

# Age-Related Brood Parasitism and Egg Rejection in Magpie Hosts

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**ABSTRACT:** When the strength or nature of a host-parasite interaction changes over the host life cycle, the consequences of parasitism can depend on host population age structure. Avian brood parasites reduce hosts' breeding success, and host age may play a role in this interaction if younger hosts are more likely parasitized and/or less able to defend themselves. We analyzed whether the age of female magpie (*Pica pica*) hosts is associated with parasite attack or their ability to reject foreign eggs. We recorded parasitism and model egg rejection of known-age individuals over their lifetime and established whether the likelihood of parasitism or egg rejection changed with age or longevity. Parasitism probability did not change with female age, and there was a trend toward longer-lived females being less likely to be parasitized. However, model egg rejection probability increased with age for each individual female, and longer-lived females were more prone to reject model eggs. Most females in the population were young, and the majority of them accepted model eggs, suggesting that brood parasites exploiting younger host individuals are benefitting from a lower defense level of their hosts. Our results stress that the intensity of selection by brood parasites may be mediated by the age structure of host populations, a to-date neglected aspect in brood parasite–host research.

**Keywords:** age-related parasitism, age-related egg rejection, brood parasitism, magpie, population age structure.

## Introduction

Parasites decrease fitness and modulate life histories of their hosts, which can select for many host adaptations that reduce the costs of parasitism (Clayton and Moore 1997). Although the negative effects of parasites on their hosts are well documented, parasite abundance or the parasitism susceptibility of hosts may vary over time or

during a host's lifetime (Clayton and Moore 1997). Moreover, the negative effect of parasitism may change during the life of host individuals. Also, parasites may affect both current and future reproductive success and/or survival of hosts, and this may have consequences for the dynamics of the interaction. However, although measuring the effects of parasites on fitness across the lifetime of hosts is of great interest, these kinds of studies are rare in comparison with short-term estimates of the prevalence and effects of parasites (e.g., Fitze et al. 2004; Benskin et al. 2009). Empirical studies across a wide range of host taxa suggest that juveniles are frequently more susceptible to pathogens than adults (see, e.g., Hudson and Dobson 1997; Sol et al. 2003; van Oers et al. 2010; Garbutt et al. 2014; Ashby and Bruns 2018 and references therein), although there are cases in which the probability of infection is positively related to age (e.g., Marzal et al. 2016). The evolutionary consequences of higher juvenile susceptibility to parasites are predicted to be a stronger expression of defenses in juveniles because hosts infected early in life suffer higher fitness costs. Despite this, it has been suggested that physiological or developmental constraints on defense expression in juveniles or trade-offs between the expression of juvenile defenses and maturation or reproduction as an adult may provide pathogens with a window of opportunity for increased disease spread and prevalence (Ashby and Bruns 2018).

Unlike endoparasitic pathogens and ectoparasites, which may affect both mortality and breeding success of their hosts (Clayton and Moore 1997; Devevey and Christe 2009; Knowles et al. 2010), avian brood parasites mainly affect reproductive success of their hosts, as they divert host resource allocation during breeding to their own benefit by laying their eggs in the nests of host species that take care of egg incubation and chick rearing (Rothstein 1990; Davies 2000; Kruger 2007; Soler 2014). Evidence suggests that host age may play an important role in the interactions between brood parasites and their hosts.

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First, host age might relate to the probability of being targeted by parasites if parasites selected hosts on the basis of traits associated with age (Parejo and Avilés 2007) or if ecological factors known to be associated with age influenced parasitism likelihood (Grim 2002). In this vein, it has been suggested that juvenile or naive hosts might be more susceptible to brood parasitism (Brooker and Brooker 1996; Grim 2002). Second, acquisition of defenses against brood parasitism by hosts may also be age dependent. In particular, it has been suggested that egg rejection, the main defense of avian host species against brood parasites (Rothstein 1990; Davies 2000), should be less frequent in young individuals because a learning period is needed to express it (e.g., Rothstein 1978; Hauber et al. 2004; de la Colina et al. 2012). For example, in great reed warblers (*Acrocephalus arundinaceus*) naive females are less likely to reject foreign eggs (Lotem et al. 1992; Moskát et al. 2014; but see Procházka et al. 2014), and in magpies (*Pica pica*) some individual hosts shift from accepting to rejecting foreign eggs when exposed to parasitic eggs several times during their lives (Molina-Morales et al. 2014).

The two longitudinal studies that have addressed the role of host age (splendid fairy wrens, Brooker and Brooker 1996; magpies, Molina-Morales et al. 2014) did not use the statistical procedures needed to adequately discriminate within- from between-individual age effects in host features, thus rendering their conclusions tentative. Van de Pol and Wright (2009) stressed the need to estimate both within- and between-subject effects because (a) when there is between-subject variation in the predictor variable (e.g., age), predictor variables in standard mixed models reflect a combination of within- and between-subject effects, and (b) significant within- and between-subject effects represent different and not mutually exclusive mechanisms and hypotheses (table 1). For instance, an age effect on egg rejection could result if host individuals that reach old ages also are more prone to reject (i.e., a between-subject effect; see table 1) or because individuals change their rejection behavior during their lifetime

(i.e., a within-subject effect; table 1). In a similar way, the susceptibility to parasitism may be related to host age because of a within-subject effect and/or a between-subject effect of age (see table 1). Both kinds of effects may have fitness consequences for the parasite: if defense levels change during the life of individual hosts (i.e., within-individual effect), parasites may benefit from selecting individuals from age classes with low defense levels. On the other hand, if host individuals differ in their rejection abilities and these abilities are related to phenotypic traits that allow them to live longer (i.e., between-individual effect), parasites may benefit by avoiding individuals bearing those traits.

Hence, a distinction between both types of effects is the only way to effectively demonstrate a genuine effect of age on the probability of parasitism or egg rejection behavior (see, e.g., Potti et al. 2014; Marzal et al. 2016; Bielański et al. 2017; table 1). Also, separating these effects may shed light on the possible existence of a genetic polymorphism in egg rejection within host populations, as has been previously claimed (Martín-Gálvez et al. 2006). The existence of a significant between-individual effect and a non-significant within-individual effect would imply the coexistence of females differing in longevity and capacity to reject eggs, which would not change through their lives. Therefore, here we separate within- from between-individual effects of age in the susceptibility to parasitism and the expression of egg rejection behavior in a host of a brood parasite. Individuals' abilities to reject parasite eggs were experimentally assessed using models resembling cuckoo eggs, as this approach provides a reliable estimate of magpie discrimination abilities selected by cuckoo parasitism (Soler and Møller 1990; Soler and Soler 2000). We use a longitudinal study of magpie females of known age, marked and monitored through their lives, in a population parasitized by the great spotted cuckoo (*Clamator glandarius*). We use these results in conjunction with accurate determination of host population age structure to discuss how demography may influence the evolutionary dynamic of magpie defenses.

**Table 1:** Hypotheses emerging from within- versus between-subject effects of age on the probability of parasitism and egg rejection of hosts of avian brood parasites

Host feature	Structure of data	Within-subject hypothesis	Between-subject hypothesis
Probability of being parasitized	Multiple breeding attempts per female in which occurrence of parasitism is recorded	Females are less likely to be parasitized when they are old	Females living longer are less parasitized
Probability of rejecting parasitic eggs	Multiple breeding attempts per female in which egg rejection of model eggs is tested	Females are more prone to reject model eggs when they are old	Female living longer are more likely to reject model eggs

## Methods

### *Study Species and Area*

The study was conducted in La Calahorra (37°10'N, 3°03'W; Granada, southern Spain) during the years 2005–2018. This is a patchy area of around 12 km<sup>2</sup> with abundant groves of almond trees (*Prunus dulcis*) where magpies preferentially build their nests (for further details, see Molina-Morales et al. 2012, 2014). Magpies are long-lived, socially monogamous passerines that start breeding at 1 (around 50% of individuals) or 2 years of age (Birkhead 1991). In Europe they are the main host of great spotted cuckoos, whose parasitism severely reduces magpie reproductive success through early hatching and effective competition for parental food delivery of cuckoo nestlings (see, e.g., Soler et al. 1996, 1997). This host-brood parasite system has selected for host recognition and rejection of cuckoo eggs and is regarded as an example of coevolution (Soler and Møller 1990; Soler and Soler 2000).

### *Individual Marking and Monitoring*

Since 2005, every fledgling (16–18 days after hatching) magpie in the population has been individually marked using a unique combination of color bands and/or alphanumeric-coded PVC bands. At the time of ringing, we took a blood sample from each individual by puncturing the brachial vein with a sterile needle. Blood samples were used to extract DNA and sex individuals using molecular markers, because males and females are not distinguishable at fledging (details of sex typing can be found in Molina-Morales et al. 2012). Many breeding magpies were also captured during nest building (using corvid traps with live magpies inside placed near the nest), bled, and color ringed. Magpie nests were monitored from the first of March to the beginning of July each breeding season. Nests were found by careful inspection of all trees in the area and GPS positioned. Each nest was observed with a telescope from a hide around 100 m away during nest building in order to assign marked birds to each nesting attempt. Nests were visited at 5-day intervals, although during egg laying and hatching the nests were visited every 2–3 days to check whether the nest was parasitized by great spotted cuckoos and to record all required data (such as clutch size or breeding success). Nests were categorized as parasitized if at least one cuckoo egg was detected in the nest. Cuckoo eggs can be easily discriminated from magpie eggs by a more rounded shape and general whiter ground color (see fig. 1 in Molina-Morales et al. 2014) as well as by their smoother texture. We are sure that this method is accurate because in more than 20 years using it we have never reported unexpected hatching of cuckoo or magpie eggs. The parasitism rate of the population was calculated as

the percentage of magpies nests parasitized out of the total nests found each year. Soler et al. (1995) estimated that rejection of naturally laid cuckoo eggs by magpies in our study area was around 5%. It is possible, however, that they did not record some rejection because that study was not experimental. In fact, in another study in which real cuckoo eggs were experimentally exchanged between magpie nests, rejection of cuckoo eggs was 12.3% (Avilés et al. 2006), so the risk of not detecting parasitized nests (because magpies rejected the cuckoo eggs quickly) is low.

We monitored 176 breeding attempts from 70 female magpies of known age over a period of 12 years (2007–2018). Most of them (62.9%) were sampled more than once (mean number of breeding attempts per female, 2.5; SD, 1.7; range, 1–7). We determined the age of magpies in two different ways. First, those fledglings ringed at the nest that recruited in the study population were of known age (exact age known, 48 females). Second, for the individuals captured at their nests, we estimated whether they were first-year individuals and then their age in successive breeding events (estimated age, 22 females) using the criteria outlined in Birkhead (1991) and Svensson (1996). In brief, first-year magpies have larger black tips at the end of their first primary feathers than adults; in our population and based on birds ringed at the nest and then of known age when captured and measured, we found statistically significant differences between first-year individuals and adults (22.5 [SD, 7.05] vs. 9.2 [SD, 2.04] mm; *t*-test,  $P < .0001$ ;  $n = 7$  and  $n = 22$ , respectively). There was, however, some overlap, with first-year individuals' tips ranging between 9.4 and 29.4 mm and adults' tips ranging between 6.6 and 13.3 mm. We decided to consider as first-year individuals only those with a black tip larger than 14.0 mm.

To estimate population age structure, we used only the data from individuals of known breeding age in the population in 2018. In this year a total of 111 breeding pairs were monitored; the majority of individuals were marked (156 of 222; 70.3%), and half of them (81 of 156; 51.9%) were individuals of known age. Thus, 36.5% of the individuals breeding in the population were of known age. This is a good estimate of the age structure of the breeding population, and in any case it is the best estimate we can use since in previous years the percentage of breeding adults of known age was smaller (31.2% in 2017, 22% in 2016, and under 20% in the other years).

### *Egg Rejection Experiments*

We tested magpie responses to model eggs made to resemble cuckoo eggs in appearance, size, and mass. Models do not perfectly match the color of real great spotted cuckoo eggs (Molina-Morales et al. 2014), and their rejection rate

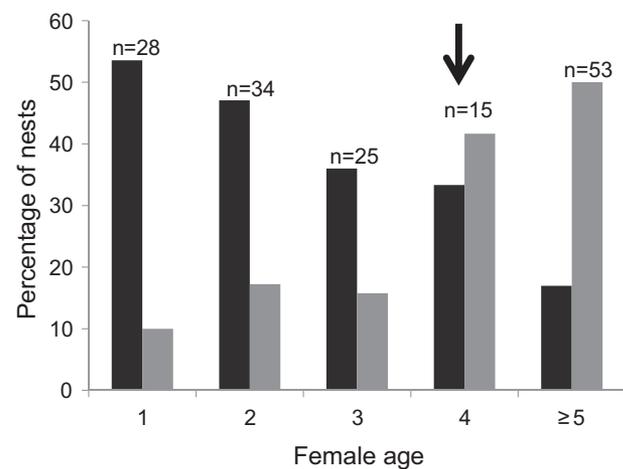
is higher than that of real cuckoo eggs. However, testing host response against model eggs is the most suitable procedure to get standardized rejection measures with cuckoo and cowbird hosts (Rothstein 1990; Davies 2000). Although by using models we are probably overestimating rejection of real cuckoo eggs, estimating rejection of naturally laid cuckoo eggs is problematic because the same female magpie might be exposed by chance to cuckoo eggs differing in mimicry from their own eggs in different years. Also, naturally laid cuckoo eggs are rarely rejected in the system; thus, gathering sufficient samples to detect an age-related pattern would be exceptionally difficult. Moreover, by using models we avoid affecting the reproductive success of magpies during the study because we do not have to visit nests every day to detect fast rejection of naturally laid cuckoo eggs. Finally, as we do not know how fast magpies can reject real cuckoo eggs (75% of models are rejected within 24 h from experimental parasitism [Avilés et al. 2004], but anecdotal evidence suggests that this may occur much faster [J. G. Martínez, M. Molina-Morales, M. Precioso, and J. M. Avilés, personal observation]), it is not feasible to study age-related patterns of rejection in a standardized way based on naturally laid cuckoo eggs. We introduced the model egg during magpie egg laying; the response to the model egg was regarded as rejection if the model egg disappeared from the nest and as acceptance if the model egg was incubated with the host's clutch when we revisited the nest. If accepted, we removed the model egg 7 days after introducing it. For further details on the procedure, see Molina-Morales et al. (2014).

### Statistical Analyses

Analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC). We proceeded in two steps. First, to test for the effect of age in the probability of parasitism and egg rejection of female magpies, we ran two generalized linear mixed models (GLMMs; hereafter, model A) in which parasitism was the binary response variable in one and egg rejection was the binary response variable in the other (link function: logit; GLIMMIX procedure in SAS), so every clutch was coded as parasitized (yes or no) or for each clutch we considered whether the female rejected the model egg (yes or no). We included in the models female age as a predictor variable, female identity as a random intercept, and parasitism rate of the population in the year of the breeding attempt as a continuous variable estimating the risk of parasitism (e.g., Thorogood and Davies 2013; Molina-Morales et al. 2014). Including population parasitism rate in the models allows us to distinguish between changes in parasitism and rejection status due to age from those due to a high or low abundance of cuckoos

in the population in different years. Second, we used the within-individual centering method described by van de Pol and Wright (2009) to distinguish between within- and between-subject effects, since females in our database are not observed or tested over the same range values of the predictor variable (age), either because of different longevities or because of incomplete sampling. For this, we ran two more GLMMs (hereafter, model B) with parasitism or egg rejection as the binary response variable in which female age was divided into two fixed effects: the mean age of each female (the between-individual effect) and the difference between the mean age of each female and the age of the female when the data were collected (delta age; within-individual effect), following van de Pol and Wright (2009; see also Potti et al. 2014; Hammers et al. 2016; Bielański et al. 2017; Clermont et al. 2018).

It could be argued that early experience with parasites may affect later probability of parasitism and rejection (Hauber et al. 2004), so that an apparent age effect may be related to the fact that some younger individuals experienced brood parasitism while others did not. We cannot include previous experience of parasitism in the above analyses because of gaps in our data set. Also, the analyses should be based on individuals reaching older ages because young ones are mostly accepters (fig. 1). To approach the question, we targeted those 19 females that lived longer than 3 years (the average age of adults in the population is 3.5 years) and for which we had information on parasitism and rejection before and after that



**Figure 1:** Black bars show the percentage of parasitized nests for each age class. Gray bars show the percentage of rejection in model egg rejection tests for each age class. Class 5 includes females aged 5 years old or older. The black arrow signals the mean age at which females start rejecting model eggs. Numbers above bars are the sample size for each age class. Data underlying this figure have been deposited in the Dryad Digital Repository (<https://doi.org/10.5061/dryad.hmgqk9c6>; Martínez et al. 2019).

threshold age. Females were classified as being parasitized or not before (early parasitism status) or after (late parasitism status) that threshold. We then used a logistic regression (GENMOD procedure in SAS) with rejection as the dependent variable and with early parasitism status and late parasitism status as categorical predictors to test whether early or late parasitism experience influences the probability of rejecting eggs at older ages. We also used a logistic regression to test whether early parasitism status influenced late parasitism status.

#### *Ethics Note*

The research presented here was conducted in accordance with all applicable laws and rules set forth by the Spanish government and Junta de Andalucía, and all necessary permits were in hand when the research was conducted.

### **Results**

#### *Age and Parasitism*

Age was significantly related with probability of parasitism in magpies after controlling for the population parasitism rate in the year of the nesting event and female identity (model A; table 2): more than 50% of nests of first-year females were parasitized, and the probability

of parasitism steadily declined to less than 20% in nests of females of 5 years old or older (table 2; fig. 1). However, we cannot ascertain the exact mechanism behind this age-related pattern because in the model in which we simultaneously consider within- and between-subject effects none of them significantly explained the probability of parasitism (model B; table 2).

#### *Age and Rejection*

Female age was positively related to the probability of rejecting a model egg once we accounted for the population parasitism rate in the year each egg rejection experiment was performed and female identity (model A; table 3): rejection probability increased from 10% in 1-year-old magpies to 50% in females of 5 years old or older (fig. 1). Model B revealed that the effect of age was due to both within- and between-individuals effects, since both terms were significant (model B; table 3). Nearly half of the females (42.9%;  $n = 63$ ) rejected at least once, and they always changed from accepting to rejecting model eggs during their lives; for those females in which we could determine the exact age at which they changed behavior, the mean age to start rejecting was 3.93 years (SD, 1.98;  $n = 15$ ). Most females (81.5%;  $n = 27$ ) that start rejecting at some point in their lives always rejected model eggs the following years.

**Table 2:** Age and parasitism in female magpies (dependent variable: probability of parasitism)

	Covariance parameter	SE	df	Z	P
Model A:					
Random effects:					
Female ID	.15	.40	...	.39	.35
	Estimate			F	
Fixed effects:					
Intercept	-1.07	.69	1,131	1.54	.13
<b>Age</b>	<b>-.21</b>	<b>.09</b>	<b>1,131</b>	<b>4.85</b>	<b>.03</b>
<b>Population parasitism rate</b>	<b>.03</b>	<b>.01</b>	<b>1,131</b>	<b>5.16</b>	<b>.03</b>
	Covariance parameter			Z	
Model B:					
Random effects:					
Female ID	.18	.42	...	.45	.33
	Estimate			F	
Fixed effects:					
Intercept	-1.04	.71	1,114	1.48	.14
Mean age (between-individual effect)	-.22	.12	1,69	3.23	.08
Delta age (within-individual effect)	.18	.14	1,130	1.89	.17
<b>Population parasitism rate</b>	<b>.04</b>	<b>.01</b>	<b>1,130</b>	<b>4.94</b>	<b>.03</b>

Note: Shown are results of generalized linear mixed models testing for the effect of parasitism rate in the population, age of the female, and female identity on the probability of parasitism of magpie females as binary dependent variables. In model A, age is one of the fixed predictors; in model B, we use the within-centering method, and delta age and mean age are used as fixed predictors (within- and between-subject effects; see "Methods"). We used the Satterthwaite method to calculate degrees of freedom. Significant terms are highlighted in boldface.

**Table 3:** Age and rejection in female magpies (dependent variable: probability of rejection)

	Covariance parameter	SE	df	Z	P
Model A:					
Random effects:					
<b>Female ID</b>	<b>1.39</b>	<b>.74</b>	...	<b>1.86</b>	<b>.03</b>
	Estimate			F	
Fixed effects:					
Intercept	-1.36	.91	1,131	1.50	.13
<b>Age</b>	<b>.35</b>	<b>.11</b>	<b>1,131</b>	<b>11.15</b>	<b>.001</b>
Population parasitism rate	-.02	.02	1,131	1.53	.21
	Covariance parameter			Z	
Model B:					
Random effects:					
<b>Female ID</b>	<b>1.42</b>	<b>.76</b>	...	<b>1.88</b>	<b>.03</b>
	Estimate			F	
Fixed effects:					
Term					
Intercept	-1.28	.97	1,130	1.32	.18
<b>Mean age</b> (between-individual effect)	<b>.32</b>	<b>.16</b>	<b>1,65</b>	<b>4.09</b>	<b>.05</b>
<b>Delta age</b> (within-individual effect)	<b>.38</b>	<b>.16</b>	<b>1,130</b>	<b>5.94</b>	<b>.02</b>
Population parasitism rate	-.02	.02	1,130	1.42	.23

Note: Shown are results of generalized linear mixed models testing for the effect of parasitism rate in the population, age of the female, and female identity on the probability of rejection of mimetic model eggs of magpie females as binary dependent variables. In model A, age is one of the fixed predictors; in model B, we use the within-centering method, and delta age and mean age are used as fixed predictors (within- and between-subject effects; see "Methods"). We used the Satterthwaite method to calculate degrees of freedom. Significant terms are highlighted in boldface.

### Population Age Structure

The mean age of adults in the population in 2018 was 3.5 years (SD, 2.88;  $n = 81$ ), and the age structure was clearly biased toward younger age classes, with the majority of individuals (71%) being 4 years old or younger. The pattern was similar for males and females (maximum likelihood  $\chi^2 = 10.83$ ,  $df = 10$ ,  $P = .37$ ;  $n = 31$  females and 41 males), and 67.7% of the females breeding in the population in 2018 were 4 years old or younger (fig. 2).

### Previous Parasitism Experience

Neither early ( $\chi^2 = 0.76$ ,  $df = 1$ ,  $P = .38$ ) nor late ( $\chi^2 = 0.27$ ,  $df = 1$ ,  $P = .60$ ) parasitism explained rejection at older ages in magpies. However, we found a trend suggesting an effect of early parasitism on late parasitism ( $\chi^2 = 3.68$ ,  $df = 1$ ,  $P = .06$ ), so that females parasitized at least once during the first 3 years of their life had a higher probability of being parasitized at older ages (8 of 16 [50%], whereas none of the 3 females [0%] not parasitized at young ages were later parasitized).

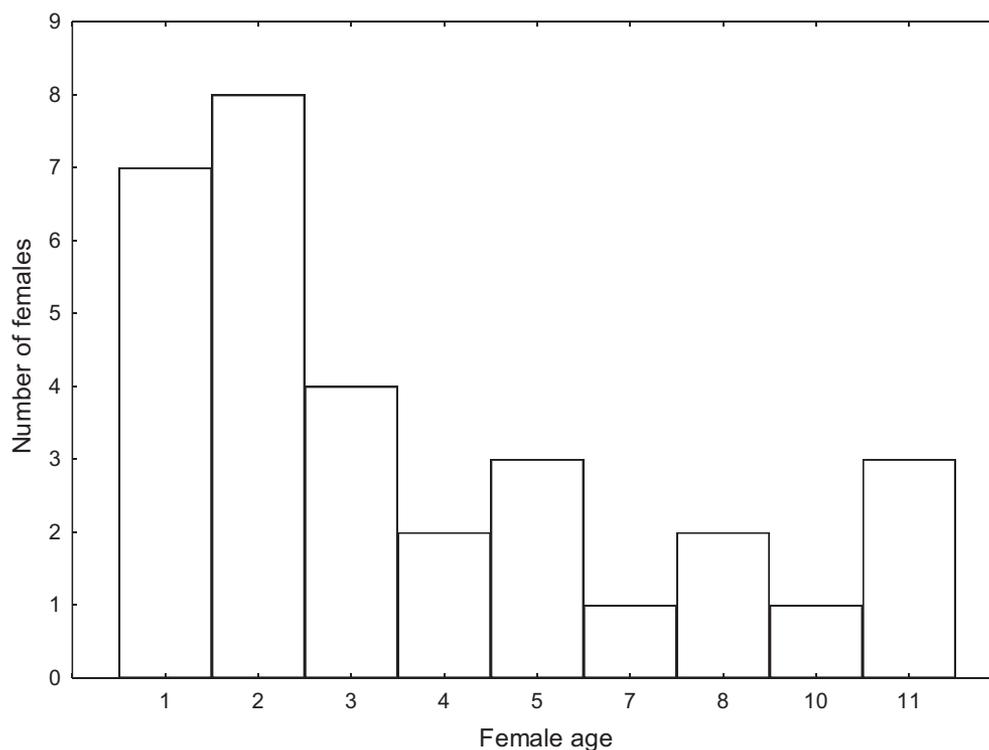
### Discussion

We used a longitudinal study of individual magpie hosts of known age to explore the potential role of host age in brood parasite–host interactions. Mixed models includ-

ing age as a fixed predictor showed significant age effects on both response variables (model A, not considering between- and within-subject effects, in tables 2 and 3), after controlling for individual identity (female ID). This suggests that older magpies are less likely to be parasitized and more likely to reject model eggs (fig. 1). However, because we sampled females with a different range of ages and longevities, this analysis cannot determine whether such a pattern is due to changes in the probability of being parasitized or rejecting eggs during the lifetime of individual hosts, to an effect of longevity on both variables, or to a combination of them (see table 1; van de Pol and Verhulst 2006; van de Pol and Wright 2009).

### Parasitism

The association between age and parasitism suggested by model A cannot be due to a change in the likelihood of parasitism of individual females as they age, since the within-individual effect in model B was not significant. However, the between-individual effect in model B ( $P = .08$ ; table 2), though nonsignificant, suggests that the age-related pattern of parasitism observed in figure 1 could be the consequence of a relationship between longevity and probability of parasitism, with longer-lived females being able to escape parasitism more often. Two non-mutually-exclusive



**Figure 2:** Histogram showing the number of females of each age class in the population in 2018.

mechanisms may explain this pattern. First, it is possible that older magpies are more likely to reject eggs quickly and are therefore less likely to be counted as parasitized. Our results with model eggs show that older female magpies are more likely to be egg rejecters than younger ones (see fig. 1), which would agree with this possibility. Against this possibility, however, analyses based on females that lived longer than 3 years showed that females parasitized early in their lives tended to have a greater probability of being parasitized at older ages. Although the rate of rejection of real cuckoo eggs by magpies reported before is very low (see above), there was no information about the age of individuals in those studies. Therefore, future studies should evaluate whether rejection of natural cuckoo eggs varies with age in magpie hosts.

Alternatively, it could be that the lower parasitism likelihood of long-lived females resulted from the parasites not targeting those individuals. A few studies with other cuckoo hosts have suggested a higher probability of being parasitized for young or naive hosts (Brooker and Brooker 1996; Øien et al. 1996; Grim 2002). However, in the great spotted cuckoo-magpie system, previous work has shown that magpie features likely to be targeted by searching cuckoos are repeatable (Molina-Morales et al. 2013) and that emerging parasitism patterns in relation to host features are contingent on yearly cuckoo abundance (Molina-Morales et al. 2016; see also table 1),

meaning that there would be a low chance for a pattern of parasitism due to active choice of younger hosts. Indeed, as noted above females parasitized early in their lives had a high probability of being parasitized at older ages, again suggesting a between-individual effect for parasitism. Nonetheless, our results would support a pattern of structured cuckoo parasitism based on differences in longevity between host individuals. Such a pattern was previously shown within the same magpie population, but it was based on phenotypic traits of magpie individuals of unknown age (Molina-Morales et al. 2012). Hence, the pattern found in that study is likely due to the association between longevity and phenotypic traits. From an evolutionary perspective, this would imply that great spotted cuckoo parasitism selects for longer magpie lifespan in parasitized populations. Indeed, magpies in non-parasitized English populations have shorter lifespan (2.4 years, Högstedt 1981; 2.8 years, Birkhead 1991) than in our population (3.5 years; see “Results”). However, further studies in different parasitized and nonparasitized host populations are clearly needed to infer selection on longevity by brood parasites.

#### *Egg Rejection*

Within-individual effects of age on the probability of rejecting model eggs (table 3) indicate that females change

their response from acceptance to rejection during their lifetime, in accordance with previous results in magpies (Molina-Morales et al. 2014) and other studies suggesting that naive or young hosts are less able to reject parasitic eggs (e.g., Lotem et al. 1992; Moskát et al. 2014). The between-individual effect suggests that birds that live longer are also rejecters. Two non-mutually-exclusive explanations for this pattern are possible. First, the pattern emerges due to the unbalanced nature of data (see above) and to the fact that because individuals are more prone to reject as they age, long-lived individuals are more likely to reject and short-lived individuals (or those sampled only at the beginning of their lives) are more likely to accept. Alternatively, the pattern could be the consequence of a genetic polymorphism whereby some birds reject and some do not, and among the birds that do reject, there is an effect of age. Although we do not have genetic data to distinguish between these two possibilities, we found that 63.33% of females living 5 years or more were rejecters. That is, almost 37% of females do still accept despite reaching the oldest ages, which would be consistent with the second possibility. Our results, however, do not support a clear-cut genetic polymorphism in rejection (i.e., acceptors vs. rejecters), which has been suggested in previous work (Martín-Gálvez et al. 2006), because although we found a significant effect of female ID on rejection (see table 3), the significant within-individual effect implies that female magpies may change their rejection behavior through their lives (see also Molina-Morales et al. 2014).

Including between- and within-individual effects in the analyses and testing females of known age several times during their lifetime make the conclusions on age patterns of rejection particularly sound. First, we have shown directly that rejection changes with age. Second, by studying a wide range of ages we have been able to determine that most females need several breeding events (years) before they start rejecting (the mean age for starting rejection was 3.93 years). There is a widely accepted idea that some kind of learning mechanism must be involved in the expression of egg rejection (see Rothstein 1978; Lotem et al. 1992; Stokke et al. 2007; de la Colina et al. 2012), and although our data do not allow us to identify mechanisms, they do support a prolonged learning mechanism (*sensu* Stokke et al. 2007).

Moreover, our results suggest that some level of acceptance may be expected in the populations due to their age structure. The age-related pattern of rejection we found suggests that females with a short lifespan would more likely be accepters and that rejection should be mainly carried out by long-lived females. In our population, 32.3% of breeding females were older than 4 years in 2018, consistent with a population rejection rate of 23.17% we recorded in that year and an overall rejection rate of around

30% in the population during the whole period of study (the average annual rejection rate during the study period was 29.72% [SD, 8.06,  $n = 11$ ]; see also Avilés et al. 2014; Molina-Morales et al. 2014). Thus, the age structure of host populations might contribute to variation in egg rejection rates among populations of many host species, adding to previous ecological and evolutionary factors known to be related to parasitism (see reviews in Davies 2000; Kruger 2007; Soler 2014). Nonetheless, current and previous findings still show that some host females likely never reject (Molina-Morales et al. 2014), which would suggest that a fraction of females would still accept irrespective of the age structure of the population.

The scope of our results on egg rejection should be considered in light of experimental parasitism with model eggs, which we assume correlate with rejection of naturally laid cuckoo eggs in magpies (see the introduction). Experiments with model eggs provide most of the empirical basis for the current understanding of the evolution of host rejection behaviors in most brood parasite–host systems (Rothstein 1990; Davies 2000). Future studies should ideally try to determine whether age-related patterns in rejection hold when assessed with real cuckoo eggs.

Summing up, our results suggest that cuckoo parasitism can depend on host age, implying that parasitism might be a powerful selective agent on host longevity that is worth exploring in future studies. Also, our results suggest that the age pattern of host defenses at the population level may arise as a result of both changes in defense through the life of host individuals and differences between individuals in their proneness to reject eggs, which is linked to longevity. Our results thus suggest that brood parasites exploiting younger host individuals would benefit from a lower defense level of their hosts and that the intensity of selection by brood parasites may be mediated by the age structure of host populations. Hence, our results stress the importance of considering the age structure of host populations to fully understand both patterns and mechanisms of parasitism and defense at the host population level.

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### Data and Code Availability

Data are available from the Dryad Digital Repository (<https://doi.org/10.5061/dryad.hmgqnk9c6>; Martínez et al. 2019).

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A few cuckoo chicks around 5 or 6 days of age. Photo credit: Mercedes Molina-Morales.