A Mosaic of Chemical Coevolution in a Large Blue Butterfly

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SOM Text
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A Mosaic of Chemical Coevolution in a Large Blue Butterfly

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Mechanisms of recognition are essential to the evolution of mutualistic and parasitic interactions between species. One such example is the larval mimicry that *Maculinea* butterfly caterpillars use to parasitize *Myrmica* ant colonies. We found that the greater the match between the surface chemistry of *Maculinea alcon* and two of its host *Myrmica* species, the more easily ant colonies were exploited. The geographic patterns of surface chemistry indicate an ongoing coevolutionary arms race between the butterflies and *Myrmica rubra*, which has significant genetic differentiation between populations, but not between the butterflies and a second, sympatric host, *Myrmica ruginodis*, which has panmictic populations. Alternative hosts may therefore provide an evolutionary refuge for a parasite during periods of counteradaptation by their preferred hosts.

Social and brood parasites often use mimicry to exploit their hosts (*1*, *2*). These parasites typically affect a small proportion of host populations, so that selection for costly defensive discrimination between kin and parasites by the host (*2*, *3*) may be weak (*4*). This allows social parasites of ants, bees, and wasps to parasitize multiple host species (*5*–*7*). However, when parasites are common enough, selection on hosts to avoid being parasitized fuels coevolutionary arms races, in which parasites evolve better mimicry and hosts improve their recognition of parasites (*8*, *9*).

The dynamics of parasite density and distribution can be explained by geographic mosaic models of coevolution (*10*), which allow different degrees of interaction and adaptation between local populations. In these models, mutual coadaptation is restricted to sites of intense and lasting interactions (hotspots), whereas parasites and hosts may evolve independently in other populations (coldspots). Theoretical studies have explored geographic mosaic models (*11*, *12*), but there have been few empirical tests of ecological systems in which the mechanisms of coevolution and the patterns of interaction were known (*10*).

The Alcon blue butterfly, *Maculinea alcon*, is socially parasitic on two species of *Myrmica* ants in Denmark (*13*). The butterfly's caterpillars initially develop on marsh gentian plants, *Gentiana pneumonanthe* (Fig. 1A), before being "adopted" by a foraging *Myrmica* worker (Fig. 1B). Once inside the host ant nest, caterpillars are fed by the ants in preference to their own larvae (*14*), reducing host fitness, particularly in small colonies (*15*) (Fig. 1C and fig. S2). The overlap in distribution of the widespread host ants and the rare host plant is small, both locally and regionally, and there is geographic variation in the abundance and use of the two host ant species (*13*). Populations of the Alcon blue are therefore patchy, and only a small fraction of host ant populations are parasitized and potentially subject to selection for resistance. The parasite is absent from most host populations, which are therefore coevolutionary coldspots (*10*, *11*). In much of Europe, a third ant species, *Myrmica scabrinodis*, is also a host of the Alcon blue (*16*), but infection of this species has never been observed in Denmark, despite its abundance on Danish *M. alcon* sites (*13*).

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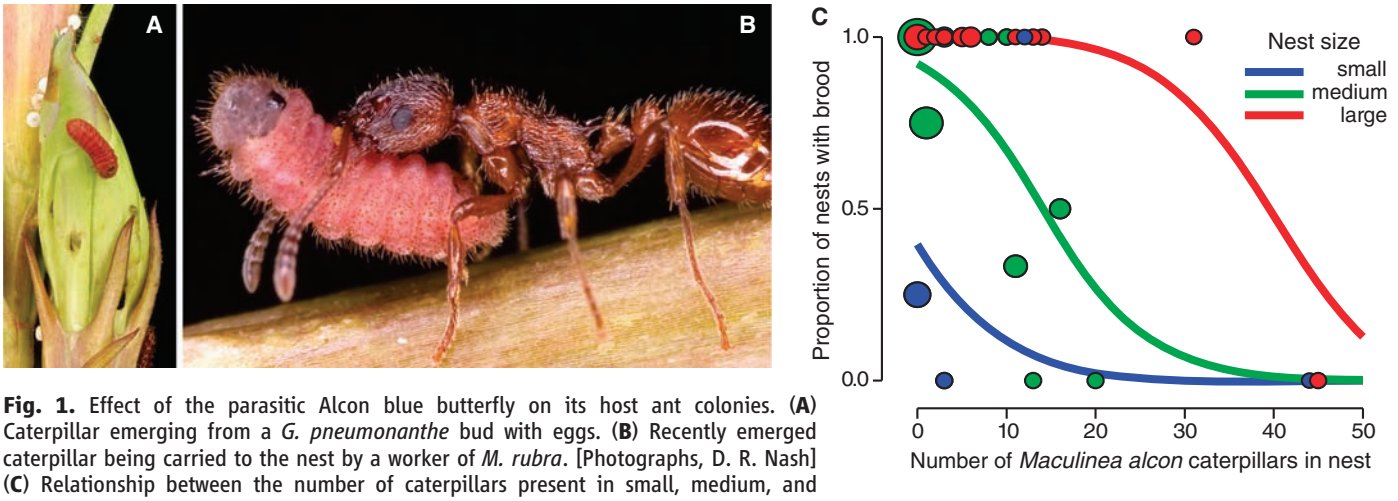

Fig. 1. Effect of the parasitic Alcon blue butterfly on its host ant colonies. (**A**) Caterpillar emerging from a *G. pneumonanthe* bud with eggs. (**B**) Recently emerged caterpillar being carried to the nest by a worker of *M. rubra*. [Photographs, D. R. Nash] (**C**) Relationship between the number of caterpillars present in small, medium, and large *M. rubra* nests (SOM text) in late spring and the probability of ant brood being present. The area of each symbol is proportional to the number of nests observed with that number of caterpillars. Lines are fitted logistic regressions.

In previous cross-infectivity experiments (*17*), Alcon blue caterpillars were adopted more rapidly by host ants from the populations that they infect than by the alternative host species present in the same area (sympatric). However, local parasite maladaptation (host resistance) was also found, because the most rapid adoption of caterpillars was by *Myrmica rubra* colonies from distant (allopatric) populations (*17*). Adoption time is a good measure of infectivity of the parasite that combines the speed of retrieval of caterpillars and initial integration into the ant colony (*18*).

Maculinea caterpillars are thought to infect *Myrmica* nests by mimicking the surface chemistry of the ant brood (*19*, *20*). We collected samples of pre-adoption parasite caterpillars and host ant larvae from the same three populations that were previously used for the cross-infection experiments and examined their cuticular chemistry (*15*, *17*). Qualitatively, the caterpillars of *M. alcon* and the larvae of *M. rubra* and *Myrmica ruginodis* shared the same set of surface compounds, whereas the nonhost ant *M. scabrinodis* did not (Fig. 2A, fig. S1, and table S1). Quantitatively, however, the relative abundances of the shared compounds varied between populations of *M. alcon* (Fig. 2B) and its two ant hosts (figs. S1 and S3). Chemical similarity was a significant predictor of infectivity, explaining 62% of the variation in adoption time for *M. rubra* and 78% for *M. ruginodis* (Fig. 2, C and D).

Local selection on hosts should favor heritable [Supporting Online Material (SOM) text] changes in ant larval surface chemistry if this allows discrimination between ants and *Maculinea* caterpillars (*21*). Because the patchy *Maculinea* populations are expected to counter host resistance, coevolutionary hotspots are created. Therefore, we hypothesized that the ants and caterpillars are in a coevolutionary arms race in degree of hydrocarbon profile matching and that there are different trajectories in change in surface chemistry between hotspots. However, if substantial gene flow occurs between infected host subpopulations and nearby uninfected subpopulations, any parasite-induced selection on the ants to change recognition compounds is likely to be ineffective, resulting in a coevolutionary cold-spot. Previous work suggested that *M. rubra* has levels of gene flow much lower than those of *M. ruginodis* (*22*).

We compared the surface chemistry of ant larvae from uninfected nests of *M. rubra* and *M. ruginodis* from the three sites with *M. alcon* and from three sites where the butterfly has never been recorded (*15*) (Fig. 3A). The chemical profiles of *M. rubra* (Fig. 3B) differed significantly between the six populations (Wilk's $\lambda_{10,40} = 0.159$, $P < 0.0001$) because of divergent chemical profiles among the infected populations ($\lambda_{4,18} = 0.204$, $P = 0.0047$) rather than differences between uninfected populations ($\lambda_{4,20} = 0.899$, $P = 0.891$). In contrast, *M. ruginodis* (Fig. 3C) did not have significantly different surface chemical profiles between populations ($\lambda_{10,48} = 0.612$, $P = 0.414$), and differences between infected populations ($\lambda_{4,22} = 0.960$, $P = 0.976$) were no greater than those between uninfected populations ($\lambda_{4,24} = 0.852$, $P = 0.735$).

We estimated Wright's F_{ST} (*23*), a measure of genetic differentiation between populations, by using three variable microsatellite loci (*15*) to examine whether differences in chemical profiles between and among parasitized *M. rubra* and *M. ruginodis* reflected genetic structure (*22*). Populations of *M. rubra* were strongly genetically differentiated, with an overall F_{ST} of 0.136 [$P < 0.0001$; all loci produced highly significant F_{ST} values (table S3)], whereas populations of *M. ruginodis* were not ($F_{ST} = 0.004$, $P = 0.473$). Our F_{ST} estimate for *M. rubra* over distances >100 km was similar to that found in Finland (*22*) over a few hundred meters, confirming that this species has highly viscous populations with little local gene flow. We also measured Wright's F_{IS}, a measure of inbreeding, and found it was similar for the two ant species, with *M. ruginodis* [$F_{IS} = 0.187$ (table S4)] being somewhat more inbred than *M. rubra* ($F_{IS} = 0.136$). Thus, inbreeding is not associated with the higher hydrocarbon profile differentiation and variability in *M. rubra*. The corresponding values for *M. alcon* were $F_{ST} = 0.182$ and $F_{IS} = 0.132$, estimates very similar to those for *M. rubra*. These data indicate that all three species have patchy populations but that migration is higher in *M. ruginodis* than in either *M. rubra* or *M. alcon*.

We found a relatively constant infection rate for *M. ruginodis* nests (range of 8 to 40%) across all sampled populations (*13*), whereas infection rates of *M. rubra* nests varied from 0 to 72%. For *M. rubra*, the infection rate increased with the prevalence of *M. rubra* (logistic regression, $r^2 = 40.86$, d.f. = 5, $P < 0.0001$), whereas no such relationship existed for *M. ruginodis* ($r^2 = 4.59$, d.f. = 7, $P = 0.71$). This is consistent with the Alcon blue becoming locally adapted to *M. rubra* but not to *M. ruginodis* as host populations become denser.

The changes in host surface chemistry in parasitized *M. rubra* populations indicate that the *M. alcon*–*M. rubra* combination forms a geographic mosaic of coevolutionary hotspots, with a continuing arms race in chemical mimicry, whereas the sympatric *M. alcon*–*M. ruginodis* interaction does not. An alternative interpretation, that the changes in hydrocarbon profiles of *M. rubra* reflect environmental differences between infected and uninfected sites, seems unlikely given the arbitrary direction of profile divergences and the lack of divergence in uninfected populations. If anything, we expect uninfected populations to be more environmentally variable than infected populations because they occur in a range of habitats, whereas infected populations

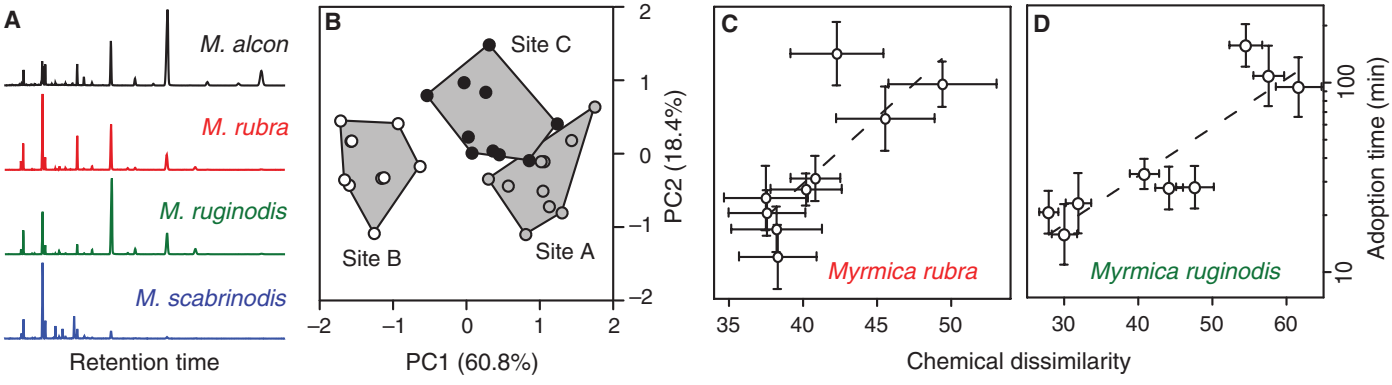

Fig. 2. Chemical mimicry of *Myrmica* ants by Alcon blue caterpillars. (**A**) Representative gas chromatograms for surface extracts of caterpillars of *M. alcon*, larvae of the host ants *M. rubra* and *M. ruginodis*, and larvae of the sympatric nonhost *M. scabrinodis*. (**B**) Ordination plots showing the first two principal components of the chemical profiles of *M. alcon* caterpillars from the three sample sites. Markers show the chemical profiles for individual caterpillars. The data for each study population are enclosed by a minimum convex polygon. (**C** and **D**) Relationship between adoption time (log scale) and dissimilarity in chemical profiles (Mahalanobis distance) between *M. alcon* caterpillars and larvae of *M. rubra* (C) and *M. ruginodis* (D). Each point is the mean ± SE of five observations for each of the nine combinations of butterflies and ants from the three infected sites (*17*). Lines are major axis regressions: for *M. rubra*, $r^2 = 0.62$, $P = 0.011$; for *M. ruginodis*, $r^2 = 0.78$, $P = 0.002$.

must overlap the niche of *G. pneumonanthe*. Lack of profile variation between uninfected populations has previously been demonstrated for *M. rubra* over much larger distances (*20*). The within-population variation in cuticular hydrocarbon profiles was consistently higher in infected populations than in uninfected populations of *M. rubra* (sum multivariate test; $F_{1,4} = 10.56$, $P = 0.034$) but not *M. ruginodis* ($F_{1,4} = 0.17$, $P = 0.455$), which further supports our interpretation.

M. alcon is a virulent parasite of *Myrmica* ants (Fig. 1C and SOM text) that will reduce the size and density of host colonies. This, coupled with the density-dependent infection rate for one of its hosts, *M. rubra* (Fig. 3D), is likely to lead to dynamic shifts in host use over time. Although *M. rubra* is not a host at site A (*13*), we observed strong divergence in its chemical profiles at this site (Fig. 3B), which may reflect former use of this host. The coevolutionary coldspots provided by *M. ruginodis* may thus allow *M. alcon* to persist during the emergence of resistant forms of *M. rubra* (SOM text), but this may also put an ultimate limit on how far *M. alcon* can diverge from the relatively static chemical profile of *M. ruginodis*. *M. rubra* and *M. ruginodis* are very similar to each other chemically (*20*), so it is unlikely that such a system could exist for parasites that use more distinct hosts.

Our findings are consistent with geographic mosaic models for coevolution (*24*) and confirm that restricted gene flow is a crucial prerequisite for local coevolution (*25*). Our results also have important implications for the conservation of the Alcon blue, which is increasingly threatened throughout much of its range (*26*). Other *Maculinea* species have been reintroduced to areas where native species have gone extinct without prior screening of similarity in genetics or cuticular chemical profiles (*27*, *28*). Our data suggest that this is reasonable for *Maculinea* species that rely on relatively panmictic species of *Myrmica* host ants because successful reintroductions can likely be made from any source population that uses the same host ant. However, for *Maculinea* species that rely on genetically differentiated host ants, the match between the cuticular chemical profiles of caterpillars and ant larvae should be carefully considered.

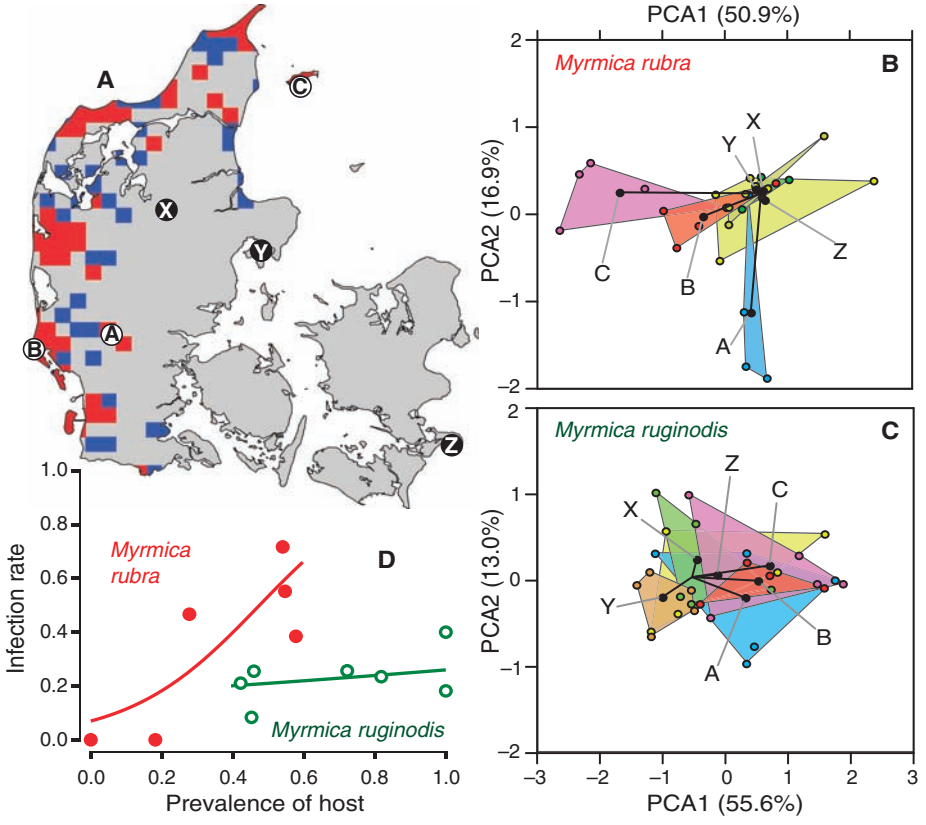

Fig. 3. Adaptation of *Myrmica* host ants to infection by Alcon blue caterpillars. (**A**) The current (after 1990, red) and historical (before 1990 but not since, blue) distribution of *M. alcon* in Denmark plotted on a 10 km–by–10 km grid. The locations of studied infected (points A, B, and C) and uninfected (points X, Y, and Z) populations are marked. (**B** and **C**) Ordination plots showing the first two principal components of the chemical profiles of *M. rubra* (B) and *M. ruginodis* (C). Colored markers show the chemical profiles for individual nests, and the data for each study population are enclosed by a minimum convex polygon. The centroid of each population (black dot) is labeled and linked by a line to the overall centroid of the three uninfected populations. (**D**) Relationship between the infection rate (proportion of nests infected) and the prevalence (proportion of the total host nests that are of that species) for the two host ant species present in each of seven populations of *M. alcon* examined in Denmark (*13*). Lines are fitted logistic regressions.

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