Relaxed selection diminishes social memory and expression of host defences against cuckoos

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Abstract

Behavioural defences are a key adaptation across animals, but confirming decay under relaxed selection is challenging. If expression involves decision-making, then the defence may only be expressed when the right environmental cues are available (cryptic plasticity). Even if defences wane, they could be quickly restored once selection resumes as animals can learn by observing others (social memory). Here we tested cryptic plasticity and social memory under relaxed selection in a cuckoo host, the Common Reed Warbler, by manipulating perceived parasitism risk with social information in geographically-distinct areas differing in allopatry (100 to 1000 years). Despite predicting social information of a cuckoo vs. a control would elicit otherwise cryptic behavioural defences, only birds that mobbed model cuckoos prior to manipulation upregulated this defence, and only in the more recently allopatric population. Social information also had no effect on low-to-absent rates of foreign egg rejection, a second line of defence common to cuckoo host species. These results contrast with parasitised locations where similar methods provoke mobbing and increase egg rejection ten-fold. Our study therefore demonstrates that, even when manipulating information to account for reaction norms, behavioural defences may degrade rapidly under relaxed selection, and thus facilitate a geographic mosaic of coevolution.

Main Text

Introduction

As species' ranges expand or contract, their exposure to enemies and selection pressures can also change (Gilman *et al.* 2010). Where selection relaxes (i.e. with allopatry), traits that were previously adaptive are expected to reduce (e.g. due to drift), particularly if maintaining the trait is costly (Lahti *et al.* 2009). However, while behavioural defences are common across the animal kingdom (Candolin & Wong 2015), whether these respond to relaxed selection is rarely tested (Foster 2013; Lahti *et al.* 2009). Behaviours are assumed unlikely to be lost due to complex genetic architectures (pleiotropy) and underlying trait variation that can be co-opted in response to other selective pressures (Lahti *et al.* 2009). However, another key issue is that loss of behaviour can be challenging to measure. Behavioural responses depend on prior experience and availability of information about the relative costs or benefits of taking action [i.e. information ecology (Dall *et al.* 2005)]. Therefore, the absence of a response does not necessarily indicate a lack of enemy recognition but rather cryptic plasticity (Foster 2013). Taking an informational approach (Gil *et al.* 2018; O'Connor *et al.* 2019) and manipulating cues in allopatry, however, makes it possible to approximate the retention of behavioural reaction norms in the wild.

Social 'memory' could play a key role in determining whether behaviours are lost or retained. For example, prey and hosts observe the behaviour of others to learn about novel threats (Feeney & Langmore 2013; Langmore *et al.* 2012; Rowell *et al.* 2020) and assess risk of predation or parasitism (Gil *et al.* 2018), while predators use social information to find suitable prey (Hämäläinen *et al.* 2022). Therefore, how individuals adjust their behaviour in response to social information [i.e. social responsiveness; (de Groot *et al.* 2023)] is likely to affect both their own fitness and the strength of selection on interacting parties (Cantor *et al.* 2021; Thorogood & Davies 2012). Social responsiveness may also determine whether populations are resilient to environmental change (Gil *et al.* 2019; Thorogood *et al.* 2018), especially as the utility of social information depends on the availability of more-informed 'demonstrators'. For example, gene flow among populations experiencing variable selection pressures (i.e. trait re-mixing) is thought to determine how quickly a host population responds to shifts in the strength of selection by parasites [Geographic Mosaic Theory of Coevolution; (Thompson 2005)]. If experienced individuals from 'hotspots' of current selection also introduce social information to naïve 'cold spots' of relaxed selection, social learning could speed up the recovery of defences in host populations that are later reinvaded by a previous enemy. However, while recent experimental evolution studies show that social behaviours can diminish very rapidly after selection is removed (Bladon *et al.* 2023), the role of social cues in

maintaining behaviours in ‘cold spots’ is still largely unknown (Lahti *et al.* 2009; Langmore *et al.* 2012).

Avian brood parasitism provides an ideal system to test if behavioural defences and social responsiveness decline in allopatry, altering the potential for hosts to respond to future (re)invasion. Virulent brood parasites lay eggs in the nests of host species and can exert strong selection for defensive behaviours such as mobbing or rejecting suspicious eggs. However, many brood parasites are aggressive or Batesian mimics (Feeney *et al.* 2015; Welbergen & Davies 2011), selecting for hosts to adjust defences according to local risk and minimise costly mistakes (Lindholm & Thomas 2000; Thorogood & Davies 2013). Parasitism can also vary in time and space with some host populations or species escaping parasitism for many generations (Grim & Stokke 2016). Over time in allopatry, plasticity is expected to be lost with hosts either becoming resistant to reinvasion (i.e. fixation of defences) or susceptible to extinction if defences wane (Soler 2014) but it is unclear which trajectory will occur. Some species long-separated from brood parasites (i.e. hundreds of thousands to millions of years) show persistent (experimentally-induced) egg rejection (Bolen *et al.* 2000; Briskie *et al.* 1992; Chaumont *et al.* 2021; Moskát, C. *et al.* 2002; Rothstein 2001), but this common defence appears to reduce in others (Briskie *et al.* 1992; Davies & Brooke 1989; Soler & Møller 1990; Yang *et al.* 2015, 2021). Until recently, mobbing of adult brood parasites has received less attention (Feeney *et al.* 2012), but this frontline defence also appears to degrade (Briskie *et al.* 1992; Lawson *et al.* 2023) or be preserved (Briskie *et al.* 1992; Chaumont *et al.* 2021; Kuehn *et al.* 2016) under recent [e.g. lost in < 100 years; (Hale & V. Briskie 2007; Lawson *et al.* 2020)] or longer allopatry. Conversely, apparently naïve species can also obtain host defences very rapidly upon invasion (Nakamura 1991) but whether these changes represent the cryptic persistence of defences in the population, gene flow from parasitised areas, or novel learning is unknown.

Here we use allopatric populations of Common reed warblers (*Acrocephalus scirpaceus*) at the northern- and southernmost extents of their European breeding range to test for the retention of behavioural defences and social responsiveness to brood-parasitic Common cuckoos (*Cuculus canorus*). Reed warblers colonised Finland ca.100 years ago (Järvinen & Ulfstrand 1980), most likely expanding from the southwest (Bergman *et al.* 2025) and away from regularly parasitised areas in the range core (Avilés *et al.* 2006). Cuckoos in Finland use different host species, away from reedbeds. In Sicily, reed warblers are likely to have escaped parasitism by cuckoos for much longer – cuckoos only occur on mountainsides while reed warblers are largely restricted to coastal areas, and mentions of both species in historical records are not in association with each other (Arnott 2007). At parasitised sites in their range-core, reed warblers upregulate mobbing

(Campobello & Sealy 2011; Davies & Welbergen 2009) and egg rejection (Thorogood & Davies 2016) defences after observing their neighbours attack cuckoos. Among populations not currently exposed to parasitism, however, egg rejection rates vary widely [e.g. from 0–40%, (Thorogood & Davies 2013)] and mobbing responses are reduced [e.g. 25% of pairs; (47)]. However, as in other studies of brood-parasite host defences in allopatry (see also Rothstein 2001), perceived parasitism risk has not yet been manipulated (e.g. via social information) so the extent to which host defences reduce under relaxed selection remains unclear.

Therefore, we experimentally manipulated social information and simulated parasitism in field experiments to test if defences and/or social responsiveness decay in allopatry. We predicted that if defences are retained, then providing social information should induce plasticity (i.e. social responsiveness) and lead to an increase in mobbing (propensity and intensity) and egg rejection, as previously shown in range-core populations. However, if defences are not upregulated in response to our manipulated cues, then this would provide evidence that these behaviours are being lost under relaxed selection. Additionally, we expected any loss of defences or weakening of social responsiveness to be more pronounced in Sicily as reed warblers there are likely to have been in allopatry for longer.

Materials and Methods

Study areas and host populations

We studied unparasitised populations of Common reed warblers breeding in May-July around Helsinki, southern Finland (30 discrete reed-lined bays along 100km of coastline, 60.19848N 24.07305E to 60.34039N 25.71162E, in 2019 and 2020) and in the Fiume Ciane nature reserve near Siracusa, Sicily, Italy (37.04285N 15.24273E, in 2022), avoiding re-use of areas across years in Finland to avoid contamination of experience or pseudoreplication. Reed warblers nest at relatively low densities in Helsinki (Tolman *et al.* 2021) but are more numerous in Fiume Ciane (median distance to closest nest = 36 m, inter-quartile range = 24-53 m). We found nests by cold-searching or monitoring singing males and recorded location and distance to the nearest potential cuckoo vantage point [tree >3 m in height, a possible indirect cue of parasitism risk; (Oien *et al.* 1996; Welbergen & Davies 2009)] using handheld GPS (accuracy ± 1 m).

Experimental protocols

To compare results to previously-studied parasitised populations in the range-core, we adopted published protocols (Davies & Welbergen 2009; Thorogood & Davies 2016) as follows. Experiments started on the day the fourth egg was laid [clutch sizes = 3-5 eggs, incubation begins

with the penultimate egg; (Cramp & Brooks 1992)]. At each nest we measured the change in mobbing response to a cuckoo after presentation of social information of either a cuckoo or control. We used the same 3D-printed model cuckoos (painted by an artist, see 50 for details) in Finland and Sicily but controls differed. In Finland we used a commercially-available plastic magpie (Live Decoys) to assess whether changes in mobbing may be due to nest predators or specific to cuckoos, and in Sicily we used plastic female teal hunting decoys (Flambeau Outdoors), similar to previous range-core experiments using innocuous controls (Davies & Welbergen 2009). Although this difference precludes comparisons of responses to controls, our focus was on probing the generality of previous findings in the context of relaxed selection, and at parasitised sites there has been similar variation in controls and presentation orders (Campobello & Sealy 2011; Davies & Welbergen 2009; Thorogood & Davies 2012, 2016). To also compare how reed warblers at each site responded to a benign control, we presented teal models at 48 additional nests in Finland in 2018-2020. Model cuckoos elicit similar responses as taxidermy specimens (Chen *et al.* 2022; Welbergen & Davies 2008) and ours were printed using the same 3D template as independent work (e.g. Marton *et al.* 2019).

To assay mobbing behaviour [bill snaps and rasping calls (Welbergen & Davies 2008)], we placed a model adjacent to the nest rim and recorded latency of the first bird to arrive within 3 m (using a stopwatch, seconds). We filmed each trial with a small action camera on a pole 1 m from the nest while observing from 3-5 m away, depending on visibility, and counted mobbing vocalisations for 5 min (mobbing intensity). Three observers carried out experiments in Finland, each with a different set of models. One observer also performed trials in Sicily. There was high consistency among observers' counts of bill snaps [ICC: 0.92 (95%CI 0.80–0.98), $p < 0.001$] and rasps [ICC: 0.98 (95%CI 0.95–1.0), $p < 0.001$], as assessed using the Intraclass Correlation Coefficient (ICC) in the *irr* package in R (see Statistical analyses below) from data collected later using 10 videos and blind to field-collected data.

We used simulated neighbour presentations to provide social information 10 m from the focal nest (cf. active neighbours < 40 m in Davies & Welbergen 2009). A previously-collected reed warbler nest was attached to reeds at a similar height to the focal nest, with either a model cuckoo or control adjacent to it (treatments assigned alternately). We then broadcast one of 5 playback tracks of reed warblers mobbing a model cuckoo [recorded for range-core experiments; (Davies & Welbergen 2009; Thorogood & Davies 2016)] for 10 min from a speaker placed under the dummy nest. Focal nests were at least 40 m from other nests or experimental neighbour playbacks to avoid contamination of information. Live mobbing by neighbours does not influence social responsiveness to playbacks (Davies & Welbergen 2009; Thorogood & Davies 2012) and simulated

vs. active nests elicited similar responses to social information treatments in previous experiments (Campobello & Sealy 2011; Thorogood & Davies 2016). In Sicily, where densities were higher, we used active neighbouring nests for social information presentations in 4 trials (3 cuckoo and 1 teal; < 20m from the focal nest) but none of these focal pairs mobbed a cuckoo.

In Finland, we always presented the cuckoo model at the start of the experiment (P1) and after (P3) presentation of social information (SI) of either a magpie control or a cuckoo (Fig. 1A). To avoid pairs in the cuckoo SI treatment being exposed to cuckoos more (i.e. P1, SI, P3) than in the magpie SI treatment (P1 & P3 only), we included a second presentation (P2) of either a cuckoo (magpie SI treatment) or magpie (cuckoo SI treatment) before SI (Fig. 1A). This design also allowed us to compare whether any changes in behaviour were due to repeated exposure rather than the SI treatment. To rule out order effects, in Sicily (Fig. 1D) we followed the same design as Davies & Welbergen (Davies & Welbergen 2009): here each nest was presented with a cuckoo and control (teal) in random order before (P1 & P2) and after (P3 & P4) social information (SI). We waited at least 1.5 h between presentations at both sites and conducted trials between 10:00 and 18:00 local time as cuckoos tend to lay in the afternoon. As such, presentations were split over 2 days for each nest.

In trials where mobbing responses were very weak (i.e. fewer than 20 vocalisations), we later confirmed sounds from video recordings of the trial and excluded five cases where three observers could not discern or agree on sound categorisation. We also excluded 4 nests (1 in Finland and 3 in Sicily) where birds failed to appear at one or more cuckoo presentations at their nest (field observations confirmed by video). In total, we analysed mobbing from 83 Finnish nests (Cuckoo treatment n = 42; Magpie treatment n = 41) and 40 Sicilian nests (Cuckoo treatment n = 22; Teal treatment n = 18).

Measuring egg rejection defences

After the final model presentation, we coloured one egg at random from the focal nest with 40 equally-spaced brown dots using a Staedtler permanent felt tip pen (Lumocolor® 314-7) to measure rejection (as in Thorogood & Davies 2016) of a pseudo reed warbler-gens ‘cuckoo egg’ (Finland: Cuckoo treatment n = 24, Magpie treatment n = 25; Sicily: Cuckoo treatment n = 22, Teal treatment n = 18). Due to a low response (see Results), for trials conducted in Finland in 2020 we then used custom 3D-printed eggs with the same dimensions and colour (Citadel© blue acrylic paints, custom mixed) as a highly non-mimetic redstart-gens cuckoo egg (Cuckoo treatment n = 29, Magpie treatment n = 27). In all trials, nests were checked 3-6 days later for removal of the ‘cuckoo egg’ (reed warblers most often reject within 3 days; Davies & Brooke 1988) or evidence of damage (reed

warblers were not able to pierce and remove 3D-printed models despite vigorous pecking: 4/25 rejection trials filmed in a subsequent year, unpublished data). Finally, in Sicily we also assessed non-mimetic egg rejection without any information of cuckoo presence (n = 11) and with personal information of parasitism risk (i.e. by presenting a cuckoo model at the nest; n = 12) by colouring one egg in the nest entirely with a blue felt-tip pen (Copic Ciao B).

Statistical analyses

All analyses were conducted in R v.4.3.2 (R Core Team 2023). First, we used GLMs to check for bias of potential indirect cues (i.e. distance to potential cuckoo vantage points, see Supporting Information S1) and to confirm that initial responses to cuckoos (i.e. P1 in Finland, or P1|P2 in Sicily) were similar in the treatment groups and among years of the study (in Finland). We then used GLMMs with a random effect for nest identity when modelling change in response across multiple presentations (i.e. P1 vs. P3, or P1 vs. P2) as implemented in the package *glmmTMB* (Brooks *et al.* 2017). To investigate whether social information led to a change in mobbing propensity or intensity, we only included responses to the first and last presentation of a cuckoo at the nest (i.e. P1 and P3 in Finland). However, due to a large proportion of zeroes and lack of suitable fit for a zero-inflated model structure, we adopted a hurdle approach to model mobbing propensity (i.e. binary variable, binomial error structure and logit-link function) and intensity (i.e. number of calls in at least one presentation > 0, quasi-poisson (*nbinom1*) distribution) separately in Finland. For mobbing data from Sicily, we used Fisher's Exact tests to compare treatment effects on mobbing propensity and quasi-binomial GLMMs to investigate changes in mobbing intensity across all pairs, due to the smaller sample size. To investigate whether social information affected egg rejection decisions, we used a GLM (binomial error structure, logit-link function) which included clutch size as a covariate predictor. Despite data being collected across 30 discrete sites in Finland, including location as a random effect did not improve model fit (Akaike Information Criterion > 2) so it was not part of our final models.

Finally, we investigated latency to approach the nest during experiments to assess more subtle differences in reaction to cuckoos (i.e. cryptic recognition without a mobbing response). We used separate GLMMs with Tweedie distributions to test if change in latency to approach across presentations differed (i) between treatments and (ii) between mobbing versus non-mobbing pairs as we lacked power to test a Presentation order*SI treatment*Mobbing status 3-way interaction. We used QQ-normality plots, residuals vs. predicted plots, and dispersion and outlier tests using package *DHARMa* (Hartig 2022) to assess assumptions and fits of all models, and we present estimated mean effect sizes (± standard error), z-statistics, and p-values. Summary tables of all models are available in Supporting Information S2.

Results

Mobbing defences

When first presented with a cuckoo at the nest, 36% (30/83) of pairs in Finland and 13% (5/40) of pairs in Sicily showed a mobbing response (Fig. 1). This propensity to mob did not differ between social information (SI) treatment groups (Finland, GLM: estimate = 0.16 ± 0.46, z = -0.35, p = 0.73; Sicily, Fisher's Exact test: p = 1.0; Table S2.1 in Supporting Information) or among years of the study in Finland (GLM: estimate = 0.25 ± 0.48, z = 0.52, p = 0.60; Table S2.1).

In Finland, providing social information about the risk of parasitism in the local area did not induce significantly more reed warblers to mob cuckoos than pairs exposed to information about magpies (GLMM Presentation order * SI treatment: estimate = 3.99 ± 2.39, z = 1.67, p = 0.099, Fig. 1B, Fig. 2A; Table S2.2) and this lack of interaction was not because social information about magpies also induced increased mobbing propensity (Fig. 1C, Fig. 2A).

Social information about cuckoos did, however, increase mobbing intensity (i.e. number of signals) of pairs who responded (across treatments n = 36 pairs with calls >0, Fig. 1B,C; GLMM Presentation order * SI treatment: estimate = 0.97 ± 0.45, z = 2.14, p = 0.032, Fig. 2B; Table S2.2) compared to those who received social information about magpies where there was no significant change among presentations (GLMM estimate = -0.28 ± 0.33, z = -0.84, p = 0.40, Fig. 2B; Table S2.2). Instead, these pairs were just as likely to increase or reduce mobbing intensity by the third presentation (P3; Fig. 1C).

In Sicily, social information about cuckoos had no effect on either mobbing propensity (across treatments n = 10 pairs with calls >0, Fisher's exact test: p = 1.0, Fig. 2A) or intensity (GLMM Presentation order * SI treatment: estimate = -1.20 ± 0.88, z = -1.36, p = 0.17, Fig. 2B; Fig. 1E, F; Table S2.3). This meant that, overall, reed warblers in Finland were more likely to mob cuckoos presented during our experiments (19/42, 45%) than pairs in Sicily (5/22, 23%; GLM: estimate = -1.03 ± 0.42, z = -2.45, p = 0.014; Table S2.4), and at a greater intensity (n = 24, GLM: estimate = 1.44 ± 0.72, z = -2.01, p = 0.044; Table S2.4). These differences between the northern and southern

areas are likely to be specific to cuckoos, as teal were mobbed at similar intensities between both populations (n = 96, GLM: estimate = -0.39 ± 0.31, z = -1.27, p = 0.21; Table S2.5).

Egg rejection defences

Reed warblers did not reject 'mimetic' brown-spotted eggs in either Finland (Cuckoo treatment = 0/24; Magpie treatment = 0/25; Fig. 3) or Sicily (Cuckoo treatment = 0/21; Teal treatment = 0/19), and there was no rejection of non-mimetic blue eggs in Sicily (no cuckoo presentation = 0/11, after cuckoo presentation at nest = 0/12). Blue eggs were however rejected at low rates in Finland, although this did not differ between social information treatments (GLM: estimate = 0.11 ± 0.64, z = 0.17, p = 0.97; Fig. 3; Table S2.6).

Recognition of cuckoos vs. response

In Finland, reed warblers became faster to approach their nests across subsequent presentations of cuckoos (i.e. P1 vs P3; GLMM estimate = -0.45 ± 0.19, z = -2.37, p = 0.018; Fig. 4A; Table S2.7), regardless of social information (i.e. difference in SI treatment at P3; GLMM estimate = 0.30 ± 0.27, z = 1.12, p = 0.26; Fig. 4A; Table S2.7). Birds were, however, slower to approach a magpie than a cuckoo at P2 (i.e. difference in SI treatment at P2; GLMM estimate = 0.72 ± 0.26, z = 2.81, p = 0.005; Fig. 4A; Table S2.8) even if they never mobbed (P2 data only, GLM mobbing status * SI treatment: estimate = 0.11 ± 0.38, z = 0.29, p = 0.78; Fig. 4A; Table S2.8) suggesting that latency was specific to the threat at the nest. Mobbing birds were quicker to approach cuckoos at first (P1 data only, GLM estimate = -0.42 ± 0.19, z = -2.29, p = 0.022; Fig. 4B; Table S2.9), but non-mobbing birds reduced their latencies more across presentations (GLMM Presentation order * mobbing status interaction: estimate = 0.54 ± 0.28, z = 1.96, p = 0.049; Fig. 4B; Table S2.10) so that there was no difference by the end of the experiment (GLM estimate = -0.12 ± 0.21, z = 0.58, p = 0.56; Fig. 4B; Table S2.9). Reed warblers in Sicily approached cuckoos at the nest more quickly than in Finland (GLM estimate = -0.47 ± 0.20, z = -2.37, p = 0.018; Table S2.11), but latencies did not change over the experiment, either overall (GLMM Presentation order: estimate = 0.20 ± 0.21, z = 0.95, p = 0.34; Table S2.11) or differently according to social information treatments (GLMM Presentation order * SI treatment: estimate = 0.67 ± 0.42, z = 1.58, p = 0.12; Table S2.12). They also approached teal as quickly as they did cuckoos (estimate = 0.065 ± 0.168, z = 0.39, p = 0.67; Table S2.13).

Discussion

A wealth of prior research in parasitised areas has revealed that reed warblers' defences predictably match local brood parasitism risk, including responding to social information of cuckoos

318 by consistently increasing mobbing and egg rejection rates (Campobello & Sealy 2011; Davies &
319 Welbergen 2009; Thorogood & Davies 2016). But, theory also predicts that the loss of defences in
320 non-parasitised populations is a key part of determining coevolutionary outcomes (Soler 2014;
321 Thompson 2005). Although behaviours are assumed hard to lose under relaxed selection, by using
322 social information to simulate increased brood parasitism risk, we found that defences and social
323 responsiveness of a common host of cuckoos are degrading in areas of allopatry. At the recent
324 range front in Finland, only pairs that mobbed a cuckoo increased their mobbing intensity after
325 receiving social information. The other two-thirds of birds largely remained quiet, although they
326 differentiated cuckoos from magpies by approaching the nest more quickly, suggesting some
327 recognition was retained. Our findings in Finland are enhanced by our results from the Sicilian
328 population, where reed warblers have been in allopatry for longer. There, they showed an even
329 greater reduction in defences and no evidence of social information use. Importantly, mimetic
330 'cuckoo' eggs remained in nests, and although non-mimetic eggs were rejected at low rates in
331 Finland, social information had no influence on this defence either. This suggests that both
332 allopatric populations would be unlikely to express plasticity or mount significant resistance should
333 cuckoos invade.
334
335 Our results are likely evidence for the decay of ancestral defences rather than a methodological
336 failure to detect responses, for three reasons. First, providing social information experimentally led
337 to an increase in mobbing intensity in Finland, even if not in Sicily. Second, we followed very similar
338 methods to previous studies conducted with reed warblers at parasitised sites, where social
339 information heightened near-absent egg rejection of similarly manipulated eggs ten-fold
340 (Thorogood & Davies 2016) and induced mobbing of cuckoos (Campobello & Sealy 2011; Davies
341 & Welbergen 2009; Thorogood & Davies 2012), including for pairs that showed no initial response
342 [e.g. 54% of quiet pairs mobbed cuckoos after social information: (Davies & Welbergen 2009)].
343 Third, the differences we detected were specific to cuckoos as responses to a control teal and nest
344 predator (magpie) were similar at our sites and to parasitised areas (Welbergen & Davies 2008;
345 Campobello & Sealy 2010; Tolman *et al.* 2021; Welbergen & Davies 2012) despite large ecological
346 differences (i.e. climate, nesting density). By using consistent experimental designs to previous
347 studies, testing a large number of reed warblers nesting across a broad range of environmental
348 variation, and considering social responsiveness explicitly, we can therefore be confident that the
349 weak defences in our allopatric study populations are not the result of cryptic plasticity (Scheiner &
350 Levis 2021). Instead, they provide compelling evidence that behavioural defences may sometimes
351 be lost in allopatry from cuckoos.
352

Given that studies of animal culture across a range of taxa demonstrate that the spread of even arbitrary behaviours is possible (Whiten 2021), it is surprising that we did not detect evidence for social learning among 'naïve' reed warblers (i.e. those that did not mob cuckoos). For example, juvenile superb fairy wrens begin to mob cuckoos after observing adults do so (Feeney & Langmore 2013) and naïve individuals of several species can be trained to recognize novel predators [e.g. introduced species (Rowell *et al.* 2020)] when paired with mobbing calls. Often social learning of threats requires only one exposure (i.e. similar to our experimental design; reviewed by Crane & Ferrari 2013) and can even be effective when using arbitrary cues (Hämäläinen *et al.* 2022). Why then did so many birds not mob cuckoos or reject eggs? One possible explanation is that ontogenetic experience or repeated exposure may be necessary before behaviours are expressed, as in the antipredator responses of several fish and mammals (Kelley & Magurran 2003; Mineka *et al.* 1984) and personal encounters with avian or piscine brood parasites (Blažek *et al.* 2018; Hauber *et al.* 2006; Stokke *et al.* 2007). While we cannot exclude this possibility it seems unlikely to explain the lack of response we detected here: we provided three encounters with cuckoos at or near their nests, yet most birds still did not respond. Perhaps with relaxed selection, there is an initial loss of recognition that then limits hosts' potential for observational learning.

Temporal and geographical variation in host defences has typically been explained by plasticity (Langmore *et al.* 2012; Lindholm 2000; Welbergen & Davies 2012), particularly in reed warblers where individuals track local parasitism risk during egg-laying and adjust defences accordingly (Thorogood & Davies 2013). However, here we found only limited evidence for plasticity in our allopatric populations in response to either indirect environmental or experimentally-manipulated cues of heightened parasitism risk. This is surprising given that in a previous study at an unparasitised site closer to the range core (Llangorse lake, Wales) and presumed to have been allopatric for at least 130 years [i.e. similar to Finland; (Lindholm 1999)], reed warblers responded more aggressively to cuckoo models if nesting closer to vantage points for cuckoos, if they were present (Welbergen & Davies 2012). We found no evidence for this indirect cue use: instead, birds nesting further from vantage points in Finland were *more* likely to mob, while mobbing in Sicily was unaffected (Supporting Information S1). Nevertheless, of the birds that mobbed, their initial intensities were similar across all three allopatric sites [Finland: 225 ± 43 calls per 5-min, Sicily: 280 ± 120, Llangorse: 281 ± 64; (Welbergen & Davies 2012)], suggesting that while the frequency of birds retaining recognition may vary across allopatric populations, they can respond similarly (see Welbergen & Davies 2012). In Sicily, a site we assume to have been in allopatry for much longer, there was no social responsiveness, suggesting that individual reaction norms may weaken in allopatry, and potentially lead to the eventual loss of defences in the absence of cuckoo parasitism (Soler 2014). If we were to revisit the Llangorse or Finnish populations in future, would

their responses have diminished further and look more like those in Sicily? Quolls' antipredator behaviours have been found to decay on a predator-free island over even fewer generations than have existed of reed warblers in Finland (Jolly *et al.* 2018), suggesting gene flow mediates the speed at which behaviours can be lost. There is already some genomic evidence from reed warblers to suggest local adaptation to the strength of parasitism (Smith *et al.* 2025), but whether the decay in defences we detected is accompanied by genetic change is yet to be determined.

Overall, our results suggest that it may be possible for host populations freed from parasitism to lose expression of defences, rendering them vulnerable to future reinvasion. The recovery of resistance is unlikely to be enhanced through social transmission, because even if there are individuals who retain recognition of cuckoos, our results show that social responsiveness may also be lost. Such a reduction of defences under relaxed selection is one of the key components of geographic mosaic theory explaining the maintenance of coevolution across time and space: rather than the arms race continually escalating, parasites are hypothesized to recurrently invade host populations, or host species, whose defences have waned in their absence. These so-called coevolutionary cycles (Rothstein 2001) are thought to only exist during 'intermediate phases' of host-parasite interactions, before defences reach fixation (Soler 2014), but the rapidity with which a brood parasite host species can acquire (or lose) successful resistance has received little attention. Our results suggest that range expansions and a geographic mosaic of coevolution may moderate the arms race, with defences decaying within even relatively short time frames [e.g. approximately 35 generations in Finland (Ceresa *et al.* 2015)], allowing the longer-term persistence of parasites until host switching becomes more favourable (Nuismer *et al.* 2003). As both social information use and behavioural defences against enemies are common across the animal kingdom, and species' ranges are changing rapidly, it is now crucial to determine how socially-mediated plasticity degrades under relaxed selection in the wild.

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Figures and Tables

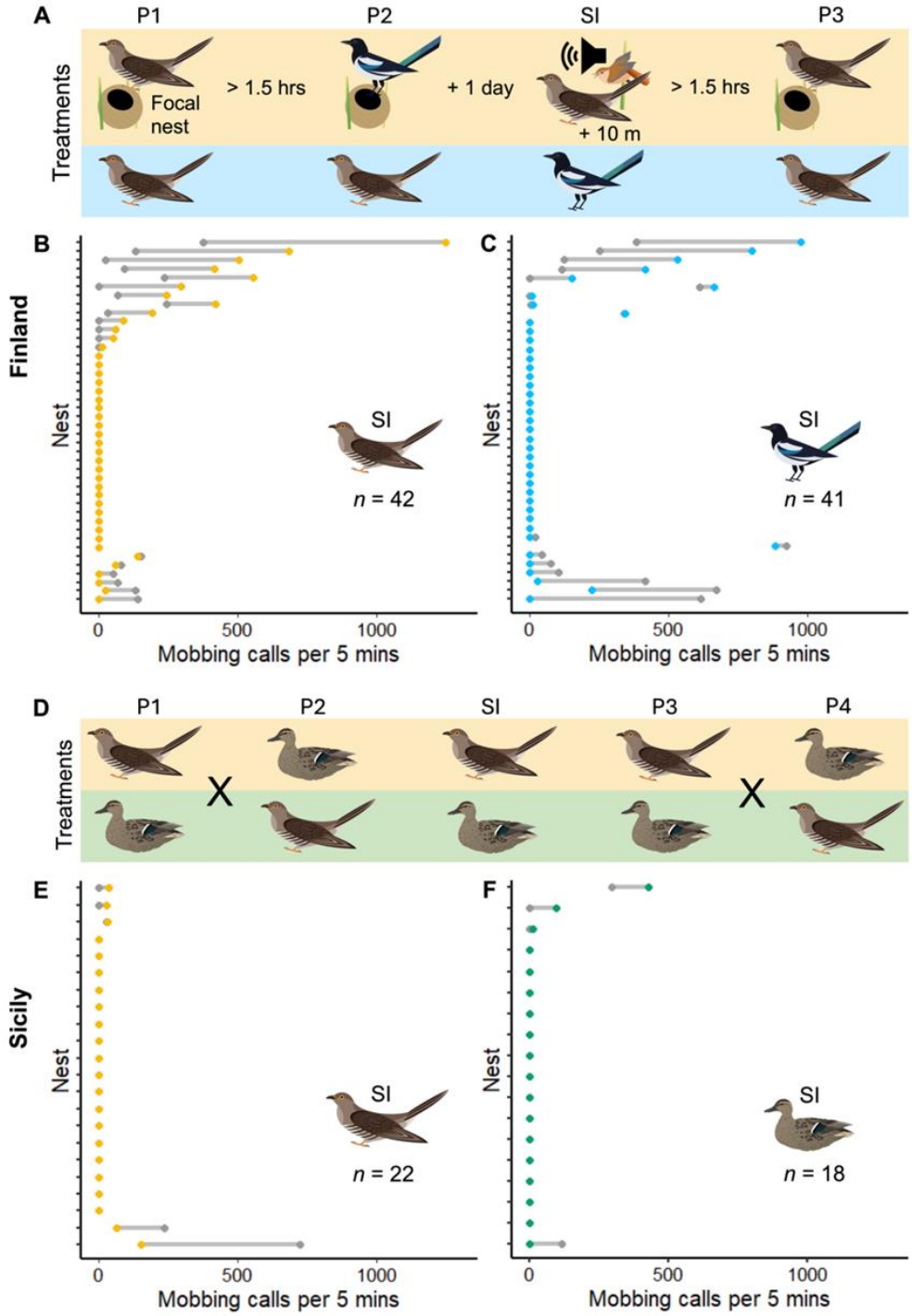

Figure 1. Reed warbler mobbing defences (calls per 5 minutes) in allopatry (Finland: A-C, Sicily: D-F) before (grey dots) and after (coloured dots) experimental manipulation of social information

605 (‘SI’) about cuckoos (B, E) versus a control (C – magpie, F – teal). Details of experimental design
606 are shown in A, presentation order (‘P’) of models was alternated in D.
607

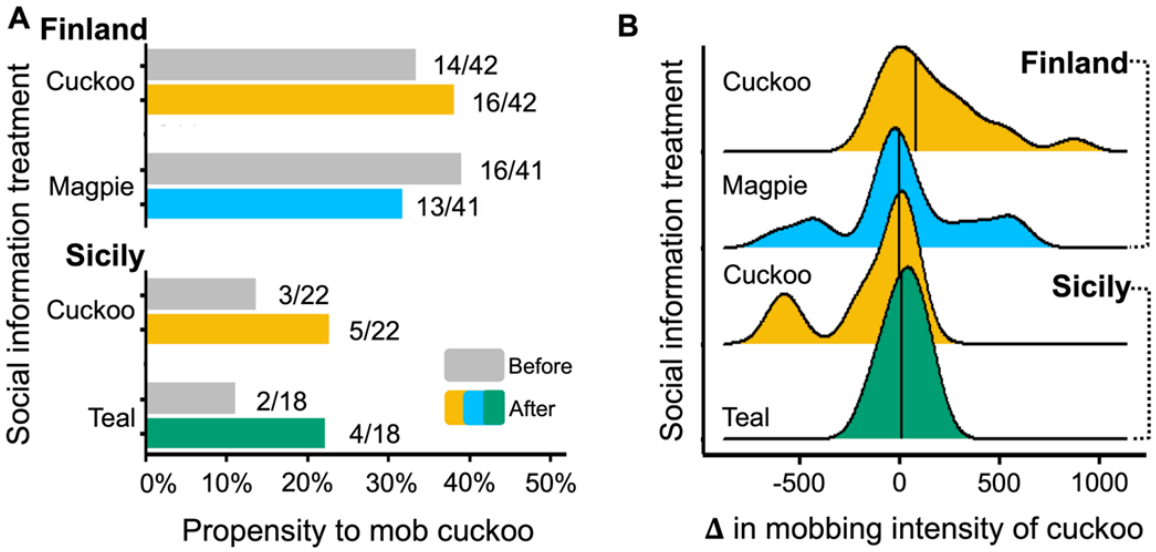

Figure 2. (A) Propensity to mob cuckoos did not change after social information about cuckoos or a control in Finland or Sicily. (B) Mobbing pairs showed social responsiveness (i.e. increased intensity) to cuckoos, but only in Finland (distributions of changes in mobbing intensity, vertical lines = medians).

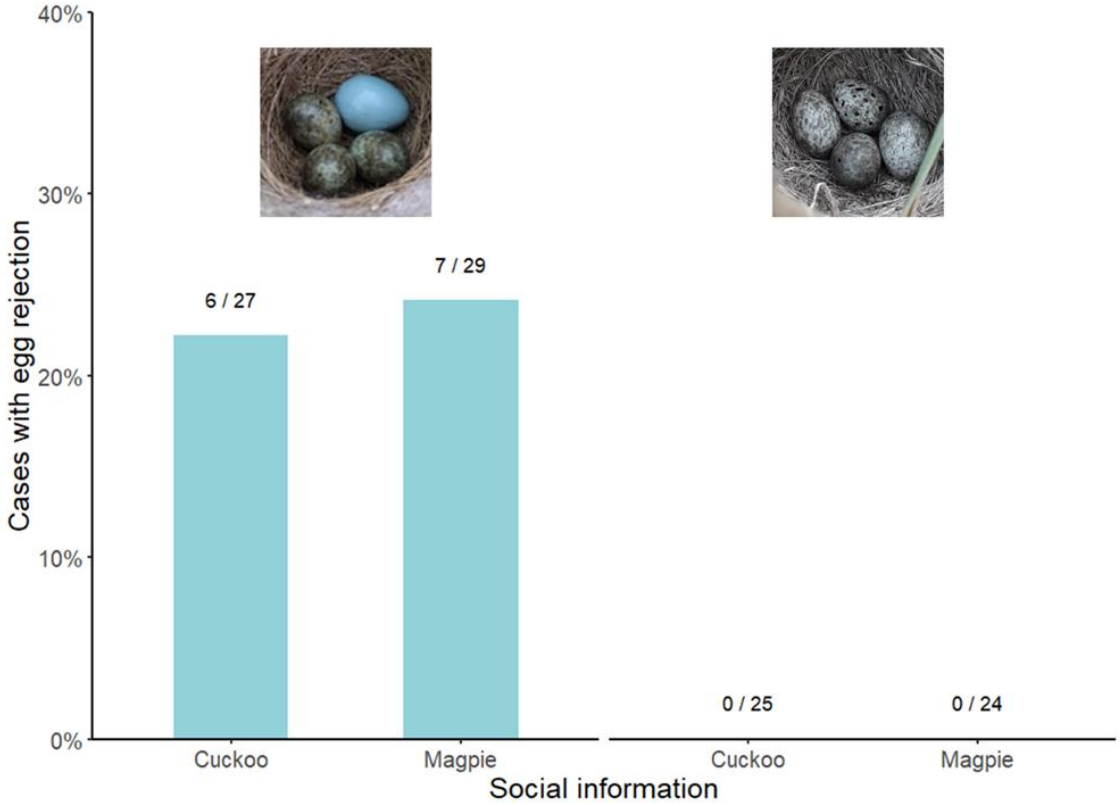

Figure 3. Rejection rates of experimental eggs in Finland (inset photos). Blue non-mimetic eggs (left) were rejected at the same rate regardless of whether social information was provided about cuckoos (24%) or magpies (22%). Mimetic brown-spotted eggs (right) were not rejected.

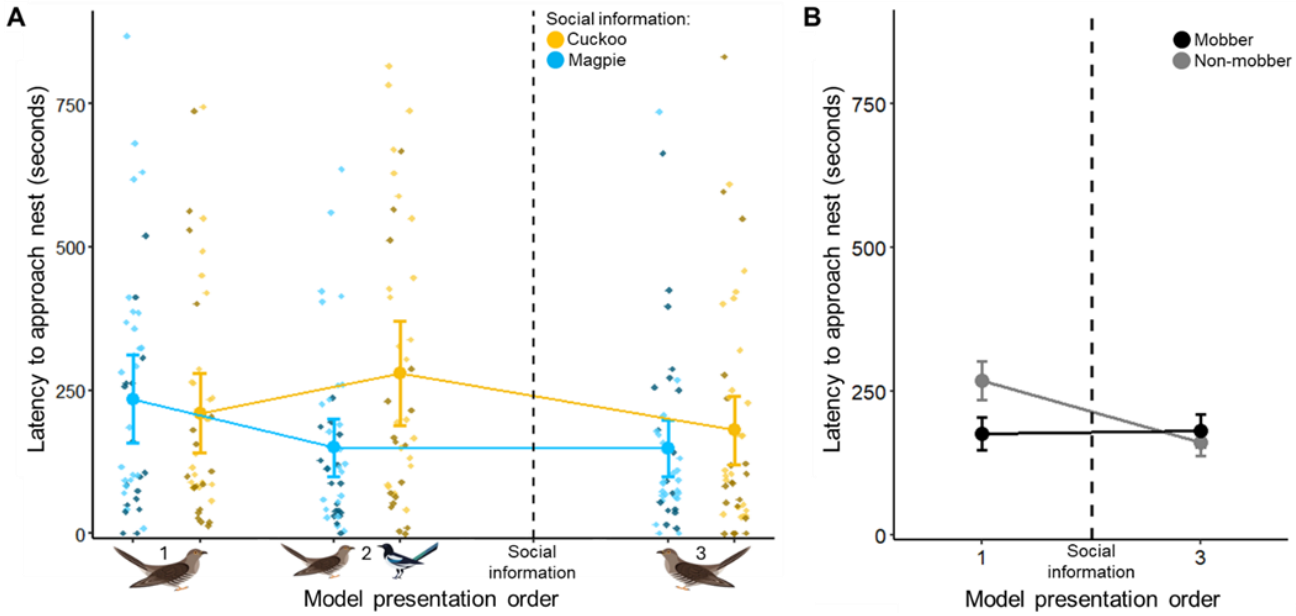

Figure 4. (A) Latency of birds to approach presentations of models at their nest (indicated by icons) before and after social information (dashed line) about cuckoos (yellow) or a magpie control (blue) in Finland. Raw data are plotted as diamonds, with darker symbols indicating pairs that mobbed cuckoos. Predicted mean values (± standard error) from a GLMM are plotted as circles and bars, with connecting lines indicating change across model presentation order (P1-P3). (B) Latency of birds to approach presentations of models at their nest according to whether they mobbed a cuckoo. Predicted mean values (± standard error) from a GLMM are plotted as circles and bars, with connecting lines indicating change across model presentation order (P1-P3).