckoos (i.e., "mafia behavior" [Soler et al. 1999b]).

LABORATORY WORK

Genomic DNA was isolated from blood using the ammoniumacetate precipitation method (adapted from Bruford et al. [1998]). We used 11 polymorphic microsatellite loci. Four of them were isolated from magpies: Ppi1, Ppi2, Ppi3 (Martínez et al. 1999), and Ppi4 (Martínez et al., EMBL accession number: PPI272377); three from Seychelles warbler (Acrocephalus sechellensis): Ase12, Ase18, and Ase64 (Richardson et al. 2000); one from indigo bird (Vidua chalybeata): Indigo28 (Sefc et al. 2001); one from western crowned-warbler (Phylloscopus occipitalis): Pocc1 (Bensch et al. 1997); and two from house sparrow (Passer domesticus): Pdo5 and Pdo6 (Griffith et al. 1999). Details of the primers used in this study can be found on the Sheffield Molecular Genetics Facility Passerine primer cross-utility database, accessed via http://www.shef.ac.uk/misc/groups/molecol/deborahdawson-birdmarkers.html. Because we previously detected (Martín-Gálvez et al. 2006) a statistical association between Ase64 genotypes (the 457bp allele) and recognition and ejection behavior of foreign eggs (see Introduction), we used the frequencies of this allele in different subpopulations in the statistical analyses as a genetic trait related to defensive behavior.

Genotypes for these 11 microsatellites were obtained during 2002 as follows. Polymerase chain reactions (PCRs) were performed with the forward primer of each marker labeled with a fluorescent dye. The reaction profile for each locus was 94°C for 120 sec, followed by 35 cycles of 94°C for 30 sec, the annealing temperature (depending of each locus) for 30 sec, and 72°C for 30 sec; and then 72°C for 5 min. Volumes of PCR reactions were 10 μ l, containing approximately 10 ng of DNA, 1.0 μ M of each primer, 0.2 mM of each dNTP, 1.5 mM MgCl₂, and 0.05 units of *Taq* DNA polymerase, in the manufacturer's buffer. PCR products were electrophoresed through an ABI Prism 377 DNA sequencer (Applied Biosystems, Foster City, CA). The output was analyzed using ABI Genescan software (ver. 3.1.2) and Genotyper DNA fragment analysis software (ver. 2.5).

STATISTICAL ANALYSES

Genetic differentiation between magpie subpopulations

STRUCTURE 2.1 (Pritchard et al. 2000) was used to infer the genetic structure of the Guadix magpie population, by assigning each individual genotyped to one or some clusters in relation with its genotype for all microsatellite loci but Ase64. In this way, we

are able to determine whether different plots of breeding magpies within the Guadix population are genetically different due to a limited gene flow among them, or because of different rates of immigration of individuals coming from other distant magpie populations (see, e.g., Martínez et al. 1999). In short, five different runs were performed for each number of subpopulations (k = 1 to k = 7), with a length of burn-in period oscillating between 10,000 and 50,000, and with the number of MCMC repetitions after burnin between 500,000 and 900,000. In all simulations, we assumed that allelic frequencies are correlated between subpopulations and that a certain individual genotype can be drawn from two or more genotypes coming from different subpopulations (i.e., model with admixture).

Allelic frequencies for each microsatellite locus and for each subpopulation were calculated using GENEPOP 3.3 (Raymond and Rousset 1995). Allelic frequencies of the 10 loci (all but Ase64) were then used to estimate the matrix of genetic distances (Cavalli-Sforza's chord distance) between subpopulations, a computation carried out by the program GENDIST included in the package PHYLIP 3.57c (Felsenstein 1993). In addition, we built matrices of differences in the frequency of copies of the 457bp allele of Ase64 between subpopulations, because the genetic association previously found between Ase64 and rejection behavior was mainly due to the fact that this allele is more frequent in the genotypes of chicks of rejecter pairs (Martín-Gálvez et al. 2006).

Finally, we also obtained the paired Fst-values between subpopulations using GENEPOP 3.3 to determine whether there is a significant correlation between (Fst/1 – Fst) values and geographical distances between plots and, thus, whether there is significant genetic structure in Guadix due to geographical isolation (Rousset 1997).

Comparisons between magpie subpopulations

Values of some phenotypic and environmental variables differed between years. Briefly, rejection rate was 51.28% (N = 156 nests) versus 35.93% (N = 177 nests) in 2000 and 2001, respectively $(\chi_1^2 = 8.97, P < 0.05)$ (Table 1); parasitism rate varied between 26.61% (N = 263) in 2000 and 41.24% (N = 194) in 2001 (χ_1^2 = 10.82, P < 0.05) (Table 1). With respect to the variables related to magpie productivity (Table 2), although we found that laying date (April 1 = day 1) in 2000 was on average 10 days later (29.40 \pm 0.70, N = 220) than in 2001 (19.11 \pm 0.79, N = 168) ($F_{1,386} = 94.01$, P < 0.001), the mean values in both years for clutch size (first year: 6.71 \pm 0.08, N = 198; second year: 6.68 ± 0.09 , N = 161) and number of hatchlings per nest (first year: 4.75 ± 0.19 , N = 68; second year: 5.09 ± 0.27 , N = 34) did not differ significantly ($F_{1.357} = 0.06$, P = 0.80 and $F_{1,100} = 1.02, P = 0.31$, respectively). Thus, for standardizing values of each subpopulation in each study year, we subtracted the mean values of the entire population in that year, obtaining

Zone	Laying date (1=1 April)		Clutch size		Number of hatchlings	
Year	2000	2001	2000	2001	2000	2001
FE	25.86 ± 1.76 (36)	_	6.69 ± 0.20 (35)	_	5.30 ± 0.52 (10)	_
FA	19.39 ± 2.49 (18)	_	6.93 ± 0.32 (14)	_	5.00 ± 0.82 (4)	-
LA	39.42 ± 3.05 (12)	16.64 ± 1.90 (22)	$6.45 \pm 0.36 (11)$	7.05 ± 0.25 (19)	4.17 ± 0.67 (6)	5.20 ± 0.69 (5)
CA	$32.07 \pm 1.40 (57)$	21.80 ± 1.16 (59)	6.73 ± 0.15 (60)	6.71 ± 0.14 (62)	4.94 ± 0.40 (17)	4.92 ± 0.45 (12)
CH	30.34 ± 1.28 (68)	17.52 ± 1.17 (58)	6.48 ± 0.17 (50)	6.47 ± 0.16 (45)	4.50 ± 0.30 (30)	4.50 ± 0.55 (8)
HU	28.45 ± 1.96 (29)	18.57 ± 1.94 (21)	7.07 ± 0.22 (28)	6.79 ± 0.25 (19)	6.00 ± 1.64 (1)	6.00 ± 0.89 (3)
CC	_	19.13 ± 3.15 (8)	-	$6.56 \pm 0.27 \ (16)$	_	5.67 ± 0.63 (6)

Table 2. Mean \pm SE of variables related to magpie productivity in each subpopulation. Values in parentheses refer to number of magpie nests.

in that way year-independent contrasts of different variables for each subpopulation. Afterward, matrices of differences between subpopulations were estimated using differences between mean contrast-values estimated for each subpopulation. We kept the signs in the matrices of differences obtained for each phenotypic and environmental variable, both when comparing them and when comparing these with the matrices of differences in frequency of the 457bp allele of Ase64. Therefore, we were able to discern the directionality of change between these variables. However, because the values of genetic and geographical distances were always positive, we used the absolute values of the differences of phenotypic and environmental variables when comparing them with genetic and geographical distances between subpopulations.

Associations between matrices were tested by means of Mantel's tests and partial Mantel's tests using FSTAT 2.9.3.2 (Goudet 2001), with estimation of *P*-values after 2000 randomizations. Further, we used STATISTICA 7.0 (Statsoft 2004) to carry out other statistical analyses. All statistical tests were two-tailed.

Results differences between magpie subpopulations

There was no significant correlation between paired Fst/(1-Fst) values and geographical distances between plots in Guadix (Mantel's test: r = 0.08, $R^2 = 0.01$, P = 0.74), which suggests a lack of genetic differentiation due to distance. Accordingly, results from the simulations performed by STRUCTURE 2.1 using 10 polymorphic microsatellite loci indicated that the Guadix population is not made up of genetically distinct subgroups. Instead, all simulations invariably suggested a single genetic group in Guadix. Therefore, these results indicate that plots of breeding magpies in Guadix do not represent genetically differentiated subpopulations, as might be expected for bird subpopulations close to each other. In any case, because the study population is geographically separated magpie breeding areas within the entire Guadix population as "subpopulations."

Parasitism rate significantly differed among subpopulations in both years (2000: $\chi_5^2 = 66.92$, P < 0.001; 2001: $\chi_4^2 = 15.16$, P < 0.05, Table 1). With respect to variables related to the level of defense against brood parasitism, we did not detect significant differences among subpopulations for either frequency of individuals with the 457bp allele ($\chi_6^2 = 4.77$, P = 0.57), or rejection rate (2000: $\chi_5^2 = 2.44$, P = 0.79; 2001: $\chi_4^2 = 4.41$, P = 0.35, Table 1). However, the coefficients of variation were considerable for both variables (frequency of individuals with the 457bp allele, CV = 44.5%; rejection rate, CV = 26.6% and 24.8% for years 2000 and 2001, respectively).

Regarding those variables related to plot productivity, subpopulations varied significantly only for laying date in 2000 (2000: $F_{5,214} = 7.09$, P < 0.001 vs. 2001: $F_{4,163} = 2.25$, P = 0.065, Table 2). With respect to clutch size, although we did not find significant differences among subpopulations (2000: $F_{5,192} = 1.10$, P = 0.36; 2001: $F_{4,156} = 1.12$, P = 0.35, Table 2), mean values ranged from 6.5 to 7.1 (CV = 3.6% and 3.4% for years 2000 and 2001, respectively). Finally, although subpopulations did not differ in number of hatchlings per nest (2000: $F_{5,62} = 0.70$, P =0.63; 2001: $F_{4,29} = 0.81$, P = 0.53, Table 2), the coefficients of variation of mean values were larger than 10% (12.8% and 11.3% for years 2000 and 2001, respectively).

ASSOCIATION BETWEEN GEOGRAPHICAL AND GENETIC DISTANCES AND EGG REJECTION BEHAVIOR

Absolute differences in rejection rate between subpopulations were not explained by genetic distances calculated for 10 microsatellite loci (Mantel's test: r = -0.02, $R^2 < 0.01$, P = 0.93), nor by geographical distances between subpopulations (Mantel's test: r = 0.03, $R^2 < 0.01$, P = 0.89). Interestingly, the differences in the frequency of the Ase64 457bp allele (statistically associated with rejection behavior, see Introduction), significantly explained among-subpopulation differences in rejection rates (Mantel's test: r = 0.91, $R^2 = 0.82$, P < 0.001, arrow 1 in Fig. 2). Thus, the frequency of the 457bp allele can be used to control statistically,



Figure 2. Relationships between variables of interest inferred from our results. According to the association between the 457bp allele and rejection behavior (Martín-Gálvez et al. 2006), (1) among-subpopulation differences in allele frequencies of 457bp allele were explained by differences in rejection behavior. Amongsubpopulations differences in parasitism rate (2, 3) and amongsubpopulation differences for some variables related to magpie productivity (laying date and hatchlings) (4,5) correlated not only with phenotypic (egg rejection, 2, 4) but also with genotypic (457bp allele, 3, 5) traits associated with defensive behaviors against cuckoo parasitism. Nonetheless, our results suggest that the association between parasitism rate and rejection behavior (2), and the association between productivity and rejection behavior (4), could be mainly explained by the association between these variables with the frequencies of 457bp allele (3 and 5, respectively). On the other hand, (6) among-subpopulation differences in parasitism rate were associated to those differences calculated for some variables related to magpie productivity (clutch size and hatchling success).

at least partially, the genetic component of rejection behavior in subsequent analyses.

ASSOCIATION BETWEEN PARASITISM RATE AND EGG REJECTION BEHAVIOR

Magpie subpopulations that suffered higher parasitism rates than average also showed higher rejection rates than average and those that suffered low parasitism showed lower rejection rates (mean of standardized values per subpopulation and year of study; Mantel's Test: r = 0.62, $R^2 = 0.39$, P = 0.006, arrow 2 in Fig. 2). A similar positive correlation was found for the differences in the frequency of the 457bp allele and differences in parasitism rate (Mantel's test: r = 0.68, $R^2 = 0.46$, P = 0.002, arrow 3 in Fig. 2). When both rejection rate and frequency of the 457bp allele were included in the same model, the former no longer explained among-subpopulation differences in parasitism rate (partial Mantel's tests, partial correlation coefficient = 0.02, $R^2 = 0.83$, P =0.94). Therefore, allele frequencies of the Ase64 allele associated with the expression of egg rejection were related to the parasitism rate suffered in different subpopulations.

ASSOCIATION BETWEEN VARIABLES RELATED TO MAGPIE PRODUCTIVITY, PARASITISM RATE, AND DEFENSIVE ABILITY

Magpie subpopulations that initiated laying relatively early had relatively higher egg rejection rates (mean of standardized values for each subpopulation and study year; Mantel's test: r = -0.49, $R^2 = 0.24$, P = 0.025, arrow 4 in Fig. 2). However, this relationship disappeared after controlling for differences in the frequency of the 457bp allele (partial Mantel's tests, partial correlation coefficient [average laying date] = $-0.09, R^2 = 0.83, P = 0.71$). This was because differences in average laying date between subpopulations were also correlated with differences in the frequency of the 457bp allele (Mantel's test: r = -0.45, $R^2 = 0.20$, P =0.035), which predicted differences in rejection rate (see above). Moreover, subpopulations with high frequencies of the 457bp allele had, on average, more hatchlings per nest (Mantel's Test: $r = 0.50, R^2 = 0.25, P = 0.024$), but no correlation was found between the frequency of the 457bp allele and clutch size (Mantel's test: r = 0.22, $R^2 = 0.05$, P = 0.35). Therefore, the frequency of the allele associated with the expression of egg rejection was related to among-subpopulation differences in some of the variables used as estimates of territory quality and productivity of magpies (arrow 5 in Fig. 2).

Finally, subpopulations that had relatively higher parasitism rates also had relatively larger clutch size (Mantel's test: r = 0.55, $R^2 = 0.30$, P = 0.012) and higher number of hatchlings per nest (Mantel's test: r = 0.85, $R^2 = 0.73$, P < 0.001), although there was no significant relationship with laying date (Mantel's test: r = -0.28, $R^2 = 0.08$, P = 0.21), suggesting a positive relationship between the selection pressure due to parasitism and host productivity (arrow 6 in Fig. 2).

Discussion association between parasitism rate and defensive traits

Presumably because of their proximity, the geographically distinct magpie subpopulations at Guadix showed no evidence of genetic structure. Proximity allows exchange of individuals between subpopulations and thus a considerable amount of gene flow that prevents isolated and independent trajectories of magpie subpopulations in their coevolutionary interaction with their brood parasites (Thompson 1994; Nuismer et al. 1999). Nonetheless, magpies breeding at different localities (i.e., subpopulations) experience different rates of parasitism and different environmental conditions (see Soler et al. 1994, 1998b; Soler and Soler 2000, and Fig. 1 and Tables 1 and 2) that, independently of the amount of gene flow between subpopulations, predict variation in the magnitude of the coevolutionary interactions across plots (Thompson 2005). Indeed, the risk of parasitism (i.e., parasitism rate) and

the variables related to the frequency of defensive individuals in different subpopulations (i.e., rejection rate and frequency of individuals with the 457bp allele) were positively related (arrows 2 and 3 in Fig. 2), and this relationship was independent of geographical and genetic distances. Importantly, our results on the strong relationship between rejection rate and the frequency of the allele associated with rejection (457bp allele, arrow 1 in Fig. 2) suggest that the relationship between rejection rate and frequency of parasitism was not a consequence of rejection being inducible (sensu Tollrian and Harvell 1999). Finally, when among-plot differences in rejection rate and frequency of the 457bp allele were both included in a multiple regression analysis as independent variables explaining rates of parasitism, the differences in risk of parasitism no longer covaried with the differences in rejection rate. Therefore, because differences in the level of rejection behavior between subpopulations were explained by a genetic trait, even after statistically correcting for differences in parasitism rate in a multiple autocorrelation analysis, our results suggest the existence of a coevolutionary mosaic and, therefore, an ongoing evolutionary process at the local geographical scale of the Guadix magpie population.

DOES MAGPIE PRODUCTIVITY PREDICT THE INTENSITY OF THE COEVOLUTIONARY INTERACTION?

Hochberg and Van Baalen (1998) suggested that selection for investment in defenses will be positively related to host productivity and, therefore, host productivity should be related to a gradient in the strength of coevolutionary interactions (Thompson 2005). Moreover, Vance (1978), using multispecies population models, showed that resistant prey species should differentially inhabit high-productivity areas because they can support a larger population of predators. In the case of parasite-host systems, defensive genotypes should be more common in areas with territories of better quality because parasitism is less costly for defensive individuals than for nondefensive individuals. Therefore, this asymmetry in the costs of parasitism for different phenotypes would confer a competitive advantage for defensive over nondefensive phenotypes (e.g., Holt 1977), the latter being more common in the least productive plots (Hochberg and Van Baalen 1998).

Laying date reflects territory and individual quality in magpies (see Birkhead 1991; Soler et al. 1995; Sorci et al. 1997; De Neve and Soler 2002), so that mean laying date of a subpopulation may be taken as an estimate of the average quality of territories/pairs in the subpopulation. Accordingly, magpie productivity (i.e., breeding success) should be higher in subpopulations with earlier laying dates, larger clutch sizes, and higher number of hatchlings per nest. Therefore, if the magnitude of the coevolutionary interaction varies depending on the productivity of the area, the frequency of defensive genotypes should be higher in subpopulations that on average start to breed earlier, lay more eggs, and hatch more nestlings per nest. In accordance with this scenario, we found that magpie subpopulations with a high frequency of the 457bp allele initiated laying earlier and had more hatchlings (arrow 5 in Fig. 2). Not surprisingly, then, laying dates of different magpie plots also predicted rejection rates (arrow 4 in Fig. 2), although when information on the frequency of the 457bp allele was included in the analysis, only 457bp frequency, but not laying date, explained among-plot variation in rejection rate. Clutch size did not predict the frequency of the 457bp allele in different subpopulations (see Results). This is not a consequence of differences in the extent of egg damage by cuckoos at different localities, because clutch size was estimated using only nests with no trace of parasitism (i.e., broken eggs). Rather, this result together with the association between number of hatchlings per nest and the frequency of 457bp allele would suggest a negative association between the frequency of 457bp and the frequency of hatching failure, which in some species is negatively related to territory quality and, therefore, productivity (e.g., Rauter and Reyer 1997; Dearborn 2001; for magpies see Högstedt 1981).

Another alternative hypothesis that could explain the association between differences in frequencies of the 457bp allele and differences in average laying dates and number of hatchlings per nest in different magpie plots is a physical linkage between genes related to egg rejection ability and those related to these variables. Although we cannot dismiss this possibility, it is reasonable to assume that physical proximity between genes that affect laying date, hatching success, and the ability to recognize and reject parasitic eggs is a priori very unlikely. Alternatively, the 457bp allele could be associated with variables not considered here that at the same time predicted either laying date, number of hatchlings, or egg rejection decisions (e.g., those that could reduce costs associated with rejection of foreign eggs (see for instance, Davies [2000]). However, this would not be a problem for considering the 457bp allele as a genetic trait because that would indicate a genetic association between the 457bp allele and variables related to the expression of defenses against brood parasitism.

Due to the very small geographical scale used in this study, the detected relationship between variables related to plot productivity, phenotypic quality, and allelic frequencies of a marker associated with defense against brood parasitism can be explained either by host selection of breeding plots or by natural selection. First, defensive individuals may preferentially select (or migrate to) areas that permit a high level of reproduction by hosts, because territory quality should be relatively more important for defensive than nondefensive individuals. Even if parasitism is not associated with territory quality, nondefensive individuals should primarily select plots with low probability of parasitism whereas defensive individuals should select high-quality territories (Fig. 3).



Figure 3. The relationship between parasitism rate and defense level (A) could be a consequence of both (1) the preference of great spotted cuckoo for parasitizing territories of high quality (Soler et al. 1995 and present results) and (2) the occupancy of good territories (by migration or by natural selection) by rejecter pairs because parasitism is less costly there for them than for nondefensive individuals.

However, the relationship between the frequency of the 457bp allele and some variables related to magpie productivity could be a consequence of natural selection, provided that rejecter individuals become more common in productive plots as a direct consequence of a higher breeding success in these plots in comparison with nondefensive individuals (Fig. 3).

On the other hand, host productivity may also predict the intensity of coevolutionary interactions because it may affect parasitism rate. That would be the case if for instance parasites preferentially use plots in which hosts have superior reproduction (see Hochberg and Van Baalen 1998; Thompson 2005). This possibility is particularly important in brood parasite-host systems because parasitic offspring are fed by hosts and, thus, resource abundance is also important for appropriate development of parasite offspring. In this sense, it is known that great spotted cuckoos preferentially parasitize large magpie nests (Soler et al. 1995), which correspond with pairs of high parental ability (Soler et al. 1995; De Neve et al. 2004b). Because nest size and laying date of magpies are positively related (Soler et al. 1995, 1999a), it is possible that great spotted cuckoos exert a stronger selection pressure in subpopulations in which magpies, on average, start to reproduce earlier, lay larger clutches, and hatch a larger number of nestlings per nests. This scenario would therefore predict a relationship between rate of parasitism and variables related to magpie productivity in different magpie plots. Accordingly, although differences in laying date were not related to differences in parasitism rate between subpopulations, both average clutch size and brood size of different plots predicted parasitism rate (arrow 6 in Fig. 2).

In summary, through the association between defensive behavior and both phenotype (egg rejection behavior) and genotype (frequency of the 457bp allele), we found evidences of differences in the magnitude of the coevolutionary interactions between magpies and great spotted cuckoos at a small geographic scale, the magpie population of Guadix, that could be mediated by the existence of among-plot differences in host productivity.

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