Chase-away evolution maintains imperfect mimicry despite rapid evolution of mimics

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Abstract

We studied a brood parasite-host system to test the fundamental hypothesis that deceptive mimics evolve to resemble models, selecting in turn for models to evolve away from mimics ("chase-away evolution"), and whether such reciprocal evolution maintains imperfect mimicry over time. Over only 50 years, parasites evolved towards hosts and hosts evolved away from parasites, resulting in no detectible increase in mimetic fidelity. Our results reflect rapid adaptive evolution in wild populations of models and mimics, and show that chase-away evolution in models can counteract even rapid evolution of mimics, resulting in the persistence of imperfect mimicry.

Main Text

Ever since Bates observed the remarkable similarity between different South American butterfly species[1], the phenomenon of mimicry has been used to illustrate how natural selection can produce striking adaptations. For mimicry to exist, mimics must evolve to resemble models. If models benefit

from being discriminable from mimics, 'chase-away' selection should drive models to evolve away from mimics[2]. Thus, chase-away evolution could prevent the accuracy of mimetic resemblance, termed mimetic fidelity, from increasing over time[3,4]. However, few studies have examined evolutionary trajectories of *both* models and mimics simultaneously, likely because the required long-term data are difficult to obtain. To our knowledge, the only study to have examined changes in mimetic fidelity over time found that, for one of four traits studied, mimetic fidelity increased over time[5]. While this might suggest that chase-away selection was insufficient to prevent increases in mimetic fidelity over time, it is unknown whether the trait is used in discriminating between models and mimics, and thus whether observed patterns were due to selection in the context of mimicry. Here, we study an aggressive mimicry system over 50 years to test the hypothesis that chase-away selection on models prevents increases in mimetic fidelity over time.

The cuckoo finch *Anomalospiza imberbis* lays eggs which imperfectly mimic the complex and variable patterns of eggs of its host, the tawny-flanked prinia *Prinia subflava* (Methods), which reject mismatched eggs from their nests[6]. Individual prinias lay eggs with distinct colour and pattern phenotypes ("egg signatures"; Figure 1A), such that a given cuckoo finch egg will be a poor match to most prinia clutches in the population[6]. Cuckoo finch eggs (mimics) exhibit simpler patterns than prinia eggs (models), and differences in pattern complexity predict egg rejection by prinias[7]. Egg rejection therefore has fitness consequences for both hosts and parasites, and this implies that selection should favour parasites evolving towards hosts (i.e. evolving increased complexity) and hosts evolving away from parasites (i.e. also evolving increased complexity). By quantifying pattern complexity of 414 prinia and 162 cuckoo finch eggs from 1970–2020 (Methods), we tested whether host and parasitic phenotypes have changed in the predicted direction in the recent past, and whether such reciprocal evolution led to any change in mimetic fidelity over time. We measured complexity (a synthetic measure of several pattern traits; see Methods for details) on a logarithmic scale, since hosts perceive this measure of complexity according to Weber's Law[7]. Because effect sizes on logarithmic scales are unintuitive, we provide estimates as percentages where appropriate.

A linear model confirmed that in this dataset, prinia eggs are more complex than cuckoo finch eggs (Estimate = 58%, 95%CI = 30–93%, $t_{572} = 4.3$, $P < 0.001$). Complexity across both species increased slightly but significantly over 50 years (Estimated increase = 0.5%, 95%CI = 0.05–0.9%, $t_{572} = 2.0$, $P = 0.04$; Figure 1B, Extended Data Figure 1). There was no significant difference between species in the rate of increase in complexity (interaction between species and year during which the egg was laid: Estimate = -0.4%, 95%CI = -0.9–0.1%, $t_{572} = -1.4$, $P = 0.15$). Because heteroscedasticity in the data (i.e. hosts exhibiting higher variance in complexity than parasites, likely as a result of diversifying selection on host phenotypes[6,8]) may invalidate model inferences, we bootstrapped the linear model (Methods). As a further validation, we categorised eggs into those laid from 1970–2002 (historical; predominantly 1980–1990) and those from 2012–2020 (current). All results were consistent with the original model (see Methods for details). Overall, the finding that complexity increases over time suggests that parasites have evolved towards hosts, and that hosts have evolved away from parasites at a similar rate.

If chase-away evolution in hosts occurred at a similar rate to parasite evolution, as implied above, then we would expect to see limited increases in mimetic fidelity despite rapid evolution of parasites. To quantify changes in mimetic fidelity, we calculated all host-parasite complexity differences from 1970–2002 (historical) and from 2012–2020 (current) (Methods). Bootstrapped estimates of historical and current mimetic fidelity showed considerable overlap (mean historical complexity difference on a logarithmic scale = 0.46, 95% CI = 0.38–0.55; mean current complexity difference = 0.42, 95% CI = 0.39–0.45; Figure 1C). This corresponds to no significant increase in this trait-based measure of mimetic fidelity (bootstrapped estimated increase = 4%, 95% CI = -3%–12%, Figure 1C). We also independently estimated mimetic fidelity using a discriminant analysis based on complexity. The discriminant analysis for historical eggs correctly assigned 72% of eggs to the correct species (bootstrapped 95% CI = 63%–80%). There was no significant difference between this and the performance of the discriminant analysis for current eggs (mean increase in mimetic fidelity = 2%; bootstrapped 95% CI = -10%–13%), which assigned 70% of eggs to the correct species (bootstrapped

95% CI = 61%–78%). This echoes the result of comparing pairwise combinations of eggs: both measures of mimetic fidelity indicate that no observable increase in mimetic fidelity occurred, as expected given the lack of any significant difference between hosts and parasites in the rate of change of pattern complexity over time. Thus, chase-away selection driving host evolution away from parasites likely explains why mimicry of pattern complexity remains imperfect in this host-parasite system.

Although observed changes in complexity conformed to *a priori* predictions of coevolution, this study is correlational. We must therefore consider alternative explanations which could influence host and parasitic eggs in tandem, such as selection on egg pattern complexity from predation or climate. However, the main predators at our field site are snakes, which rely mostly on olfaction and infra-red, and prinia nests are enclosed, limiting egg visibility at long range[8]. Climate change also appears unlikely to select for increases in complexity, since increased temperatures are likely to select for fewer pattern markings (which absorb more heat than unmarked eggshells)[9]. Complexity is highly correlated with the number of pattern markings and weakly correlated with pattern coverage[7]; thus, increased ambient temperatures due to climate change should select for reduced complexity, contrary to our findings.

In summary, tracking model and mimic phenotypes over 50 years showed that despite rapid evolution of parasites, there was no detectible increase in their mimetic fidelity to hosts. This suggests that the coevolutionary response in hosts was strong enough to prevent increases in mimetic fidelity, and so supports the hypothesis that the persistence of imperfect mimicry can be explained by chase-away evolution in models[3,4].

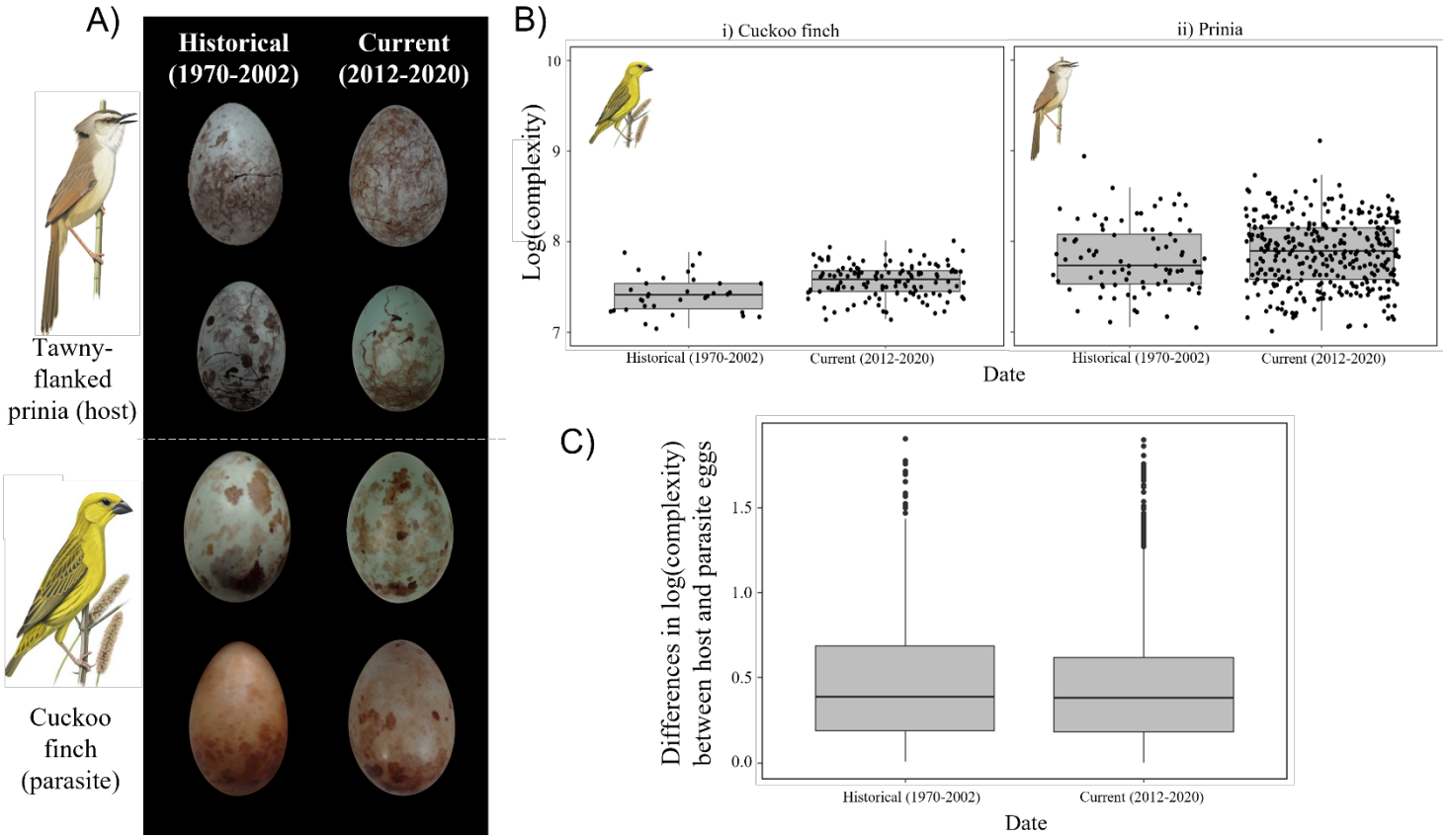

Figure 1. A) Randomly-selected host (above) and parasitic (below) eggs, from the historical (left) and current (right) samples. B) Changes in egg pattern complexity (log-transformed) over time in (i) parasites and (ii) hosts. Boxes range from the 25th to the 75th percentile and horizontal lines represent medians. All data points are shown as dots. C) Mimetic fidelity through time: no significant change in differences in log(complexity) between all historical (n=2,788) and current (n=42,496) pairs of parasites and hosts. Boxes range from the 25th to the 75th percentile and horizontal lines represent medians. Outliers are shown as dots. Individual points are excluded as they obscure the

boxplot. Bird illustrations: faansiepeacock.com.

Methods

Study species

At our study sites, on Semahwa and Musumanene Farms (around 16.74'S, 26.90'E) and surrounding areas in the Choma District of southern Zambia, the cuckoo finch currently parasitises four cisticolid warbler species[10]. Of these four species, tawny-flanked prinias are the commonest, have the most variable (and subjectively the most complex) egg patterns[11]. High inter-individual variation in prinias ("egg signatures") provide an effective defence against parasites, since egg signatures facilitate the rejection of mismatched eggs from host nests[6,12]. Although many egg signature traits may be important for egg rejection in this system[6], we focussed on complexity because quantifiable differences between hosts and parasites in this trait allow us to make clear predictions about the direction of evolution, namely that both should evolve towards higher complexity[7].

Photography of eggs

In all analyses, one egg per photographed clutch was included (prinia n = 414; cuckoo finch n = 162; from 1970–2020), with a single image considered representative of the egg's phenotype. Images of eggs collected from 1970–2002 (from the private collection of JFRCR, collected by JFRCR and LH, and deposited in the Livingstone Museum, Zambia) were taken by CNS. Most of these eggs were from the 1980s. Images from 2013 were taken by WEF, CNS, and WT; images from 2014 were taken by WT and CNS; images from 2018–2020 were taken by TD; all other images were taken by CNS. Although host and parasite eggs were also studied in 2007–2009[6], these years were excluded from this study, because images from 2007–2009 were not comparable to other images taken (due to differences in scaling and normalisation[7]). In a few years, some host eggs were not photographed or

analysed due to a specific research focus on parasitic eggs, and host eggs were not routinely photographed owing to time constraints. Parasitic eggs can be reliably distinguished from host eggs by the absence of 'scribbles' of pigment on their shells, which hosts always exhibit[13]. Images were taken in linearised RAW format, in shade with either a Nikon D90 camera with a 60 mm Micro-Nikkor lens or a Fuji Finepix S7000 camera. For eggs collected from 1970–2002, a 17% grey card was used to normalise images. For all other eggs, two grey standard squares (N6.5 and N5; reflectance values 36.2% and 19.8% respectively) of an X-rite ColorChecker Passport (X-Rite, MI, USA) were used to normalise images.

In all images except for those from 2018–2020, only 'one side' of each egg was photographed. In 2018–2020, eggs were photographed four times, rotating the egg through 90 degrees around the long axis after each image, to maximise the amount of pattern photographed[7,14]. This produced images of 'sides' a, b, c, and d, where a is opposite c and b opposite d. When determining historical changes, we used complexity values from only one side of eggs photographed in 2018–2020 ('side a'), rather than the average of a and c as used previously[7]. Complexity values for different sides of the egg are highly repeatable[7].

Image analysis

We used the MICA toolbox[15] in ImageJ to normalise and scale images to 29 px/mm, 'cut out' (i.e. remove from the background) and mask (i.e. add an artificial black background) eggs, and produce greyscale images from the green channel. The green channel was used because it corresponds closely to the sensitivity of avian double cones, thought to be involved in pattern processing[16]. Pattern features were extracted using NaturePatternMatch (NPM)[17]. NPM detects and encodes local features ('SIFT features') as 132-dimensional vectors, which loosely correspond to pattern markings. Complexity of the egg pattern was then calculated as in [7]. Briefly, six traits were measured: (i) the number of pattern features, (ii) the variation in position of features on eggs, (iii) the variation in the

scale (size) of features, (iv) the variation in the orientation of features, (v) the Redies change, a measure of how much intensity (i.e. brightness) changes across an image, and (vi) a measure of clustering tendency of features and within-cluster feature variation. All but (v) were based on features extracted using NPM. An optimisation algorithm optimised the complexity metric (defined as a linear combination of these six traits) such that the absolute complexity difference between an experimental egg and the host clutch in which it was placed would best predict rejection of the experimental egg. For full details of this quantification, see [7]. Although this metric was based on present-day rejection data, we found evidence that selection has acted on host and parasite pattern complexity in the recent past (see main text). This implies that the complexity metric is not only relevant to current host rejection behaviour, but also was relevant to rejection behaviour in the recent past.

Because perception conforms to Weber's Law[7] (i.e. hosts perceive relative, rather than absolute, differences in complexity), we quantified pattern complexity on a logarithmic scale, with estimates of percentage changes in complexity calculated as exp(Estimate).

One concern with using historical egg collections is that the background colour of eggs can fade over time, especially if they are poorly stored, which was not the case for the eggs photographed as part of this study. Old eggs were photographed in 2007 and 2009, and eggs were kept in a darkened room and collected relatively recently[8]. Furthermore, it is largely blue-green colours on eggs which fade (e.g. [18]), which has no relevance to the pattern measures we extracted, since pattern measures extracted from NPM should be unaffected by the underlying colour. In the unlikely event that fading affected the detectability of faint markings by NPM, any background colour fading on old eggs would make faint markings *more* detectable on these eggs, resulting in higher complexity scores for old eggs than fresh eggs. Our results run counter to this (see Main Text), and are therefore conservative.

A second concern with studying host and parasitic egg phenotypes more generally is that some (likely poorly-matched) parasitic eggs may be rejected from host nests before data from that nest are collected. This may mean that only closely-matched parasitic eggs are phenotyped. However, in this system this is unlikely to be a problem, since (i) hosts often take 1–4 days to reject a poorly-matched egg (particularly eggs that are poorly matched in terms of pattern, rather than colour)[7], and (ii) high variation in host egg appearance between clutches (Figure 1B) means that all cuckoo finch eggs are poor matches to the majority of the host population at any given time. Thus, there is unlikely to be a bias towards phenotyping well-matched eggs.

Testing for changes in complexity over time

All statistical analyses were conducted in R (version 4.0.2[19]). We used linear models (function lm) to quantify change in complexity over time across species, using the model Complexity ~ Species + Year + Species:Year. For example, a negative coefficient for the interaction term, with positive coefficients for the Species and Year terms would indicate that prinia complexity was greater than cuckoo finch complexity, and that complexity increased over time but cuckoo finch complexity increased more than prinia complexity.

First, we tested for continuous changes over time. Year was modelled as a continuous variable with years assigned integer values from 0 (year 1970) to 50 (year 2020). Because prinias exhibited much greater variance than cuckoo finches (thus falsifying the model assumption of homoscedasticity), we bootstrapped the model to calculate 95% confidence intervals for model coefficients using 1000 replicates. Results from bootstrapping were consistent with the initial model. Confidence intervals for the interaction term spanned zero (Estimate = -0.4%, 95% CI = -0.7–0.003%), while confidence intervals for species (Estimate = 58%, 95% CI = 35–86%) and year (Estimate = 0.5%, 95% CI = 0.2–0.7%) did not span zero, indicating that prinia eggs are more complex than cuckoo finch eggs, and that complexity increased over time. Median complexity appeared to fluctuate during

certain periods (Extended Data Figure 1), such that the overall increase in complexity was not monotonic. These fluctuations are likely due to low sample sizes of eggs from specific years, combined with very high population-wide variation in complexity. Such sampling error is especially likely during periods such as the mid- to late-1980s, in which sample sizes were low for each year; correspondingly, fluctuations in complexity were apparent in these years. However, with these data we cannot rule out other selective pressures or environmental influences driving short-term increases or decreases in complexity in one or both species. Regardless of the cause of these apparent fluctuations, they mean that we did not observe a monotonic increase in complexity in either species. Therefore, we conducted further analyses to test the robustness of the results of the linear model.

We subdivided the datasets into historical eggs (from 1970–2002; prinia n = 82, cuckoo finch n = 34) and current eggs (from 2012–2020; prinia n = 332, cuckoo finch n = 128). Results for this model were also consistent with the previous models (Species: Estimate = 34%, 95% CI = 23–45%, t_{572} = 8.4, $P < 0.001$, Year: Estimate = 16%, 95% CI = 2–32%, t_{572} = 2.3, $P = 0.02$, Interaction: Estimate = -9%, 95% CI = -22–7%, t_{572} = -1.3, $P = 0.2$). Conclusions also remained unchanged when this model was bootstrapped (Species: Estimate = 34%, 95% CI = 27–42%, Year: Estimate = 16%, 95% CI = 7–26%, Interaction: Estimate = -9%, 95% CI = -19–3%).

In summary, complexity was higher in prinias than cuckoo finches, complexity increased over time across both species, and there was no detectible difference between species in the rate of increase of complexity over time.

Testing for changes in mimetic fidelity over time

We measured mimetic fidelity using two methods. The first method calculated all host-parasite differences in each time period. Comparing all host-parasite pairs assumes that cuckoo finches lay their eggs at random in prinia nests, i.e. independently of the patterning on the prinia eggs they

234 contain. This has been shown to be a valid assumption in this system[6]. All possible pairings of
235 parasite and host eggs measured at each time point (n = 82x34 = 2,788 for historical data; n =
236 332x128 = 42,496 for current data) provides a sample from the joint distribution of parasite and host
237 pairs in the population at large during each time period. To test whether mimetic fidelity had
238 changed over time, we generated absolute differences in the logarithm of complexity between all
239 pairs of host and parasitic eggs. From these we calculated the mean absolute difference for each
240 time period and used a 2-sample bootstrap with 500 replicates to estimate its 95% confidence
241 interval (= mean ± 2*bootstrap SE). The bootstrap was necessary because the number of paired
242 differences contributing to the mean estimate far exceeded the available degrees of freedom: for
243 instance, the sample size for current data (n=42,496) was generated from 460 observations (i.e. d.f.
244 = 459). Thus, simply conducting statistical tests without a bootstrap would overestimate statistical
245 power, whereas bootstrapping allows calculation of confidence intervals which do not overestimate
246 statistical power.

247 As a second measure of mimetic fidelity, we used Flexible Discriminant Analysis (FDA;
248 function *fda* in the R package *mda*[20]) with log(complexity) as the only predictor, and with
249 uninformed (i.e. equal and unbiased) priors. A high-performing FDA would indicate low mimetic
250 fidelity, because high performance would imply that the algorithm can accurately assign eggs to
251 species. Since the performance of an FDA tends to increase with sample size, we re-sampled current
252 eggs to the same number as historical eggs (n = 82 prinia and n = 34 cuckoo finch). We ran 1000
253 iterations of the FDA for both historical and current populations to calculate confidence intervals.

254 The two measures of mimetic fidelity used here correspond to slightly different questions. The
255 mean host-parasite pairwise distance is an estimate of the average similarity (in terms of complexity)
256 of a randomly-selected host-parasite pair, for the historical and present-day subsets. This simulates
257 the visual information available to guide the behaviour of a host female, who must compare her own
258 egg(s) with the egg of a parasite. The FDA provides an estimate of the likelihood of assigning eggs

correctly to species based on their complexity, for each subset. This considers whether mimetic fidelity has changed at a population level.

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Data accessibility

Data and R code associated with this manuscript are available as supplementary material.

Competing interests

The authors declare no competing interests.

Authors' contributions

TD and CNS conceived of and designed the study. TD, WEF, WT, LH, JFRCR, and CNS collected the data. ALA, KCC, and CPT designed methods for computation of complexity; ALA conducted the computation under the supervision of CPT and TD. TD, JL, and AJCF processed and analysed the data. TD, JL, CNS, and AJCF interpreted the results. TD drafted the manuscript and CNS, JL, KCC, AJCF, WEF, WT, and CPT contributed to later versions.