Dense Sampling Phylogenomics Reveals Highly Dynamic Evolution of Batesian Mimicry Accuracy in Two Clades of Myrmecomorphic Spiders

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ABSTRACT

Batesian mimicry is an impressive example of convergent evolution driven by predation. However, the observation that many mimics only superficially resemble their models despite strong selective pressures is an apparent paradox. Here, we tested whether inaccurate mimicry represents an optimized or transitional stage at the macro-evolutionary scale by performing the hereto largest phylogenetic analysis of ant-mimicking spiders across two speciose clades, the jumping spider tribe Myrmarachnini (Salticidae) and the sac spider sub-family Castianeirinae (Corinnidae). We found that accurate ant mimicry evolved in a gradual process in both clades, by an integration of compound traits contributing to the ant-like habitus with each trait evolving at different speeds. Accurate states were highly unstable at the macro-evolutionary scale likely because strong expression of some of these traits comes with high fitness costs. Instead, the inferred global optimum of mimicry expression was at an inaccurate state. This result reverses the onus of explanation from inaccurate mimicry to explaining the exceptional evolution and maintenance of accurate mimicry and highlights that the evolution of Batesian mimicry is ruled by multiple conflicting selective pressures.

Keywords: Araneae, Castianeirinae, macro-evolution, Myrmarachnini, trait evolution

Contrary to theory (e.g., Mappes and Alatalo 1997), mimicry does not consistently involve the convergence of the full range of model traits, resulting in widespread cases of imperfect or inaccurate mimicry (Sherratt and Peet-Paré 2017). For example, many species of hoverflies are poor mimics of wasps and bees (Edmunds 2000), many species of nonvenomous kingsnakes imprecisely mimic deadly coral snakes (Kikuchi and Pfennig 2010), and many myrmecomorphic (i.e., ant-mimicking) spiders inaccurately resemble their ant model (Cushing 2012). Several non-mutually exclusive hypotheses have been proposed to explain the prevalence and maintenance of inaccurate mimics (reviewed in McLean et al. 2019). Most of these hypotheses assume that inaccurate mimics are at an evolutionary stable state and that further refinement would not provide any additional fitness benefit or may even reduce the mimic's fitness. Alternatively, inaccurate mimics may be under continuing directional selection and represent a transitional stage in an evolutionary trajectory towards a phenotypic adaptive optimum (discussed in Fisher 1930; Nur 1970; Edmunds 2000; Gilbert 2005; McLean et al. 2019).

In the 'two-step' theory of mimetic evolution (Poulton 1912), a large mutational change results in a relatively close resemblance to the model (potentially by-passing the inaccurate mimetic phenotype), with subsequent refinement by smaller mutations at several loci (Allen and Cooper 1995). Alternatively, accurate mimics may evolve through incremental trait evolution, requiring many mutational or recombination steps (Fisher 1930), building on Darwin's concept of gradualism (Darwin 1859). This idea has been termed the evolutionary lag or *perfecting* hypothesis (*sensu* McLean et al. 2019), with limited empirical studies that have found mixed results: support in ant-mimic spiders (McIver and Stonedahl 1993; Pekár 2014) and coral snake mimicry (Kikuchi and Pfennig 2010) but not in wasp-mimicking hoverflies (Holloway et al. 2002). Irrespective of the precise mechanism of transition (gradual or punctuated), testing the idea of perfecting in mimicry requires a phylogenetic approach to determine whether there are observable trends in mimic accuracy (see Pennell et al. 2014 and citations within).

Here, we test the perfecting hypothesis using myrmecomorphic spiders (Cushing 1997, 2012) who gain protection from predators by mimicking unpalatable or aggressive ants (Cushing 1997). In spiders, myrmecomorphy has evolved in at least 16 families and 85 genera (Reiskind 1971; McIver and Stonedahl 1993; Cushing 1997, 2012; Pekár 2014). While spiders are generally palatable and are readily preyed upon by numerous predators (Edmunds 1974), myrmecomorphy is effective against visually-guided predators including insectivorous birds (Moya-Laraño et al. 2013), praying mantises (Nelson et al. 2006; Ramesh et al. 2016), wasps (Uma et al. 2013) and various araneophagous spiders (Durkee et al. 2011; Huang et al. 2011; Nelson 2012).

Accurate myrmecomorphy is a remarkable modification of the typically highly stable body shape of spiders (Wolff et al. 2021; Wolff et al. 2022): from a stocky arachnid with two tagmata and eight legs into a thin ant-like habitus creating the illusion of three tagmata separated by narrow constrictions, and with two antennae and six legs (Shamble et al. 2017). Traits such as the elongation of the body, extension of the pedicel (the narrow stalk connecting the cephalothorax and the abdomen; Foelix 2011), constrictions to the body, color changes, patches of hair-like seta and thinning of all legs are integrated to produce a convincing ant-like illusion (Kelly et al. 2021). Due to the number and extent of these morphological changes, we hypothesized that the evolution of accurate myrmecomorphy occurs gradually over long timescales in spiders, and that observed cases of inaccurate myrmecomorphy represent unstable intermediate stages of a long-term trend towards mimicry perfection.

To test this, we conducted the first comprehensive macro-evolutionary study of myrmecomorphy in spiders across two separate clades that have evolved myrmecomorphy independently (Pekár 2014) – the jumping spider tribe Myrmarachnini (Salticidae) and the sac spider subfamily Castianeirinae (Corinnidae). Both clades profoundly differ in their ecology and basic body plan, offering an opportunity to test whether the evolution of Batesian mimicry follows common rules of convergent evolution. We used target-enrichment sequencing of ultraconserved elements (UCEs; Faircloth et al. 2012), a method of genome sub-sampling that is phylogenetically informative at both shallow and deep level timescales (e.g., Kulkarni et al. 2019; Hedin et al. 2019, 2020; Xu et al. 2021; also see Gustafson et al. 2020 and citations within). As many of the ant models that are mimicked by these spiders are unknown and not all our specimens have been identified to species level, we generated a matrix of myrmecomorphic traits based on a comprehensive literature analysis (Kelly et al. 2021) and used it to analyze trait distribution across the fossil-calibrated phylogenies. Support for the perfecting hypothesis was given if moderately accurate species were nested in clades of inaccurate species, and if accurate species were nested in clades of moderately accurate species. In contrast, the perfecting hypothesis was not supported if highly accurate species were nested in clades of non-mimic or inaccurate species. To test whether selection acts preferentially towards inaccurate mimicry, we fitted different evolutionary models with and without the assumption of global adaptive peaks.

METHODS

Methods described here are in brief, for extended method descriptions see online Appendix 1 (I. Extended Methods). Refer to Data Availability section for links to supplemental material.

Taxon Sampling

In total, 204 specimens of Myrmarachnini (Salticidae) and 91 specimens of Castianeirinae (Corinnidae) were included in our study. We targeted taxa to represent global diversity (including undescribed taxa) and variation in myrmecomorphy. For notes on species identification see online Appendix 1 (II. Species Identification). While we attempted a broad global coverage in our species sampling, we recognize that some geographic regions were underrepresented, such as South American Castianeirinae. Outgroups for Myrmarachnini included members of the subfamilies Hisponinae (*n* = 4) and Salticinae (*n* = 12; Maddison 2015). Outgroups for Castianeirinae consisted of representatives of the subfamily Corinninae (*n* = 10; Wheeler et al. 2017). All specimen data, including collection information, are listed in online Appendix 2 (Dataset S1 - sample information).

Trait Selection and Evaluation

Appropriate quantification of accurate mimicry is often noted as a challenging step in studies focused on mimetic variation (Penney et al. 2012; Kelly et al. 2021). The ultimate method is often intuitively considered to be via experimentation using relevant predator assessment trials or alternatively through the direct assessment of similarity between the mimic's phenotype and that of its' putative model (i.e., geometric morphometrics or linear morphometrics; see Kelly et al. (2012) for a discussion on methodological approaches for mimic assessment). However, these approaches of quantification only become feasible when considering a small number of identified mimics, a knowledge of the relevant biological predators and/or when the putative models have been identified. In studies that include a large sampling of mimic species (which is largely based on museum specimens with limited natural history data available) across a global distribution, such as the present study, these methodologies of predator trials and direct mimic/model comparison would be an unviable task as many predators and models have not been empirically validated for many mimic species, including those used here.

Here, we have elected to implement a trait-based assessment of mimetic accuracy following Kelly et al. (2021) that utilises several mimetic traits repeatedly noted in the literature as resulting in an overall ant-like morphology (e.g., Cloudsley-Thompson 1995; Cushing 1997, 2012; Deeleman-Reinhold 2001; Edmunds 2006; Durkee et al. 2011; see Fig. 1 and Table S1 for selected traits and method of trait and overall mimetic accuracy quantification). We acknowledge that this approach is largely based on human assessment, albeit expert opinion, of those traits considered important in contributing to ant-like morphology in myrmecomorphic spiders and that other phenotypic aspects may also be important cues for predator recognition, such as other morphological, behavior and olfactory traits.

Further, we acknowledge that predator vision will deviate from human perception, however, we assume that these selected traits are likely salient to potential predators and are under selective pressure which has resulted in the extreme morphological adaptations observed in myrmecomorphic species. For example, previous experiments using birds (Dittrich et al. 1993) and spiders (Nelson 2012) showed that predators may use comparable cues as humans for assessments of mimetic accuracy.

Finally, based on a former comparison of methods (Kelly et al. 2021), the use of trait-based assessment should result in a meaningful description of mimetic accuracy as it has been shown to be highly correlated with other quantification methods (e.g., geometric morphometrics and linear morphometrics) while remaining feasible for the use of large-scale museum specimens.

Specimens were filmed (alive) or photographed (preserved) for biometrical measurements (in millimeters) of selected myrmecomorphic traits (nine traits and overall mimetic accuracy score; Fig. 1) using ImageJ v1.51 (Schneider et al. 2012). Mimicry accuracy was defined as follows: accuracy score <0.15 non-mimic; 0.15 to <0.30 inaccurate mimic; ≥0.30 accurate mimic. Among the inaccurate mimics we further distinguished between: 1) low levels of mimicry accuracy (scores 0.15-0.19) and; 2) moderate levels of mimicry accuracy (scores 0.20-0.29).

DNA Extraction and UCE Library Preparation

Genomic DNA was extracted from either leg(s) or whole individuals with a maximum starting quantity of 500 ng total DNA for UCE library preparation. UCE library preparation followed the protocol of Starrett et al. (2017) and the UCE website (ultraconserved.org). Libraries were sequenced on a NovaSeq 6000 at the Bauer Core Facility at Harvard University with 150 bp paired-end reads.

Sequence Data Processing

Processing of raw demultiplexed read data was performed using the PHYLUCE v1.6.8 pipeline (available at https://phyluce.readthedocs.io/en/latest/tutorials/index.html; Faircloth 2016). UCE alignments were then imported into Geneious 11.1.5 (http://www.geneious.com; Kearse et al. 2012) and visually inspected to remove any non-homologous sequences and obvious alignment errors.

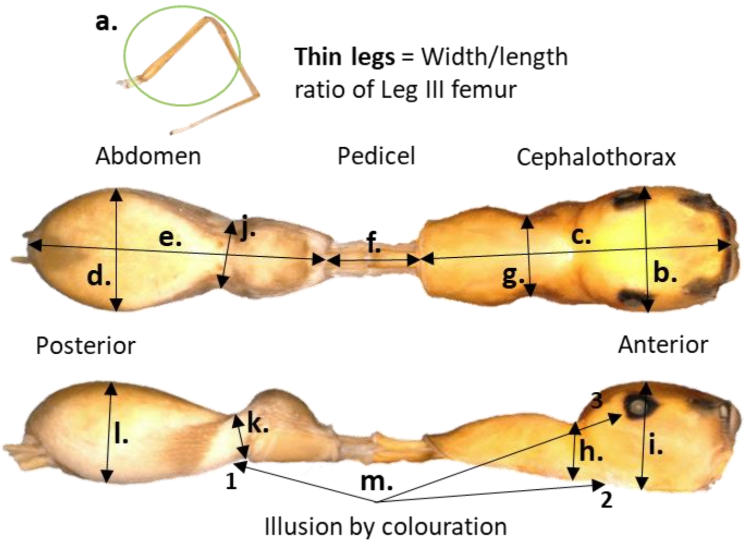

Figure 1. Traits quantified to determine myrmecomorphy, after Kelly et al. 2021: 1) Thin legs = 1 – width leg III femur / length leg III femur (a.); 2) Elongation of cephalothorax = 1 – cephalothorax width (b.) / cephalothorax length (c.); 3) Elongation of abdomen = 1 – abdomen width (d.) / abdomen length (e.); 4) Elongation of pedicel = pedicel length (f.) / total body length (c. + e. + f.); 5) Constriction of the cephalothorax (in dorsal view) = 1 – cephalothorax width at point of constriction (g.) / cephalothorax width at widest point (b.); 6) Constriction of the cephalothorax (in lateral view) = 1 – cephalothorax height at point of constriction (h.) / cephalothorax height at highest point (i.); 7) Constriction of the abdomen (in dorsal view) = 1 – abdomen width at point of constriction (j.) / abdomen width at widest point (d.); 8) Constriction of the abdomen (in lateral view) = 1 – abdomen height at point of constriction (k.) / abdomen height at highest point (l.); 9) Illusion by coloration (m) = scored based on the presence of the illusion by coloration traits 1-3 (i.e., single trait = 0.334, two traits = 0.667, and all three traits = 1.0), 1 = transverse band or stripe of lightly colored setae on the abdomen creating the illusion of a separation into a petiole (or postpetiole) and abdomen, 2 = transverse band or stripe of lightly colored setae on the cephalothorax creating the illusion of a separation into a head and thorax, 3 = darkening of the area surrounding the posterior lateral eye creating the illusion of only two large compound eyes. Overall mimic accuracy = average of all trait scores combined (i.e., all traits summed and divided by nine).

Phylogenetic Analyses

Phylogenetic inference based on maximum likelihood (ML) was performed in IQ-TREE v2.1.3 (Minh et al. 2020). Each UCE locus was treated as a separate partition; partitions were set with linked branch lengths and independent evolutionary rates. Best-fit models of molecular evolution for each partition were determined by ModelFinder (Kalyaanamoorthy et al. 2017), as implemented in IQ-TREE. Branch support was estimated with an ultrafast bootstrap approximation, using 1000 replicates (Hoang et al. 2018).

For the Bayesian phylogenetic inference (BI), all UCE loci were concatenated into a single partition. The BI analysis was performed using BEAST 2 v2.6.6 (Bouckaert et al. 2019), using the following priors: HKY + Gamma site model, a log-normal relaxed molecular clock, random starting tree, and the birth-death evolutionary model. To obtain divergence time estimates, monophyly constraints were set based on published phylogenomic information (Wheeler et al. 2017; Fernández et al. 2018), with the age prior of the common ancestor informed by fossil taxonomy or secondary calibration points from published analyses (Magalhães et al. 2020).

The inferred trees were visualized using FigTree v1.4.3 (Rambaut 2016) and a time-stamped ultrametric consensus tree was calculated from a posterior sample from each of three independent MCMC runs using *TreeAnnotator* from the BEAST package.

Comparative Analyses

The evolutionary history and dynamics of mimic accuracy and mimetic traits were inferred with a phylogenetic comparative approach in R v4.1.1 (R Development Core Team 2018) using the packages *ape* (Paradis and Schliep 2019), *phytools* (Revell 2012), *geiger* (Harmon et al. 2008), and *BBMV* (Boucher 2019). We tested the fit of the OU (model assumes evolution is stabilized around an optimal trait value, an extension of the BM model; Butler and King 2004) and EB (BM with evolutionary rate deceleration; Harmon et al. 2010) models against the default BM (unconstrained trait diffusion; Felsenstein 1973) model, as mimicry trait evolution was predicted to deviate from BM.

To infer the evolutionary trait optimum of mimicry accuracy, we further analyzed the parameter estimates of the best-fitted OU models, i.e., σ^2 (the macro-evolutionary rate of trait change), θ (the global macro-evolutionary trait optimum, or root state) and α (the ‘pull-factor’ indicating the strength of selection towards the trait optimum θ). Due to conflicting selective pressures, mimicry accuracy could also evolve towards disparate global optima; to test this, we fitted the FPK model by Boucher et al. (2018) with the *BBMV* package. See online Appendix 1 (III. Ancestral Character Estimation) for notes on ancestral character estimation methods.

To measure the phylogenetic signal of each trait, Pagel's λ and Blomberg's K were calculated in *phytools*, see online Appendix 1 (IV. Phylogenetic Signal) for details.

To test the effect of phylogenetic uncertainty on the results, for each clade all phylogenetic comparative analyses were repeated across a sample of 100 trees randomly drawn from the combined posterior of all MCMC analyses (after removal of the 30% burn-in). Data were summarised and visualized using the *sensiPhy* package (Paterno et al. 2018), based on the script in Wolff et al. (2019), see online Appendix 1 (V. Phylogenetic Sensitivity) for details.

RESULTS

Trait Variation

We used a numeric index of myrmecomorphy representing the degree of modification of the basic body-shape of modern spiders across a set of nine traits (Kelly et al. 2021). In Castianeirinae (n = 91), the mimic accuracy was relatively low (average = 0.18, min-max: 0.10-0.30, SD± = 0.04) with 21 specimens scored as non-mimetic, 69 scored as an inaccurate mimic (38 low, 31 moderate accuracy) and only one species considered to be an accurate mimic, *Myrmecium bifasciatum*. In Myrmarachnini (n = 204), overall mimic accuracy was higher (average = 0.31, min-max: 0.16-0.47, SD± = 0.07), with no specimens scored as non-mimetic, 77 scored as inaccurate (9 low, 68 moderate accuracy) and 127 specimens scored as accurate mimics.

An important myrmecomorphic trait in Myrmarachnini is the prevalence of dorsal and lateral constrictions to both the abdomen and the cephalothorax relative to Castianeirinae:

Myrmarachnini: *n* = 198 out of 204 with constriction; Castianeirinae: *n* = 15 out of 91 with constriction. The degree and prevalence of traits including the elongation of the cephalothorax, the abdomen, and the pedicel were both higher in Myrmarachnini, contributing to their higher overall mimic accuracy indices (Table 1). Illusion by coloration was more consistent within Myrmarachnini (*n* = 165) than Castianeirinae (*n* = 27). For all biometric measurements see online Appendix 3 (Dataset S2 - biometric measurements).

Sequencing Results (UCEs)

Specimen sequence data can be found in online Appendix 2 (Dataset S1 - sample information). For information on availability of UCE sequence data see Data Availability. For the Castianeirinae dataset, the number of reads pass QC ranged between 145,707 and 19,183,248 (mean = 6,955,279; SD± = 4,756,270), the number of contigs ranged between 10,855 and 1,853,713 (mean = 583,502; SD± = 419,282), and the number of UCE loci (50% occupancy matrix) ranged between 15 and 809 (mean = 636; SD± = 258). For the Myrmarachnini dataset, the number of reads pass QC ranged between 209,873 and 37,817,349 (mean = 5,780,188; SD± = 3,973,659), the number of contigs ranged between 9,924 and 5,562,935 (mean = 550,682; SD± = 475,362), and the number of UCE loci (75% occupancy matrix) ranged between 24 and 603 (mean = 542; SD± = 120).

Table 1. The minimum, maximum, mean, and sample standard deviation (±) values for the eight selected morphometric traits, and the overall mimic accuracy scores for Castianeirinae and Myrmarachnini (see Kelly et al. 2021 and Table S1 for definitions and method of measurement).

Characteristic	Minimum	Maximum	Mean	SD (±)
Castianeirinae (*n* = 91)				
Thin legs	0.59	0.89	0.71	0.06
Elongation of cephalothorax	0.17	0.66	0.33	0.09
Elongation of abdomen	0.12	0.61	0.39	0.11
Elongation of pedicel	0.00	0.11	0.03	0.02

Lateral cephalothorax constriction	0.00	0.39	0.01	0.05
Dorsal cephalothorax constriction	0.00	0.63	0.01	0.07
Lateral abdominal constriction	0.00	0.18	0.01	0.03
Dorsal abdominal constriction	0.00	0.22	0.01	0.03
Overall mimic accuracy	**0.10**	**0.30**	**0.18**	**0.04**
Myrmarachnini (*n* = 204)				
Thin legs	0.59	0.84	0.75	0.04
Elongation of cephalothorax	0.22	0.66	0.47	0.06
Elongation of abdomen	0.20	0.80	0.50	0.12
Elongation of pedicel	0.00	0.24	0.07	0.05
Lateral cephalothorax constriction	0.00	0.48	0.19	0.10
Dorsal cephalothorax constriction	0.00	0.44	0.18	0.10
Lateral abdominal constriction	0.00	0.48	0.16	0.13
Dorsal abdominal constriction	0.00	0.39	0.12	0.13
Overall mimic accuracy	**0.16**	**0.47**	**0.31**	**0.07**

Phylogenetic Analyses

Maximum likelihood (ML) analysis of both Castianeirinae (Fig. S1) and Myrmarachnini (Fig. S2) UCE datasets produced a phylogeny with the vast majority of the ultrafast bootstrap support (UBS) values ≥ 95%. The lower-supported nodes were mostly associated with historical museum specimens with highly fragmented DNA.

The topology of the BI trees for both Castianeirinae (Fig. 2) and Myrmarachnini (Fig. 3) showed high topological congruence with the tree produced by the ML analyses. We tested the *Myrmecium bifasciatum-Castianeira coquito* clade for long branch attraction by removing one of the two taxa from the dataset and re-running the analysis to determine if the placement of the taxon would change, then we repeated the process for the other taxon. Neither analysis produced any changed topologies to the tree, which demonstrates that long branch attraction is not affecting the placement of either taxa within the clade. For further discussion on topology and divergence date estimates see online Appendix 1 (VI. Topological Incongruence (I)), and Figs. S3 and S4, respectively. For a discussion on the phylogenetic topology of the reconstructed Castianeirinae and Myrmarachnini phylogenies and phylogenetic incongruence between this study and previous studies, see online Appendix 1 (VII. Topological Incongruence (II)).

Comparative Analyses

The maximum likelihood estimated adaptive landscape of fitted Fokker–Planck–Kolmogorov (FPK) models showed a single optimum for most traits, which approximates an Ornstein-Uhlenbeck (OU) model. For most traits of the alternative models Brownian Motion (BM), OU, and Early Burst (EB) distributions were best fitted with OU models (Table S2). Detailed discussion of model selection results can be found in online Appendix 1 (VIII. Results of Model Selection).

***Overall mimicry accuracy.*—**The inferred phylogenetic structure of trait expressions (Figs. 2, 3) supports the theory that accurate mimicry evolves through the gradual and accumulating changes of myrmecomorphic traits. Ancestral character estimation for both myrmecomorphic groups indicate that the moderately accurate extant species have diverged from inaccurate ancestors, and in Myrmarachnini accurate extant species have diverged from moderately accurate ancestors. In our Castianeirinae taxon sampling only a single species was highly accurate while in Myrmarachnini, high overall mimic accuracy was scattered throughout the tree with the highest frequency in two clades. Despite support for a gradual evolutionary process, parameter estimates for both OU and FPK models indicated that higher levels of mimetic accuracy are at an unstable state which was acquired and lost multiple times independently (Figs. 2, 3 insets; and Fig. S5). Therefore, the *perfecting* hypothesis does not explain inaccurate mimicry due to the unstable nature of accurate mimicry, thus we cannot infer which direction selection is driving mimic species - i.e., either maintaining mimic inaccuracy, evolving towards higher levels of accuracy or perhaps even the complete loss of mimetic traits.

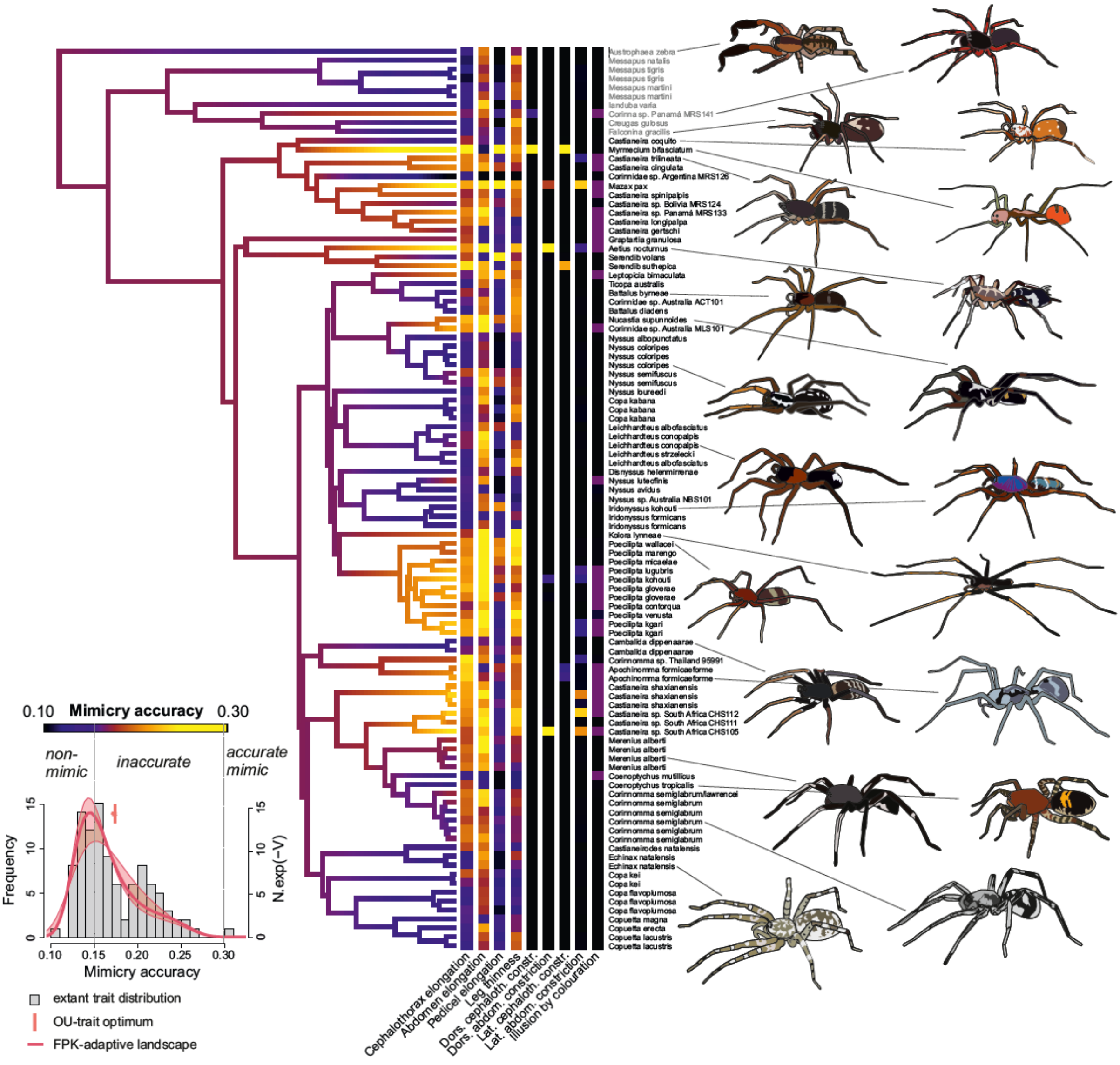

Figure 2. Evolution of ant mimicry in Castianeirinae. Colored branches indicate the evolution of myrmecomorphy strength (mimicry accuracy), with estimation of trait values at internal nodes based on OU-model fit. Colored squares next to the tips indicate the expression of nine traits contributing to the mimicry score. Spider drawings based on live photos of conspecifics or close congeners. The plot in the bottom left shows a histogram of the observed trait variation (in grey), the estimated trait optimum of the fitted OU-models across a sample of 100 phylogenies (median indicated by a vertical red bar), and the shape of the adaptive landscape of the fitted FPK model (thick red line for consensus

tree, thin lines for the most extreme parameters found across the sample of 100 trees). Outgroup taxa are in gray font, ingroup taxa are in black font.

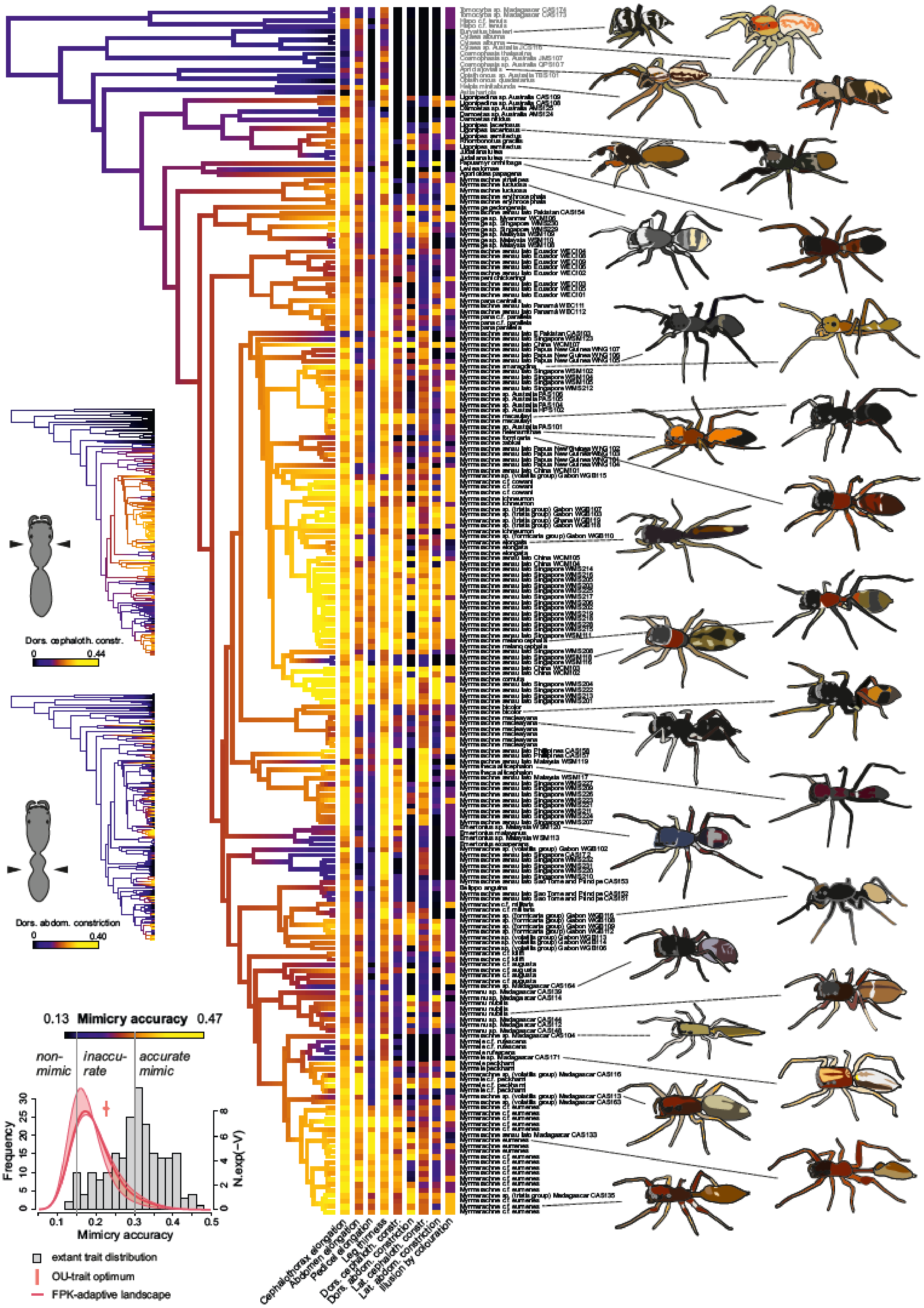

Figure 3. Evolution of ant mimicry in Myrmarachnini. Colored branches indicate the evolution of myrmecomorphy strength (mimicry accuracy), with estimation of trait values at internal nodes based on OU-model fit. Colored squares next to the tips indicate the expression of nine traits contributing to the mimicry score. Spider drawings based on live photographs of specimen or conspecifics. Drawings of Malagasy *Myrmarachne sensu lato* based on microscopy images of ethanol-preserved specimens, with live posture and coloration estimated. The two small phylogenies to the left show the inferred trait evolution for cephalothorax and abdominal constrictions. The plot in the bottom left shows a histogram of the observed trait variation (in grey), the estimated trait optimum of the fitted OU-models across a sample of 100 phylogenies (median indicated by a vertical red bar), and the shape of the adaptive landscape of the fitted FPK model (thick red line for consensus tree, thin lines for the most extreme parameters found across the sample of 100 trees). Outgroup taxa are in gray font, ingroup taxa are in black font.

Myrmecomorphic traits distribution.—Thin legs were a typical trait for all Myrmarachnini (Fig. S6) with only a few lineages scattered across *Myrmarachne sensu lato* exhibiting reversed evolution towards thicker legs. Castianeirinae showed evolution around moderate leg thickness, with separate lineages evolving towards thinner legs and others towards thicker legs. In Castianeirinae, an elongated cephalothorax evolved repeatedly in distinct lineages, often with sister clades showing opposite trends. An elongated cephalothorax emerged at the base of Myrmarachnini and evolved gradually longer towards the core clade. Abdominal elongation evolution was dynamic in both clades, often isolated in single lineages or species. In Castianeirinae, pedicel elongation was scattered across the clade, with multiple independent events in separate lineages or species. Pedicel elongation evolved patchily in Myrmarachnini, with three origins in one clade composed of *Myrmarachne sensu lato* from Southeast Asia and Africa, and another case in a distantly related clade from Madagascar.

Constrictions of the cephalothorax.—Lateral cephalothorax constriction evolved early in the evolutionary history of the *Myrmarachne sensu lato* group, has been enhanced (greater constriction) in several clades, particularly in individuals collected from Singapore, but has been lost in groups from Malaysia and Madagascar. However, in the Malagasy clade constrictions have subsequently re-evolved. In Castianeirinae, the lateral constrictions of the cephalothorax have only evolved in three lineages, most notably *Myrmecium* and *Serendib*. The lateral constrictions of the cephalothorax were closely tied with the evolution of the dorsal constriction in Myrmarachnini and two of the Castianeirinae, *Myrmecium* and *Serendib*.

Constrictions of the abdomen.—In Myrmarachnini, abdominal constrictions were more dynamic and scattered across separated lineages compared to cephalothorax constrictions with dorsal and lateral constrictions of the abdomen correlated. In Castianeirinae, abdominal constrictions evolved only in a few non-related lineages, with dorsal and lateral abdominal constrictions evolving independently, except for a *Castianeira* sp. from South Africa. For further discussion of all the individual myrmecomorphic traits see online Appendix 1 (IX. Myrmecomorphic Trait Evolution).

Phylogenetic signal

To understand the dynamics of the traits underlying mimicry accuracy, the phylogenetic signal was analyzed for each trait (Fig. S7). In Myrmarachnini, the elongated pedicel, thin legs, and the dorsal and lateral cephalothorax constrictions had strong phylogenetic signal (i.e., phylogenetically stable; Fig. S7A). In contrast, lateral and dorsal constrictions of the abdomen had low phylogenetic signal (i.e., were phylogenetically labile). Elongation of the abdomen was less stable than elongation of the cephalothorax.

For Castianeirinae, interpretation of the phylogenetic signal was limited, as some trait distributions strongly deviated from normal due to non-expression in the majority of lineages (Fig. S7C), with the exception of thin legs that were evolutionarily stable.

Phylogenetic sensitivity analysis

We further investigated the phylogenetic sensitivity of the inferred evolutionary model fit and parameter estimates and found that results were highly consistent across the tree sample. The FPK model had the best fit, followed by the OU model (Fig. S5A-F). Inspection of the inferred model parameters and the polynomial functions describing the adaptive landscape of the FPK model showed high consistency across alternative trees in both clades, with a single optimum at a mimicry accuracy score between 0.13 and 0.15 in Castianeirinae (Fig. 2 inset) and between 0.15 and 0.18 in Myrmarachnini (Fig. 3 inset). In both clades, the curve was slightly skewed towards lower accuracy, but otherwise comparable to the adaptive landscape of an OU model (Boucher et al. 2018). Compared to the FPK model, the fitted OU models exhibited slightly higher trait optima (Figs. 2, 3 insets) and slightly higher evolutionary rates (Fig. S5G,I).

The evolutionary rate (σ^2) and α of the fitted OU-models were comparable in both clades. The phylogenetic half-life calculated from α indicates how fast the trait evolves towards the trait optimum θ. With a value of 5th-4th of the total tree height, mimicry accuracy had a low phylogenetic half-life in both Myrmarachnini and Castianeirinae (Cooper et al. 2016), with slightly lower values in the latter. This indicates that mimicry accuracy is generally an evolutionary labile trait and that strong myrmecomorphy is a macro-evolutionary highly unstable state.

DISCUSSION

The evolution of Batesian mimicry converges towards inaccurate expression.

In this comprehensive macro-evolutionary study of myrmecomorphy in spiders, we tested the hypothesis that the tempo and mode of the evolution of accurate mimicry occurs via a gradual, *perfecting* process, and that inaccurate states represent unstable transitional stages (on the macro-evolutionary scale). We found evidence that accurate states, where present, evolved from inaccurate states in a gradual or step-by-step manner, by the accumulation and enhanced expression of multiple traits contributing to myrmecomorphy. This has previously been suggested for the evolution of myrmecomorphy in spiders at the family-level (Pekár 2014) and for Batesian mimicry in coral snakes (Kikuchi and Pfennig 2010).

However, we also found that the evolution of myrmecomorphy is highly dynamic and that trends towards higher mimicry accuracy are frequently reversed, similar to wasp-mimic hoverflies (Leavey et al. 2021). Our findings suggest that inaccurate states are not transitional states *per se* but may more frequently represent an evolutionary optimum. Both OU and FPK models indicated the same global pattern: that integrated selection globally favors inaccurate mimicry and that accurate mimicry is phylogenetically unstable. This aligns with the common observation of inaccurate mimicry across many taxa and the development of novel theoretical frameworks (Dalziell and Welbergen 2016). Although we found myrmecomorphy to be expressed differently in the two focal clades, the rates and modes of mimicry evolution were comparable between them (Myrmarachnini and Castianeirinae). One caveat to our overall finding is that the single accurate Castianeirinae species included in our analyses (*Myrmecium bifasciatum*) was sister group to a low accuracy species (*Castianeira coquito*) – but as both were divided by long branches and nested within a clade of predominantly inaccurate mimics, it is most plausible that *Myrmecium* evolved the high myrmecomorphy via a gradual process, but our sampling may not have included the intermediate sister species between these specimens. Alternatively, a punctuated model is also possible in this case.

Our overall results were robust against uncertainties in phylogenetic topologies and divergence time estimates, indicating that the evolution of myrmecomorphy follows the same rules in different clades of spiders that acquired ant mimicry independently. Inaccurate Batesian mimicry has been recorded frequently (e.g., spiders, hoverflies, butterflies, snakes) suggesting that the adaptive peak may be inaccurate mimicry (Ruxton et al. 2018). Our study is the first attempt at a large-scale phylogenetic comparative approach supporting that this might be a global pattern.

Ecological effects on myrmecomorphic trait expression.

***Proximity to models.*—**There is evidence of relaxed selection on mimetic traits when models are rare or absent, such as in coral snake mimicry (Hodson and Lehtinen 2017; Davis Rabosky et al. 2016). Equally, proximity to ants may play a role in mimic accuracy in myrmecomorphic spiders (Edmunds 1978; Edmunds 2006; Cushing 2012), but not to the point of predators being ant-naïve since ants are found throughout almost all terrestrial habitats.

***Clade-specific environmental context.*—**Some of the differences observed between corinnid sac spiders (e.g., Castianeirinae) and jumping spiders (e.g., Myrmarachnini) may be explained by their predominant ecology. Most jumping spiders are found in the vegetation and other above-ground habitats (Cumming and Wesołowska 2004), while most corinnid sac spiders are mostly ground-, bark- and litter-dwelling. Thus, the occupation of above-ground habitats may lead to greater exposure to predators and hence greater selective pressures to evolve defensive strategies (Perger et al. 2022), whilst crypsis might be more successful in ground- and bark-dwelling corinnid spiders. A case in point is the accurate corinnid ant mimic *Myrmecium* that is frequently found on the vegetation (Candiani and Bonaldo 2017). Evolution towards either aposematism/mimicry or crypsis depending on habitat choice is also known from other animals, such as butterflies and fish (Poole 1970; Cortesi et al. 2016;

Willmott et al. 2017).

Trade-offs.—The constrictions to the abdomen were among the phylogenetically most labile traits, possibly due to an apparent trade-off with fecundity, in terms of egg production in females (Cushing 1997; Sarikaya et al. 2017). Cephalothorax constrictions were evolutionarily more stable traits, but the magnitude of the constriction could result in a trade-off with negative effects on hemolymph hydraulics which may affect jumping capabilities (Shamble et al. 2017; Hashimito et al. 2020) and possibly brain size (i.e., cognition), which has not been tested yet. Although corinnids are not renowned for their cognition or jumping, cephalothorax constrictions and elongations were rare in this clade, but when they occur (e.g., *Myrmecium*) they are often extreme (Candiani and Bonaldo 2017) compared to Myrmarachnini.

Motion-limited discrimination.—The overall greater resemblance to ants in the salticids compared to the corinnids could be explained by differences in running speed, whereby corinnids are often noted for their fast-running speed (Perger and Pett 2022). Predators may not be able to distinguish less accurate mimics from models if they move at speed (‘motion-limited discrimination’). While one study found weak support indicating myrmecomorphic accuracy is negatively related to speed in analyses that considered corinnids, salticids, and insect ant mimics (Pekár et al. 2022), another study reported no support (McLean and Herberstein 2021). It is notable that both studies considered a range of spider and insect myrmecomorphs collectively, therefore we are unable to compare running speeds between salticids and corinnids specifically, which has intriguing potential.

The dynamics of mimicry evolution we found in our study indicate that ecology-specific selection pressures and trade-offs may act differently on the compound traits that contribute to the highly integrative phenotype of a myrmecomorphic body. The result is a dynamic adaptive landscape where locally either inaccurate or accurate mimicry provides the best fitness benefits. Setting aside that ants themselves can be morphologically variable (see Andersen 2016), potential local factors that might contribute to these dynamics include the loss of the model (e.g., Prudic et al. 2002) or changes in predation pressure due to the availability of alternative prey (Kikuchi et al. 2022).

CONCLUSION

Here we have shown that contrary to the prediction that strong selection from predators should result in increased mimic accuracy over evolutionary time, accurate mimicry is a highly unstable state on the macro-evolutionary scale and frequently converges towards inaccurate mimicry. Our multi-taxon approach revealed a complex assembly of selection targets and trade-offs, that may explain why accurate mimicry is rarely maintained.

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Author contributions

M.E.H., G.G., and G.C. designed research; M.B.J.K., S.D., C.J.G., and J.O.W. performed research; M.B.J.K., S.D., D.J.M., R.S., and J.O.W. analyzed data; M.B.J.K. and J.O.W. wrote the initial manuscript draft; and S.D., D.J.M., R.S., C.J.G., C.R.H., G.C., G.G., and M.E.H., provided comments and feedback.

Diversity and Inclusion Statement

We are strong supporters of equity, diversion, and inclusion in sciences (Rößler et al. 2020). While our list of authors is heavily male-biased, we stem from diverse cultural backgrounds and represent different stages of research careers, from PhD student to Professor and at least one of the authors identifies as a member of the LGBTIQA+ community.

Declaration of interests

The authors declare no competing interest.

Data Availability

Online Appendix 1 and Supplemental Figures and Tables are available at Zenodo [https://zenodo.org/records/10468323]. Online Appendix 2 (Dataset S1 - sample information), online Appendix 3 (Dataset S2 - biometric measurements), and the final concatenated UCE alignments used as input for our analyses is available from the Dryad Digital Repository [https://datadryad.org/stash/dataset/doi:10.5061/dryad.18931zd45].

Appendix 2, Dataset S1 - sample information

Sample information including collection data, UCE sequence statistics, voucher institutions and sample registration information.

Appendix 3, Dataset S2 - biometric measurements

Biometric measurements including raw data, calculations of trait scores, and overall mimic accuracy used in the final analyses.

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