Caves EM, Stevens M, Spottiswoode CN. 2017 Does coevolution with a shared parasite drive hosts to partition their defences among species? Proc. R. Soc. B 284: 20170272. http://dx.doi.org/10.1098/rspb.2017.0272

Received: 9 February 2017
Accepted: 19 April 2017

Subject Category: Evolution
Subject Areas: behaviour, ecology, evolution
Keywords: brood parasitism, colour, mimicry, coevolution, phenotypic space

Authors for correspondence:
Eleanor M. Caves
e-mail: eleanor.caves@gmail.com
Claire N. Spottiswoode
e-mail: cns26@cam.ac.uk

Electronic supplementary material is available online at http://dx.doi.org/10.6084/m9.figshare.c.3759560.

Does coevolution with a shared parasite drive hosts to partition their defences among species?

Eleanor M. Caves¹, Martin Stevens² and Claire N. Spottiswoode¹,³

¹Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK
²Centre for Ecology and Conservation, College of Life and Environmental Sciences, University of Exeter, Penryn Campus, Penryn, Cornwall TR10 9FE, UK
³DST-NRF Centre of Excellence at the FitzPatrick Institute, University of Cape Town, Rondebosch 7701, South Africa

When mimicry imposes costs on models, selection may drive the model’s phenotype to evolve away from its mimic. For example, brood parasitism often drives hosts to diversify in egg appearance among females within a species, making mimetic parasitic eggs easier to detect. However, when a single parasite species exploits multiple host species, parasitism could also drive host egg evolution away from other co-occurring hosts, to escape susceptibility to their respective mimics. This hypothesis predicts that sympatric hosts of the same parasite should partition egg phenotypic space (defined by egg colour, luminance and pattern) among species to avoid one another. We show that eggs of warbler species parasitized by the cuckoo finch Anomalospiza imberbis in Zambia partition phenotypic space much more distinctly than do eggs of sympatric but unparasitized warblers. Correspondingly, cuckoo finch host-races better match their own specialist host than other local host species. In the weaver family, parasitized by the diederik cuckoo Chrysococcyx caprius, by contrast, parasitized species were more closely related and overlapped extensively in phenotypic space; correspondingly, cuckoos did not match their own host better than others. These results suggest that coevolutionary arms races between hosts and parasites may be shaped by the wider community context in which they unfold.

1. Introduction

When mimicry is costly to models, selection should drive a model’s phenotype to evolve away from that of its mimic [1]. Such models include the vertebrate immune system [2,3], and the hosts of reproductive parasites including insects [4] and birds [5]. Hosts of avian brood parasites lay their eggs in the nests of other birds, and rely on deception such as egg and chick mimicry to fool the host parents into providing costly care to their young [6]. In several independent brood-parasitic systems, hosts have defended themselves by diversifying their own egg phenotypes away from those of parasites, resulting in egg ‘signatures’ that help hosts to detect mimetic parasitic eggs [7–9]. However, many brood-parasitic species have evolved multiple sympatric host-races that specialize on different hosts and show appropriate egg mimicry for each [10–12]. If individuals of a host escaping parasitism diversify their egg signatures into phenotypic space occupied by another host, they may become susceptible to pre-existing mimicry by another parasitic host-race if attempts at host-switching occur [11]. Consequently, we should expect hosts not only to be more diverse in appearance than unparasitized species [7,13–15], but specifically to diversify egg phenotypes away from those of other sympatric hosts.

In a two-host system with sympatric hosts Hₐ and H₀, parasitized by parasites Pₐ and P₀, respectively, host Hₐ can escape from mimicry by parasite Pₐ by
shifting its own egg phenotype away from \( P_a \). But if it shifts its phenotype into the area of phenotypic space occupied by host \( H_b \), it risks parasitism from parasite \( P_b \), so long as \( P_b \) is capable of switching to a new host. Adding other sympatric hosts and parasites further restricts areas of unoccupied phenotypic space. Therefore, selection should favour host individuals that reduce phenotypic overlap with hosts of other parasites, because they will be susceptible to a smaller subset of the parasitic population. There is evidence from numerous brood-parasitic systems that host-switching by parasites is a relevant selection pressure on hosts. Specialist parasites sometimes lay eggs in the nest of the wrong species; for example 12.1% of eggs laid by common cuckoos (\( n = 1397; \) [16]) were non-mimetic for the host species in whose nest they were laid, and 1.8% of nests parasitized by cuckoo finches \( A. \) hirtus (\( n = 276; \) C.N.S. 2017, unpublished data) belonged to a different species from that mimicked by the parasitic egg; both figures are probably underestimates as many mismatched eggs will have been rejected by the hosts before the nest was found. Correspondingly, host-switches have repeatedly occurred over evolutionary time, leading to the evolution of new parasitic species [17] or host-races [18,19]; indeed many host colonization events must have once begun with such events.

Parasites, in turn, are under selection to track their respective hosts through phenotypic space. As a result, greater phenotypic partitioning among hosts should drive greater specialization among parasitic host-races, resulting in parasites better matching the eggs of their own specific host than those of other co-occurring hosts. However, if hosts do not partition phenotypic space then parasites may correspondingly overlap with many hosts and operate more like generalists.

This coevolutionary scenario makes clear predictions both for the degree of phenotypic partitioning among groups of sympatric hosts, and between host–parasite pairs. The hypothesis that hosts experience selection from multiple specialized host-races of parasites, favouring phenotypic partitioning among hosts, predicts (i) that host egg phenotypes of different species should be more distinct from one another than are the egg phenotypes of related, sympatric species that are not exploited by brood parasites. If so, then (ii) parasitic egg phenotypes should be more similar to those of the host species they were found in (hereafter ‘own host’) than to those of other hosts. Alternatively, if host species overlap extensively with one another, then parasites need not match their own host any more closely than they match other hosts, and parasitized and unparasitized species should show similar levels of phenotypic partitioning.

Here, we test these predictions in two African brood-parasitic systems with different distributions of parasitism among hosts. The African warblers (Cisticolidae) parasitized by cuckoo finches provide a strong test of both predictions because parasitized and unparasitized species are dispersed across their phylogeny [14]. The weaverbirds (Ploceidae) parasitized by the diederik cuckoo \( C.\) caprius provide an interesting comparison: host species have variable but (to the human eye) largely overlapping egg phenotypes. However, only four species in our weaver dataset are unparasitized, two of which are in relatively distantly related, basal genera [20], preventing a strong comparison with parasitized species. Nevertheless, this system provides a good test of the second prediction, that close host–parasite matching is only expected when hosts are phenotypically distinct.

First, for each family we quantified the degree of phenotypic partitioning within each group of sympatric host species and compared it to that found in co-occurring species that are not currently parasitized in the study area. Second, we examined the consequent degree of phenotypic specialization of parasites both to their own host and to other hosts. We measured phenotypic partitioning and mimicry in multidimensional space, because egg signatures are comprised of multiple traits such as colour, luminance (perceived lightness) and pattern, which hosts are known to integrate when making rejection decisions [11,21]. The hypothesis assumes that parasites occasionally lay an egg in the nest of a species other than their usual specialist host, which is known for at least the cuckoo finch system (above); it also assumes that host egg appearance is not solely explained by phylogenetic relatedness, which we test.

2. Material and methods

(a) Study system

Within each bird family, some host species show marked inter-clutch variation in appearance (egg ‘signatures’; figure 1), which is at least partially matched by corresponding variation within parasitic host-races [11,22]. In each system, different parasitic host-races, their respective hosts and unparasitized species of the same family all occur sympatrically and in similar habitats in the study area [11,22]. We treated each family separately, because they make slightly different predictions (see §1) and because parasites are highly unlikely to switch between host families owing to differences in body size, habitat and timing of breeding. We measured eggs in the private collection of Major John Colebrook-Robjent (bequeathed to the Natural History Museum, Tring, United Kingdom), which were all collected in the Choma, Monze and Mazabuka Districts (primarily Choma, 16° 47' S, 26° 50' E) of southern Zambia from 1970–1990. Our dataset comprised 939 clutches from 11 warbler species (five parasitized, six unparasitized), 14 weaver species (10 parasitized, four unparasitized), five parasitic host-races of the cuckoo finch, and five parasitic host-races of the diederik cuckoo (details in [14]). We randomly selected one egg per clutch for analysis to avoid pseudoreplication.

(b) Quantifying egg phenotypes

We used reflectance spectra to quantify egg colour and digital photography to quantify egg pattern, following the methods reported in [14]. Briefly, we calculated photon catches for the double cones, and the UV, SW, MW and LW single cones, which we used as indices of luminance and colour, respectively [9]. We applied a granularity approach [23] to digital photographs to quantify five pattern traits, as previously used to quantify egg pattern [9,11,24]: predominant marking size, contribution of the main marking to overall pattern, contrast between pattern markings and background, the proportion of the egg’s surface covered by markings, and dispersion of markings across the egg.

(c) Discriminant function analysis

To quantify and visualize partitioning in phenotypic space, we used discriminant function analysis (DFA [25]; for an excellent description see [26]). First, DFA generates discriminant functions, which are linear combinations of classification variables that maximize the probability of correctly assigning observations to their pre-determined groups. Second, DFA can classify each observation into one of the groups, and assess the success rate of classification. Mathematically, DFA is identical to a single-factor MANOVA;
the arcsine-square-root transformation to reduce heterogeneity of variances among test groups, and repeated all analyses on the transformed data. Removing correlated variables and transforming the data did not change the direction or significance of our conclusions in any instance (electronic supplementary material, tables S2 and S3); therefore the results in the main text are from untransformed data with correlated traits not removed.

To further guard against any violations of DFA’s fairly restrictive assumptions, we also performed a multinomial logistic regression, which can be used to characterize observations when the response variable has more than two categories. In general, logistic regression makes fewer assumptions than DFA, but is less powerful when sample sizes are small, and when all of the assumptions of DFA are met [38]. Results are given alongside those of the DFA for our ‘groupwise’ analyses (see below).

(i) Using discriminant function analysis to examine phenotypic partitioning among sympatric hosts and non-hosts

We carried out DFA in two ways for each bird family. First, we conducted ‘groupwise’ analyses, in which we compared the accuracy (i.e. discriminant rate) of phenotypic partitioning among parasitized species, to that among unparasitized species. This gives an overall measure of phenotypic partitioning within a group of species. We used jack-knifing (above) to help to control for differences in sample size of clutches between species within a group. However, there were also different numbers of species within each group of parasitized and unparasitized species. Therefore, we also conducted a ‘pairwise’ analysis in which we compared the accuracy (i.e. discriminant rate) of phenotypic partitioning among all possible pairs of parasitized species, to that among all possible pairs of unparasitized species. These analyses provide a less realistic picture of how a community of species responds to parasitism pressure, but have the advantage of removing any bias introduced by differences between groups made up of different sample sizes of species.

Before analysis, we calculated a null hypothesis of classification rates based solely on the relative sample sizes of species within each group; therefore, this ‘expected accuracy’ was the probability that a species would be correctly assigned due to chance alone. First, this tested the assumption that DFA performs

![Figure 1. Representative egg phenotypes for each of the parasitized and unparasitized warbler (Cisticolidae) and weaver (Ploceidae) species in this study. Each egg is from a different clutch. (Online version in colour.)](image-url)
better than chance in classifying species. Second, it allowed us to apply each of our groupwise and pairwise analyses to the ‘expected’ data as well as to the ‘observed’ data. To reject a null hypothesis, an effect of parasitism status should be present in the observed classification rates (i.e. based on phenotypic traits) that is not also present in the expected classification rates (i.e. based on chance alone arising from sample size variation). As the groupwise analysis yielded a point estimate of observed and expected classification rates, respectively, we compared them using Fisher’s exact tests and calculated their binomial proportion confidence intervals [39]. The pairwise analysis yielded a distribution of observed and expected classification rates, which we compared using Welch’s (unequal variances) t-tests on ranked data [40]. We did not use Bonferroni or other similar methods to correct for multiple testing, as each test generated a discriminant rate not a p-value, and therefore was not a significance test.

(ii) Using discriminant function analysis to examine host–parasite similarity
To test whether a parasitic host-race is phenotypically more similar to its own host than to other hosts, we performed DFA between a given parasitic host-race and its own host, and between it and each other host species in turn. For each host-race, DFA yielded a measure of accuracy for an ‘own’ comparison, as well as multiple accuracies for ‘other’ comparisons. Within a given host-race, we subtracted the ‘other’ value from the ‘own’ value and took the mean of those differences to yield an average measure of how much more phenotypically similar a host-race is to its own host than other hosts. To assess significance, we used a paired t-test to examine the differences between ‘own’ and ‘other’ comparisons within each host-race. For the cuckoo finch, sample size for two of five host-races was very low (Cisticola erythropus, n = 2; C. natalsensis, n = 1). Therefore, these eggs were not included in statistical analyses, but are presented in the figures for completeness.

(d) Phylogenetic methods
To test the extent to which phylogenetic relationships [41] may have confounded the DFA, we estimated the degree of phylogenetic signal exhibited by each trait individually within each family. We used Pagel’s λ [42] to estimate the extent to which variation in a given trait is explained by phylogenetic structure, ranging from zero (no phylogenetic signal) to one (variation completely explained by phylogenetic structure). Some species in our study have either not been formally placed on a tree, or placed but with low confidence; to address this uncertainty, we used birdtree.org [43] to compile 100 trees with branch lengths for each focal family.

We then used the R package caper [44] to calculate phylogenetic signal (Pagel’s λ) in each of the 100 trees per family. For each tree, we calculated Pagel’s λ, as well as p-values for significance tests of whether λ differed significantly from zero (p₀) or from one (p₁), which would indicate no significant phylogenetic signal. We then calculated the average and standard deviation of λ, p₀, and p₁ for each trait. We found no evidence of significant phylogenetic signal in egg traits in the Cisticolidae (electronic supplementary material, table S4), as in all cases lambda differed significantly from one, but not from zero. However, in the Ploceidae, we found that both luminance (p₀ = 1.00 ± 0.00, p₁ = 0.10 ± 0.28) and UV (p₀ = 1.00 ± 0.00, p₁ = 0.08 ± 0.23) did not differ significantly from either zero or one. This indicates that phylogenetic structure is neither helpful nor unhelpful in explaining the trait distribution; this likely occurred because the phylogeny is small and, due to phylogenetic uncertainty, has large confidence intervals. However, low values for Pagel’s λ (λ = 0.15 ± 0.32 for luminance and λ = 0.08 ± 0.26 for UV) suggest that the influence of phylogeny on luminance and UV is not of large magnitude.

Complete results of the phylogenetic signal analyses are in electronic supplementary material, table S4.

3. Results
(a) Does discriminant function analysis separate species better than chance?
Within each group (parasitized and unparasitized warblers and weavers), observed accuracy of DFA based on phenotypic traits was significantly higher than expected accuracy based on chance alone. This indicates that irrespective of parasitism status, DFA performed significantly better than chance at classifying individuals to species (comparisons within columns in table 1). Similarly, in the pairwise analyses, observed accuracy within a group was always significantly higher than expected accuracy (t-test, p < 0.0001 in all cases). This, first, justified the use of DFA to quantify phenotypic partitioning between species within groups and, second, generated expected classification rates that could be applied to each analysis below, for comparison with observed classification rates.

(b) Phenotypic partitioning in warblers (Cisticolidae)
Parasitized and unparasitized groups of species did not differ in expected accuracy, i.e. the likelihood of correctly classifying eggs based purely on chance given differences in sample size between groups (Fisher’s exact test, p = 0.55; comparisons across columns in table 1). However, once we incorporated phenotypic information, using either a DFA or logistic regression, observed accuracy was significantly higher for parasitized than unparasitized warblers (Fisher’s exact test, p < 0.0001 for both DFA and logistic regression; comparisons across columns in table 1). Taken together, this indicates that DFA is indeed better able to distinguish among parasitized species than among unparasitized species (figure 2a,b), and that this is not simply an artefact of different sample sizes within and between groups, or of the statistical approach used. Correspondingly, in the pairwise comparisons, based on chance there was no significant difference in expected accuracy between pairs of parasitized (mean ± s.e. = 52.9 ± 0.01 per cent) and unparasitized species (53.3 ± 0.01 per cent; Z = 1.00, p = 0.32). Incorporating phenotypic traits, pairs of parasitized species were significantly more accurately classified (95.6 ± 1.7 per cent accurate) than pairs of unparasitized species (86.1 ± 2.6 per cent; Z = 2.58, p = 0.01). Thus, DFA was better able to distinguish pairs of parasitized species than pairs of unparasitized species (figure 2a,b). In summary, the results consistently supported our prediction of greater than expected phenotypic partitioning among parasitized warblers than unparasitized warblers.

(c) Phenotypic partitioning in weavers (Ploceidae)
The small number of unparasitized weaver species (n = 4) in the dataset undermines comparisons with parasitized species, and correspondingly we found that higher accuracy was expected for unparasitized species than for parasitized species, based purely on chance (Fisher’s exact test, p = 0.045; comparisons across columns in table 1). Similarly, when phenotypic information was incorporated, via either DFA or logistic regression, observed accuracy was significantly higher for unparasitized than parasitized species (figure 2b,d; Fisher’s
Table 1. Accuracy, expected correct, observed correct and improvement over chance in categorizing eggs to species using both jack-knife validation of discriminant function analysis, and multinomial logistic regression, within groups of parasitized and unparasitized warblers and weavers.

|                      | warbler family |        | weaver family |        |
|----------------------|----------------|--------|---------------|--------|
|                      | parasitized species | unparasitized species | parasitized species | unparasitized species |
|                      | (n = 205 clutches, 5 species) | (n = 219 clutches, 6 species) | (n = 339 clutches, 10 species) | (n = 46 clutches, 4 species) |
| **discriminant function analysis (DFA)** | | | | |
| accuracy ± 95% CI (%) | 82.4 ± 5.21 | 54.8 ± 6.59 | 63.7 ± 5.12 | 100 ± 0.00 |
| expected correct     | 44            | 42     | 46            | 12     |
| observed correct     | 169           | 120    | 216           | 46     |
| p (expected versus observed) | <0.001       | <0.001 | <0.001       | <0.001 |
| improvement over chance | 60.9%        | 36.6%  | 50.2%        | 74.6%  |
| **multinomial logistic regression** | | | | |
| accuracy (%)         | 92.2          | 66.2   | 79.4          | 100    |
| expected correct     | 44            | 42     | 46            | 12     |
| observed correct     | 189           | 145    | 269           | 46     |
| p (expected versus observed) | <0.001       | <0.001 | <0.001       | <0.001 |
| improvement over chance | 70.7%        | 47.0%  | 65.8%        | 73.9%  |

*p* Fisher’s exact test.

4. Discussion

Distinguishing self from non-self is paramount to the hosts of avian brood parasites, as it is to the victims of many other aggressive mimics. To improve their chances of detecting a parasitic mimic, hosts can diversify their own eggs into a multi-dimensional phenotypic space comprised of such traits as egg colour, luminance and pattern [8,9,14]. In this study, we asked whether such diversification by different host species can be constrained by susceptibility to other parasitic strains, such that co-occurring host species might indirectly shape one another’s coevolutionary trajectories with a shared parasitic species.

In support of this hypothesis, we found that sympatric warbler host species of the cuckoo finch are phenotypically less similar to each other than are sympatric, unparasitized warbler species (figure 2r). Thus, hosts partition egg phenotypic space much more distinctly than do related species that are not currently parasitized. Because of the high level of phylogenetic relatedness among the parasitized warblers (four of the five unparasitized species are in the genus...
Cisticola), they would in the absence of parasitism likely be more phenotypically similar to each other simply as a result of shared phylogenetic history. The fact that they are statistically more discriminable than unparasitized warblers despite their relatedness lends strength to this result, especially given that the unparasitized warblers come from four different genera. As predicted for groups of hosts that partition phenotypic space among themselves, we found that cuckoo finch host-races more closely matched their own warbler host species than other co-occurring warbler hosts, supporting a second prediction of this coevolutionary scenario.

By contrast, in the weaver family, which are hosts of the diederik cuckoo, many host species have diverse eggs, and this diversity overlaps among species. Correspondingly, we were unable to reject our null hypothesis of no difference with unparasitized species. As predicted given this lack of phenotypic partitioning, diederik cuckoo host-races were on average not specialist mimics of their own host, such that...
parasitic eggs found in the nests of a given host species sometimes spanned the phenotypic space of several other host species. Others have previously noted this in South Africa and speculated that a single host-race may exploit multiple *Ploceus* species that each lays highly diverse eggs [45].

This invites the question of why weaver hosts, unlike warbler hosts, do not partition phenotypic space among closely related species, contrary to our prediction that selection should drive them to diversify between as well as among species. We have already underlined that the four species of warblers, parasitic host-races are significantly better visual mimics of their own host species than of other co-occurring host species; by contrast, in the weavers, parasitic host-races are no better mimics of their own host species than of other hosts (see the §3d for statistical analyses). In both panels, a host species and its parasitic host-race are represented by the same shape and colour, host species with hollow symbols and parasitic host-races with filled symbols. Host species in grey indicate species for which we had little or no data regarding parasitic host-races. In panel (a), we had very low sample sizes of parasitic eggs for the parasitic host-races that parasitize *C. erythrops* (*n* = 2) and *C. natalensis* (*n* = 1); thus, we did not include these data points in statistical analysis, but show them here (in black) for completeness. The height and width of the ellipses around each group centroid represent one standard deviation of discriminant function 1 and discriminant function 2, respectively; ellipses around host-races are solid, while those around parasitic host-races are dashed. (Online version in colour.)

**Figure 3.** Phenotypic specialization of (a) cuckoo finch host-races to warbler host species and (b) diederik cuckoo host-races to weaver host species. In the warblers, parasitic host-races are significantly better visual mimics of their own host species than of other co-occurring host species; by contrast, in the weavers, parasitic host-races are no better mimics of their own host species than of other hosts (see the §3d for statistical analyses). In both panels, a host species and its parasitic host-race are represented by the same shape and colour, host species with hollow symbols and parasitic host-races with filled symbols. Host species in grey indicate species for which we had little or no data regarding parasitic host-races. In panel (a), we had very low sample sizes of parasitic eggs for the parasitic host-races that parasitize *C. erythrops* (*n* = 2) and *C. natalensis* (*n* = 1); thus, we did not include these data points in statistical analysis, but show them here (in black) for completeness. The height and width of the ellipses around each group centroid represent one standard deviation of discriminant function 1 and discriminant function 2, respectively; ellipses around host-races are solid, while those around parasitic host-races are dashed. (Online version in colour.)

Parasitic eggs may experience weaker selection from brood parasites at the egg stage, if their front-line defences against laying cuckoos are superior owing to communal vigilance and nest defence in colonial species [47]. Third, intra-specific brood parasitism is common among weavers, which may select for phenotypic diversity within a species irrespective of interspecific parasitism, and thus confound any signal of cuckoo parasitism [48]. Finally, we might speculate that the arms race between weavers and the diederik cuckoo may be younger than that occurring between warblers and the cuckoo finch, which is known to be an exceptionally ancient species [49]; greater coevolutionary advancement should be associated with more sophisticated host defence. Each of these potential explanations might add noise to our results, blurring differences among groups of hosts and non-hosts.

Much research on species interactions has focused on cases where the relationship is mediated by a single trait in each species [reviewed in [50]]. In nature, however, the majority of antagonistic interactions between species are governed by multiple traits [51–53]. For example, wild parsnip resistance to
parasitic webworms is influenced by both flowering phenol
ogy and at least two different chemical defence compounds
[54], and parasitism by monogeen and copepod parasites on
teleost fish is mediated by both mucosal barriers and bioci-
dal secretions [55]. In the brood-parasitic systems in this study,
host defence is based on multiple visual traits that parasites
need to mimic adequately in order to be accepted [11,21],
and we therefore tested our predictions in a multi-dimensional
trait space comprised of colour, luminance and pattern. This
is important because theoretical work has shown that hosts can
achieve an advantage over their parasites when host–parasite
coevolution is mediated by multiple traits, and that the host’s
advantage increases as the number of traits governing the
system increases [52]. This arises because successful parasites
must overcome all of the defences produced by a host, lend-
ing hosts more options for escaping from parasitism. Such
theoretical work underlines that parasites may find it easiest
to switch between hosts with similar defensive phenotypes,
and especially when such host defences comprise multiple
traits and hence are hardest to overcome. Work with gallwasp
(Cynipidae) lends support to this hypothesis, as specialized
parasitoid wasps are more likely to switch between gallwasp
hosts that induce phenotypically similar galls [56].

In summary, our results suggest that the evolution of sig-
nature-like defences against parasitism may be tempered by
suscptibility to closely related parasitic strains. Similar pro-
cesses might shape other antagonistic interactions where
distinguishing self from non-self is crucial and has led to
signature-like diversification in host traits, such as olfactory
signatures in the hosts of insect social parasites [4], and mol-
cular signatures in the adaptive immune system [2]. In
support of recent calls to consider the community context
of coevolutionary interactions [57,58], our results imply that
when multiple host and parasitic lineages coexist, host–host
interactions must be considered in tandem with host–
parasite interactions to obtain a complete picture of the
selection pressures driving host defences.

Data accessibility. Data are available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.dg58v [59].

Authors’ contributions. E.M.C. and C.N.S. wrote the manuscript with con-
tributions from M.S.; E.M.C. and C.N.S. collected the data; M.S. and
E.M.C. conducted visual modelling and image analysis; E.M.C. con-
ducted statistical analyses; all authors contributed to study design
and gave final approval for publication.

Competing interests. We declare we have no competing interests.

Funding. E.M.C. was funded by the Pomona College–Downing
College Student Exchange Scholarship, and thanks Downing College,
Cambridge. M.S. was supported by a BBSCD David Phillips Fellow-
ship (BB/G022887/1). C.N.S. was supported by a Royal Society
Dorothy Hodgkin Fellowship and BBSCD David Phillips Fellowship
(BB/JO14109/1) and the DST-NRF Centre of Excellence at the
FitzPatrick Institute.

Acknowledgements. We thank the late Major John Colebrook-Robjent for
his remarkable egg collection; Emma and Ian Bruce-Miller for their
hospitality in Zambia; Jeroen Koorevaar for assistance with photo-
graphing eggs; Rose Thorogood and Nicholas Brandley for helping
to write custom R codes; and Nick Davies and two anonymous
referees for helpful comments.

References

1. Fisher RA. 1930 The genetic theory of natural
selection. Oxford, UK: Clarendon Press.

2. Borghans JAM, Beltman JB, De Boer RJ. 2004 MHC
polymorphism under host–parasite coevolution.
Immunogenetics 55, 732–739. (doi:10.1007/
s00251-003-0630-5)

3. Finlay BB, McSadden G. 2006 Anti-immunology:
evasion of the host immune system by bacterial
and viral pathogens. Cell 124, 767–782. (doi:10.1016/j.
cell.2006.01.034)

4. Martin SJ, Helantera H, Drijfhout FP. 2011 Is
parasite pressure a driver of chemical cue diversity
in ants? Proc. R. Soc. B 278, 496–503. (doi:10.1098/rspb.
tb101047)

5. Davies NB, Brooke ML. 1989 An experimental study
of co-evolution between the cuckoo, Cuculus
canorus, and its hosts. I. Host egg discrimination.
J. Anim. Ecol. 58, 207–224. (doi:10.2307/49955)

6. Davies NB. 2000 Cuckoos, cowbirds, and other
cheats. London, UK: T. and A.D. Poyser.

7. Olen UI, Moksnes A, Raskha E. 1995 Evolution of
variation in egg color and marking pattern in
European passersines: adaptations in a revolutionary
arms race with the cuckoo, Cuculus canorus. Behav.
Ecol. 6, 166–174. (doi:10.1093/beheco/6.2.166)

8. Lahit DC. 2006 Persistence of egg recognition in the
absence of cuckoo brood parasitism: pattern and
mechanism. Evolution 60, 157–168.

9. Spottiswoode CN, Stevens M. 2010 Visual modeling
shows that avian host parents use multiple visual
cues in rejecting parasitic eggs. Proc. Natl Acad.
Sci. USA 107, 8672–8676. (doi:10.1073/pnas.
0910486107)

10. Davies NB, Brooke ML. 1988 Cuckoo versus reed
warblers: adaptations and counteradaptations.
Anim. Behav. 36, 282–284. (doi:10.1016/0003-
3472(88)90269-0)

11. Spottiswoode CN, Stevens M. 2011 How to evade a
coevolving brood parasite: egg discrimination versus
egg variability as host defences. Proc. R. Soc. B 278,
3566–3573. (doi:10.1098/rspb.2011.0401)

12. Spottiswoode CN, Stojković K, Quader S,
Colebrook-Robjent JFR, Sorenson MD. 2011 Ancient
host specificity within a single species of brood
parasitic bird. Proc. Natl Acad. Sci. USA 108,
17738–17742. (doi:10.1073/pnas.1109630108)

13. Medina I, Troschiano J, Stevens M, Langmore NE. 2016
Brood parasitism is linked to egg pattern diversity
within and among species of Australian passerines.
Ann. Zool. 187, 351–362. (doi:10.1686/0864627)

14. Cavas EM, Stevens M, Iversen ES, Spottiswoode CN.
2015 Hosts of avian brood parasites have evolved
egg signatures with elevated information content.
Proc. R. Soc. B 282, 20150598. (doi:10.1098/rspb.
tb2015.0598)

15. Davies NB, Brooke ML. 1989 An experimental study
of co-evolution between the cuckoo, Cuculus
canorus, and its hosts. II. Host egg markings, chick
discrimination and general discussion. J. Anim. Ecol.
58, 225–236. (doi:10.2307/49956)

16. Moksnes A, Raskha E. 1995 Egg-morhps and
host preference in the common cuckoo (Cuculus
canorus): an analysis of cuckoo and host eggs
from European museum collections. J. Zool. Lond.
265, 625–648. (doi:10.1111/1469-7998.1995.
tb20736.x)

17. Sorenson MD, Seef KM, Payne RB. 2003 Speciation
by host switch in brood parasitic indigobirds. Nature
424, 928–931. (doi:10.1038/nature01863)

18. Gibbs HL, Sorenson MD, Marchetti K, Brooke ML,
Davies NB, Nakamura H. 2000 Genetic evidence for
female host-specific races of the common cuckoo.
Nature 407, 183–186. (doi:10.1038/33205305)

19. Fossey F, Antounov A, Moksnes A, Raskha E, Vikan
JR, Moller AP, Stykoff JA, Stokke BG. 2011 Genetic
differentiation among sympatric cuckoo host races:
male matter. Proc. R. Soc. B 278, 1639–1645.
(doi:10.1098/rspb.2010.2090)

20. De Silva TN, Townsend Peterson A, Fernando SW,
Bates JM, Marks BD, Girard M. 2017 Phylogenetic
relationships of weaverbirds (Aves: Ploceidae); a first
robust phylogeny based on mitochondrial and
nuclear markers. Mol. Phylogenet. Evol. 109,
21–32. (doi:10.1016/j.ympev.2016.12.013)

21. Lahit DC, Lahit AR. 2002 How precise is egg
discrimination in weaverbirds? Anim. Behav.
63, 1135–1142. (doi:10.1016/anbehav.2002.0309)

22. Colebrook-Robjent JFR. 1984 The breeding of the
didric cuckoo Chrysococcyx caprius in Zambia. In
Proc. 5th Pan-African Ornithological Congress,
Lilongwe, Malawi 1980 (ed. John Ledger), pp. 763–777. Johannesburg, South Africa: Southern African Ornithological Society.

23. Barbosa A, Mathger LM, Buresch KC, Kelly J, Chubb C, Chiao C-C, Hanlon RT. 2008 Cuttlefish camouflage: the effects of substrate contrast and size in evoking uniform, mottle or disruptive body patterns. *Vis. Res.* **48**, 1242–1253. (doi:10.1016/j.visres.2008.02.011)

24. Stoddard MC, Stevens M. 2010 Pattern mimicry of host eggs by the common cuckoo, seen through a bird’s eye. *Proc. R. Soc. B* **277**, 1387–1393. (doi:10.1098/rspb.2009.2018)

25. Fisher RA. 1936 The use of multiple measurement in taxonomic problems. *Ann. Eugen.* **7**, 179–188. (doi:10.1111/j.1469-1809.1936.tb02137.x)

26. Quinn GP, Keough MJ. 2002 *Experimental design and data analysis for biologists*, 1st edn. Cambridge, UK: Cambridge University Press.

27. Stevens M, Lown AE, Wood LE. 2014 Camouflage and individual variation in shore crabs (*Carcinus maenas*) from different habitats. *PLoS ONE* **9**, 1–31. (doi:10.1371/journal.pone)

28. Teasdale LC, Stevens M, Stuart-Fox D. 2013 Discrete colour polymorphism in the tawny dragon lizard (*Ctenophorus decresii*), 140–147. (doi:10.1111/j.1469-1809.2009.11.009)

29. Dechaume-Moncharmont F, Monceau K, Cezilly F. 2011 Sexing birds using discriminant function analysis: a simulation study. *Metod. Zv.*, 143–161.

30. Morrison ML. 1984 Influence of sample size on discriminant function analysis of habitat use by birds. *J. F. Ornithol.* **55**, 330–335.

31. Manly BFJ. 1994 *Multivariate statistical methods: a primer*. London, UK: Chapman and Hall.

32. Efron B. 1982 *The jackknife, the bootstrap, and other resampling plans*. Philadelphia, PA: Society for the Industrial Application of Mathematics.

33. James F, McCulloch CE. 1990 *Multivariate analysis in ecology and systematics: panacea or Pandora's box?* *Ann. Rev. Ecol. Syst.* **21**, 129–166. (doi:10.1146/annurev.ecolsys.21.1.129)

34. Picard RR, Berk KN. 1990 *Data splitting*. *Am. Stat.* **44**, 140–147. (doi:10.1016/j.neunet.2009.11.009)

35. Tabachnick B, Fidell L. 2007 *Using multivariate Statistics*, 5th edn. New York: Allyn and Bacon.

36. Tabachnick B, Fidell L. 2007 Evaluating outlier identification tests: Mahalanobis D squared and Conroy Dk. *Multivariate Behav. Res.* **32**, 189–202. (doi:10.1207/s15327906mbr2302_4)

37. Jarrell MG. 1994 A comparison of two procedures, the Mahalanobis distance and the Andrews–Pregibon statistic, for identifying multivariate outliers. *Res. Sch.*, 1, 49–58.

38. Pohar M, Blas M, Turk S. 2004 Comparison of logistic regression and linear discriminant analysis: a simulation study. *Metod. Zv.*, 1, 49–58.

39. Sereno MD, Payne RB. 2001 A single ancient origin of brood parasitism in African finches: implications for host–parasite coevolution. *Evolution* **55**, 2550–2567. (doi:10.1111/j.0014-3820.2001.tb00768.x)

40. Abrams PA. 2000 The evolution of predator–prey interactions: theory and evidence. *Annu. Rev. Ecol. Syst.* **31**, 79–105. (doi:10.1146/annurev.ecolsys.31.1.79)

41. Berenbaum M, Zangerl A, Naito JK. 1996 Constraints on chemical coevolution: wild parsnips and the parsnip webworm. *Evolution* **40**, 1215–1228.

42. Jones SR. 2001 The occurrence and mechanisms of innate immunity against parasites in fish. *Dev. Comp. Immunol.* **25**, 841–852. (doi:10.1016/S0145-305X(01)00039-8)

43. Speed MP, Fenton A, Jones MG, Ruxton GD, Brockhurst MA. 2015 Coevolution can explain defensive secondary metabolite diversity in plants. *New Phytol.* **208**, 1251–1263. (doi:10.1111/nph.13560)

44. Orme D, Freckleton R, Thomas G, Petzoldt T, Fritz S, Isaac N, Pearse W. 2013 *caper*: Comparative Analyses of Phylogenetics and Evolution in R. R package version 0.5.2.

45. Smilowitz MD. 1988 Evaluating outlier identification tests: Mahalanobis D squared and Conroy Dk. *Multivariate Behav. Res.* **32**, 189–202. (doi:10.1207/s15327906mbr2302_4)

46. Sexton JO, Fortin MJ, Stewart MB, Hébert JD. 2010 Movement ecology: linking organismal movements to population processes. *Annu. Rev. Ecol. Evol. Syst.* **41**, 37–68. (doi:10.1146/annurev.earc.30.110808.143530)

47. Fox J, Weisberg S. 2011 *An R companion to applied regression*. London, UK: Sage Publications Ltd.

48. Jackson WM. 1992 Relative importance of parasitism and individual variation in shore crabs (*Carcinus maenas*) from different habitats. *PLoS ONE* **9**, 1–31. (doi:10.1371/journal.pone)

49. Dahlgren S, Pettersson B, Sundh P. 2012 Parasite-free offspring: a common assumption for empirical studies. *Philos. T. Roy. Soc. B* **367**, 2047–2057. (doi:10.1098/rstb.2011.0109)

50. Broderick AO. 2007 Coevolution can explain defensive secondary metabolite diversity in plants. *New Phytol.* **208**, 1251–1263. (doi:10.1111/nph.13560)

51. Dunn OP, Oshinski JM, Golladay S. 2010 The use of multiple measurement in taxonomic problems. *Ann. Eugen.* **7**, 179–188. (doi:10.1111/j.1469-1809.1936.tb02137.x)

52. Quinn GP, Keough MJ. 2002 Experimental design and data analysis for biologists, 1st edn. Cambridge, UK: Cambridge University Press.

53. Stevens M, Lown AE, Wood LE. 2014 Camouflage and individual variation in shore crabs (*Carcinus maenas*) from different habitats. *PLoS ONE* **9**, 1–31. (doi:10.1371/journal.pone)

54. Picard RR, Berk KN. 1990 *Data splitting*. *Am. Stat.* **44**, 140–147. (doi:10.1016/j.neunet.2009.11.009)

55. Tabachnick B, Fidell L. 2007 Using multivariate statistics, 5th edn. New York: Allyn and Bacon.