

Explaining Imperfect Ant mimicry

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Abstract

Many species have evolved to mimic unprofitable models in order to avoid attack by predators. Mimics are most likely to deceive predators if they resemble their models very accurately, so the existence of many poor mimics is a puzzle. Currently, there is no shortage of hypotheses to explain the persistence of poor mimicry, but comparative evidence supporting them is largely lacking. In this study, I first review the literature regarding imperfect mimicry hypotheses and evidence, then address several hypotheses using ant mimics as study subjects. Ants are avoided by many predators, and ant mimics are widespread, abundant and diverse, making them effective subjects for mimicry studies. Utilising morphometric analysis to quantify body shapes and statistical analysis of walking trajectories as a measure of behavioural mimicry, the accuracy and variation of mimicry in ant-mimicking spiders is compared with that of ant-mimicking insects, and used to assess several imperfect mimicry hypotheses. We find some evidence that poor visual mimicry in spiders may result from developmental constraints, but no evidence that selection is relaxed for smaller mimics. Additionally, ant-like walking neither reinforces nor compensates for visual mimetic accuracy.

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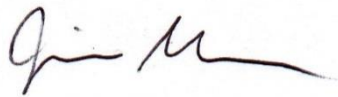
Declaration

I wish to acknowledge the following assistance in the research detailed in this report:

Mariella Herberstein: input on study design, data analysis and writing

Ajay Narendra: input on study design

All other research described in this report is my own original work.

A handwritten signature in dark ink, appearing to read 'Jim McLean', with a stylized, flowing script.

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Introduction

Abstract

Mimicry is the phenotypic resemblance of an organism (the mimic) to another organism (the model). When a third party mistakes the mimic for the model it confers selective benefits on the mimic. To maximise the likelihood of success of the deception, mimics are expected to resemble their models very closely. Therefore, the existence of many poor mimics, which do not accurately resemble their models, is considered an unanswered evolutionary puzzle. Here, we review the current hypotheses and supporting evidence, then evaluate how best to address this gap in our evolutionary understanding by investigating what types of mimicry systems have been studied in the past, and the desirable characteristics of mimicry systems for future studies. We conclude that the diversity, variation and abundance of ant mimics makes them highly suitable subjects for comparative mimicry studies.

Understanding Imperfect Mimicry

Mimicry is a deceptive phenotypic resemblance to another organism (the *model*) which confers a fitness advantage on the mimic [1]. The organism being deceived may be referred to as the operator [2], selective agent [3] or dupe [4]. Mimicry theory predicts that mimics gain maximum benefit from their mimicry when they accurately resemble their models, since accurate resemblance yields the highest probability of successful deception [5-8]. Consequently, mimics should be subject to stabilizing selection that results in a narrow range of mimetic accuracy, centred on perfect resemblance (Figure 1a). It is surprising, therefore, that many mimics appear to bear only a crude resemblance to their models. Studies over the last couple of decades have begun to address the greater than expected occurrence of persistent imperfect mimicry, but so far have failed to establish a consensus as to its causes [9].

In this review we identify and classify a large number of imperfect mimicry hypotheses, and assess which of the hypotheses are currently supported by empirical evidence. We explicitly identify various origins of variation in mimicry and consequently the characteristics of mimicry systems which make them suitable for comparative studies. We analyse the literature to determine the mimics and models that are currently being studied, and discuss how best to approach studies of imperfect mimicry, including the value of ant-mimicry as a study system.

The Study of Imperfect Mimicry

Henry Walter Bates was the first to recognise the adaptive significance of mimicry, as well as the existence of imperfect mimicry and the fact that it required an explanation [10]. Later studies of mimicry investigated whether mimics and models were locked in an evolutionary arms race, since the presence of mimics reduces the efficacy of model aposematism – the chase-away hypothesis [11-13]. More recently, it has been recognised that the abundance and diversity of poor mimics must result from evolutionarily stable mechanisms which are not addressed by conventional theory, so attention has shifted to identifying and describing these mechanisms [14].

Any study of imperfect mimicry must objectively and reliably quantify mimetic accuracy. This has been approached in a number of ways that can generally be categorised as either operator responses or trait measurements. Operator responses include human rankings [15] and pigeon or predator responses to mimics and models [16-19]. Trait measurements include morphometrics to quantify and compare body shapes [15, 20, 21], measurements of colour and/or pattern [22-25], and quantitative behavioural analysis [26, 27]. Difficulties in quantifying accuracy are caused by the need to identify both the model [28] (which need not be a single species [14, 29]) and the traits that are salient to the operator(s) [30-33], and by the unknown interactions between multiple components of mimicry such as appearance and behaviour [32].

Hypothesis Descriptions

There is a large and ever-increasing number of imperfect mimicry hypotheses (Table 1). They vary widely in their levels of empirical support and the level of interest they generate in mimicry literature. Some hypotheses are mutually exclusive, many are not. The human perception hypotheses (not mimics and eye-of-the-beholder) argue that no mimics are imperfect when judged by operators, so no further explanation is necessary. The perfecting and mimetic breakdown hypotheses argue that imperfect mimics are in an intermediate evolutionary stage, while the remaining hypotheses can be grouped into four categories according to the evolutionary landscapes they describe (Figure 1, c-g). Currently, some of the hypotheses are not clearly delineated or differentiated within the literature. Further research is required to identify the predictions that can be used to accept or reject different hypotheses, and the traits to which they can be applied. For instance, are there predictions which can be used to differentiate eye-of-the-beholder from the cone of protection hypotheses? Similarly, is eye-of-the-beholder simply a prediction of another hypothesis such as stimulus salience, since both describe a situation where

predator responses do not correspond with human assessments of mimetic accuracy, while only the latter provides an evolutionary explanation?

Table 1. Summaries of the hypotheses offered to explain imperfect mimicry, grouped by evolutionary characteristics.

Hypothesis	Description	References
Human Perception		
Not mimics	The observed resemblance is not a result of mimicry, but rather convergent evolution, coincidence or a perceived similarity.	[3, 16]
Eye-of-the-beholder	Mimics that humans judge to be imperfect appear accurate to operators, which therefore respond in the same way to both poor mimics and models.	[16]
Evolving		
Perfecting	Mimics are in the process of evolving towards greater accuracy. This may result in persistent imperfection if mimics are unable to evolve faster than the rate at which models evolve a dissimilar appearance (the chase-away hypothesis).	[6, 34]
Mimetic breakdown	Mimicry no longer serves any purpose due to loss of models or operators, and the breakdown occurred before accurate mimicry had evolved or precise mimicry has since been degraded by a lack of direct selection for mimic fidelity. Mimetic breakdown may result in persistent imperfection if the remaining mimetic resemblance is selectively neutral, or the resemblance is maintained by gene flow from regions where mimicry is still active.	[10, 35, 36]
Developmental Constraints		
Developmental Constraints	Lack of phenotypic variation limits further development of mimicry, even though more accurate mimics would be better adapted.	[14, 37, 38]
Trade-offs		

Selection trade-offs	Inaccurate mimicry is the result of trade-offs for other functions such as thermoregulation, camouflage or traits that are under sexual selection.	[24, 39]
Multiple predators	Mimics are subject to predation from multiple predators: predators of non-mimics and predators of models. Each set of predators selects on different morphological aspects of the mimic, resulting in the mimic not resembling the model accurately.	[40]
Multiple models	Imperfect mimics are generalised mimics of multiple different models.	[3, 41]
Kin selection	At high relative frequencies of mimics to models, or for weakly aversive models, there is a net cost to improving mimetic accuracy since it increases total predation in a population of close kin.	[42]
Character displacement	Phenotypic similarity with models results in increased competition with models (e.g. foraging at the same time and place), whereas phenotypic divergence reduces competition.	[43]
<hr/> Relaxed Selection <hr/>		
Cone-of-protection	When models are very toxic and/or much more abundant than mimics, or alternative prey are very abundant, predators are assumed to optimise the cost/benefit ratio of prey selection by erring on the side of caution, avoiding even poor mimics.	[27, 44-47]
Speed/accuracy trade-offs	Predators trade-off accuracy for speed when choosing prey.	[48, 49]
Stimulus salience	Predators use only one or a few salient traits such as colour, shape or smell to discriminate between models and mimics. Accordingly, mimics are only under selection for the traits that are utilised by operators for discrimination.	[17, 30, 44, 48, 50]

Information limitation	The costs of learning result in less than optimal discrimination of mimics by predators, due to incomplete information.	[51, 52]
Increased deception	Poor accuracy in one modality, such as visual resemblance, is compensated for by mimicry in one or more other modalities, such as behaviour.	[27, 32, 53, 54]
Other factors	Selective pressure for accurate resemblance varies depending on the environment, the sensory system of the operator, operator preferences, or prey characteristics that are unrelated to mimicry (such as prey size or speed of locomotion).	[15, 27, 55, 56]
Satyrical mimicry	The mixed and/or conflicting signals in imperfect mimics slow down or lengthen the “perceptual process” required by the predator to interpret the signal, giving the mimic time to escape.	[57, 58]
Aide mémoire	A predator will be deterred from attacking prey if they are reminded of a past encounter with negative consequences.	[59, 60]
Perceptual or Cognitive Exploitation		
Perceptual bias exploitation	Imperfect mimics exploit a <i>perceptual bias</i> in predators, whereby to operators they appear to be <i>more</i> accurate than their models. Unlike eye-of-the-beholder, operators are able to differentiate mimics from models, however mimics benefit from the differential response.	[61, 62]

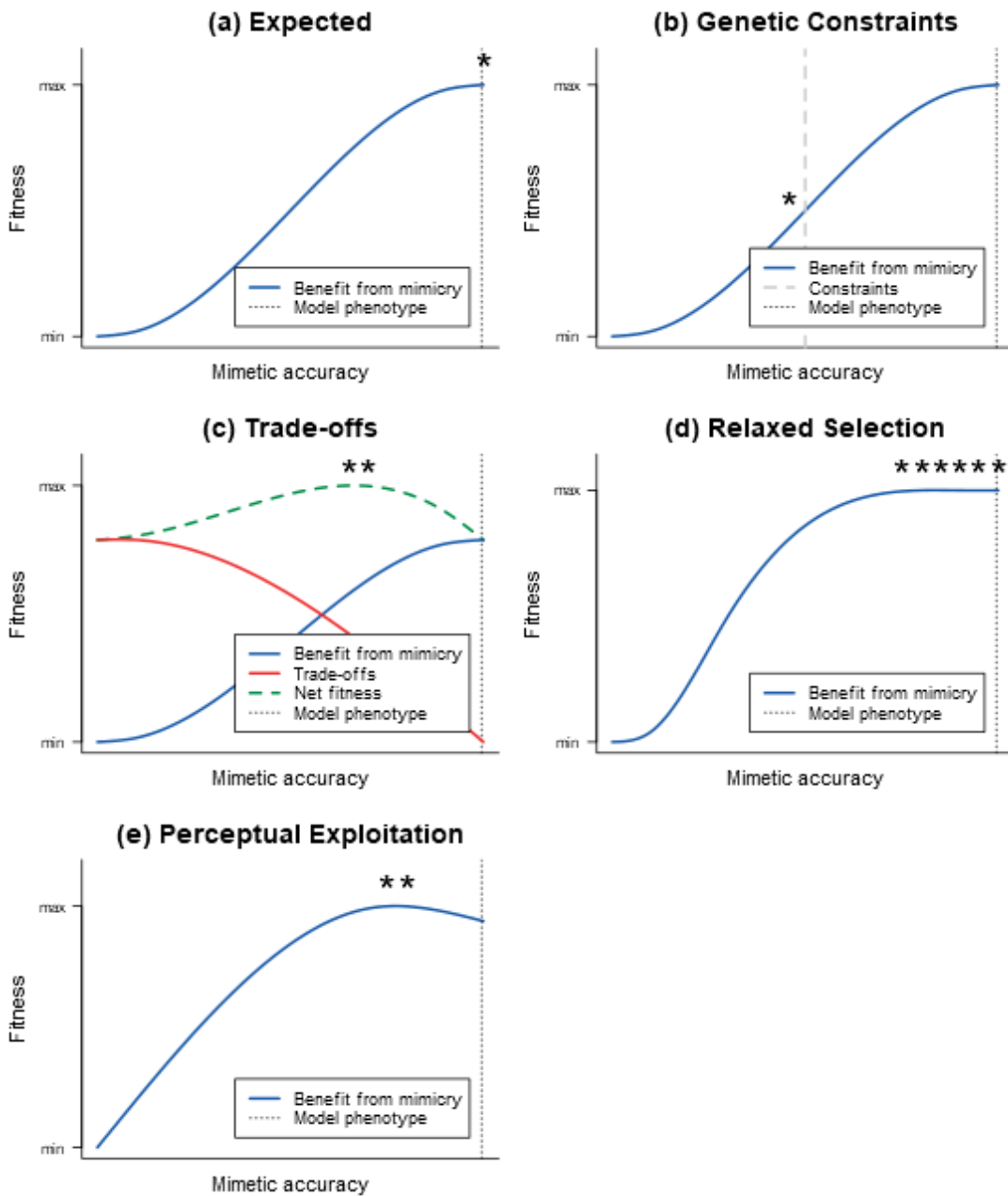


Figure 1. Fitness landscapes relating to imperfect mimicry hypotheses. Each plot represents a relationship between a single phenotypic dimension, mimetic accuracy, on the x-axis (with perfect accuracy, i.e. the model phenotype, indicated by a vertical dotted line at the right-most extent of the axis), and increasing fitness on the y-axis [63]. Organisms are expected to evolve to maximum possible fitness. Asterisks mark the stable values of mimetic accuracy. (a) The fitness landscape predicted by conventional mimicry theory, which does not predict the existence of imperfect mimics. Selection is expected to drive ever improving mimetic accuracy, resulting in all mimics being accurate mimics [5]. (b) Under the constraints hypothesis, organisms are unable to attain optimal theoretical fitness due to their current structure, character, composition, or developmental dynamics [37]. The dashed vertical line in the plot represents a developmental barrier to the evolution of improved accuracy. (c) Under trade-off hypotheses [e.g. 39, 40, 43], selective pressure for greater accuracy is competing with conflicting selective pressure for reduced

accuracy. The result is a stable fitness peak at less than full accuracy. (d) The relaxed selection hypotheses argue that there is no benefit to greater mimetic accuracy beyond a certain threshold. (e) Under the perceptual exploitation hypotheses, imperfect mimics are fitter than accurate mimics, since predators avoid them more than they avoid accurate mimics [57, 61].

Hypothesis Assessments

Human Perception

The human perception hypotheses suggest that the “problem” of imperfect mimicry is rather a problem of human perception, as imperfect mimics are either not mimics [3, 16, 64, 65], or they are accurate mimics as perceived by operators [16]. Assumed instances of mimicry may in fact be examples of convergent evolution, exploitation of perceptual bias or even merely coincidence [64, 65], for example, it is widely assumed that spiders and insects that resemble ants are Batesian mimics [66], despite this assumption having only been tested in a limited number of cases [3, 8, 67-71]. Of the studies which have tested for mimicry, most have tested accurate mimics; very few have tested the efficacy of mimicry in imperfect mimics [18, 19, 27]. The existence of Batesian mimicry can be demonstrated indirectly, by showing that the required behavioural and ecological conditions hold for putative mimics, or directly, by showing that operators respond in the same way to mimics as to models [72]. Direct, experimental evidence of mimicry is stronger than indirect evidence, which is more persuasive for species-specific mimicry than for more “generalised” mimicry [3].

Poor mimicry may be in the eye-of-the-beholder: humans may be better than operators at identifying poor mimicry, so operators do not discriminate between models and poor mimics [16]. There is evidence that pigeons do not discriminate between models and some inaccurate mimics (hoverflies which mimic wasps) [16]. However, other studies have found that various predators are able to discriminate between perfect and imperfect mimics [15, 19]. While there is overwhelming evidence that mimics (and poor mimics) do exist, the alternatives should always be assessed when examining any particular case of putative mimicry.

Evolving

Mimics which are in the process of evolving from poor to good or from good to poor mimicry are covered by the two evolving hypotheses, perfecting and mimetic breakdown. Both hypotheses assume a traditional selective landscape for mimicry: that selection will drive ever improving mimetic accuracy (Figure 1a). The perfecting hypothesis predicts that poor mimics are ancestral to good mimics [34], and that populations of accurate mimics should contain lower levels of

phenotypic variation since they have been subject to selection for increased accuracy for a longer period of time [38]. Evidence supporting the perfecting hypothesis comes ant-mimics [34, 72], whilst it has not been supported in coral snake mimics [73] or hoverflies [38]. 'Chase-away' describes a possible outcome of the evolving hypothesis. It argues that mimics and models are locked in an evolutionary arms race, with the fitness of models decreased by the presence of mimics, resulting in selective pressure on models to decrease the resemblance between mimic and model. The result is that imperfect mimics cannot evolve fast enough to catch their models [6, 74]. No evidence of chase-away was found in a study of ant-mimicking jumping spiders [7], and it is now generally discounted for theoretical reasons [6, 14].

Mimetic breakdown occurs when the protection afforded by mimicry breaks down due to loss of predation [10], loss of models [75], or changes in relative abundance of mimics and models [35] and can be demonstrated by showing the appropriate ecological conditions apply [36]. Mimetic breakdown will result in high levels of phenotypic variation, since selection for mimicry has been relaxed [38]. The evidence for this idea is mixed with support from coral snake mimics [36], but a lack of support in hoverflies [38].

Developmental Constraints

Developmental constraints on mimics may result in a lack of the phenotypic variation required to evolve a closer resemblance to their models, hence preventing the development of accurate mimicry (Figure 1c). Mimics that are imperfect due to developmental constraints are predicted to exhibit little phenotypic variation since they are expected to be subject to strong purifying selection [37]. Imperfect hoverfly mimics show large phenotypic variation, rejecting genetic constraints as a mechanism [38]. Developmental constraints are not generally considered to be a stable mechanism for maintaining imperfect mimicry [14], since given sufficient time and strong selection, such constraints can be overcome [37].

Trade-offs

If the cost of mimicry increases with mimetic accuracy, then some level of inaccuracy may be more adaptive than greater accuracy (Figure 1d) [39]. Evidence for the cost of mimicry exists in a number of systems. Mimicry can lead to reduced mating success, presumably due to a lack of recognition of suitable mating partners [76, 77]. Phenotypic convergence can lead to increased competition for resources between mimic and model, as similar body structures may lead to similar diet and habitat requirements [43]. Morphological constraints, such as the changes in body shape required by spiders for ant-mimicry, can limit the number of eggs a female can produce [14,

78]. Hoverflies in temperate regions may need to compromise between mimicry and thermo-regulation [24]. Similarly, model-specific predators may impose costs on mimetic accuracy, selecting for less accurate resemblance, as poor accuracy reduces detection by model specific predators while still avoiding model averse predators [40]. Other costs may include constraints on mimic specific foraging and mating behaviours which result from emulating model behaviour [66].

Relaxed Selection

Half of the identified hypotheses describe specific circumstances under which selection for greater accuracy is relaxed as operators do not discriminate between good and poor mimics (Figure 1e). Reasons for relaxed selection include operator cognition (which covers speed/accuracy trade-offs in operator decision making and generalisation of models by operators using salient traits), models for which the cost of mistaken identity is particularly high, operator preferences unrelated to mimicry (such as size preferences in predators) and so on (see Table 1). High levels of variation in accuracy may be used as an indicator for relaxed selection [38], or it may be detected using circumstantial evidence [15]. Empirical support for relaxed selection has been found in hoverflies, where selection was found to be relaxed for smaller flies due to a preference by predators for larger prey [15], and for hoverfly mimics of very noxious models [38], although a later study failed to support relaxed selection since they found no evidence of greater variation in poorer mimics [24]. There is empirical support for relaxed selection in poison frog mimics [79], coral snake mimics [80-82], and ant-mimics [27, 83]. Various forms of relaxed selection hypotheses have been addressed many times in the literature, so appear to be the most seriously regarded of the various alternatives.

Perceptual Exploitation

Operators drive mimicry by selecting for similarity to the operator's cognitive image or *percept* [84]. If the percept does not match the phenotype of the model, selection will drive the phenotype of the mimic away from the phenotype of the model [61], resulting in imperfect mimicry [62] (Figure 1f). There is disagreement as to whether this phenomenon should be classified as mimicry or as a related but more general phenomenon, exploitation of perceptual biases [85]. Perceptual bias exploitation has been demonstrated to result in imperfect resemblance in sexually deceptive orchids. The orchids mimic signals of female insects so that male insects attempt to mate with the flowers, resulting in pollination of the flowers [61]. The scent stimulus emitted by the flowers imperfectly mimics that of the insects, yet it elicits a stronger response in male insects than the

scent of the model itself, possibly due to a male bias for novel signals [61]. The flower-mimicking orchid mantis *Hymenopus Coronatus* mimics flowers in order to prey on the pollinators that are attracted to it, and it has been shown to attract pollinators at a higher rate than surrounding flowers, possibly due to pollinator's preferences for larger flowers [62].

Supporting Evidence

Most of the imperfect mimicry hypotheses have been subject to experimental testing, with the exception of speed/accuracy trade-offs, satyric mimicry and aide mémoire (Table 2). Evidence was often conflicting, with only two hypotheses (trade-offs and cone-of-protection) consistently supported by empirical evidence. The cone-of-protection hypothesis was the most extensively tested and consistently supported of the hypotheses to date. We included support from ant-mimicry systems within Table 2 to support later evaluation of ant mimicry as a study system. Within ant-mimicry systems, only five of the 17 hypotheses were supported by empirical evidence from natural systems, three were tested but not supported, while the remainder have not been tested.

Table 2 Summary of evidence for and against imperfect mimicry hypotheses, divided into evidence within general mimicry systems (excluding ant-mimicry) and evidence within ant-mimicry systems.

Hypothesis	General evidence	Ant-mimicry
Not mimics	Not supported	Not supported
Eye-of-the-beholder	Conflicting	Not supported
Perfecting	Not supported	Supported
Mimetic breakdown	Conflicting	Not tested
Constraints	Not supported	Not tested
Selection trade-offs	Supported	Supported
Multiple predators	Not tested	Supported
Multiple models	Not supported	Supported
Kin selection	Conflicting	Not tested
Character displacement	Potentially Supported	Not supported
Cone-of-protection	Supported	Supported
Speed/accuracy trade-offs	Not tested	Not tested
Stimulus salience	Supported	Not tested
Increased deception	Supported	Not supported

Other factors	Not tested	Not tested
Satyrlic mimicry	Conflicting	Not tested
Aide mémoire	Not tested	Not tested
Perceptual bias exploitation	Supported	Not tested

Origins of Variation

Variation in mimicry derives from multiple origins that should be considered in studies of imperfect mimicry. The different life stages of an individual can vary in mimetic accuracy, by mimicking different models (transformational mimicry) [72, 86], and by changing from mimetic to non-mimetic [87]. This variation may result from differing predation pressures, suitability of models due to changes in body sizes [15, 86], or the requirements of one life-stage may impose developmental constraints on another [72]. Accuracy of mimicry can vary between individuals of the same form or species, with the magnitude of variation in a trait within a population or species negatively related to the strength of selection for that trait [24, 38]. Different forms of a species can mimic different models (polymorphic mimicry) [28, 72], which can lead to different predation pressures. Within sexually dimorphic species, the sexes can differ in accuracy, in their models, and whether they are mimetic and non-mimetic [72, 88], so are subject to different selective and developmental constraints. Different populations of the same species can vary in mimetic accuracy [36], and may be subject to different selective pressures. Between mimetic species (and higher taxa) there is variation in mimetic accuracy, and differences in models, developmental and selective constraints. Since the different classes of imperfect mimicry hypothesis predict different types of variation, it is possible to identify the origins of variation predicted by each hypothesis class (Table 3).

Table 3. Origins of variation in mimetic accuracy, predicted by different classes of hypothesis.

	Between individual developmental stages	Between individuals of a population	Between sexes in sexually dimorphic species	Between forms of polymorphic species	Between conspecific populations	Between species or higher taxa
Perfecting		✓				✓
Mimetic breakdown		✓			✓	✓
Constraints	✓		✓			✓

Trade-offs			✓	✓	✓	✓
Relaxed selection	✓	✓	✓	✓	✓	✓

Comparative Hypothesis Testing

In order to evaluate the proposed hypotheses, they must be directly compared. Since results from one test system cannot always be generalised, the most effective method is to test multiple hypotheses within a single mimicry system [9]. Mimetic systems suitable for testing multiple hypotheses should possess a number of characteristics. It must be possible to quantify mimetic accuracy; currently, visual similarity is most easily quantified. The system should contain multiple origins of variation, ideally ranging from variation between individuals through to variation between species and higher taxa [9]. Mimics and models should be abundant, hence making it easy to locate specimens, and larger populations are more likely to provide the desired variation. Ideally, the models and mimics should be well known and studied.

Subjects for Imperfect Mimicry Studies

To assess at what level of variation mimicry is currently being studied, we conducted a literature analysis. We queried the Web of Science (<http://apps.webofknowledge.com>) for articles published after 1987 with the topic “batesian mimicry”, which returned 583 records. The records were shuffled into random order and we analysed the first 60 relevant articles. Articles such as reviews or opinion pieces, or on unrelated topics, were excluded from the analysis. For each relevant article, mimic and model taxa and type of operator were recorded (supplementary material, Table S1).

It is clear that mimicry is mostly studied in insects (Figure 2), of which a significant proportion were the *Heliconius* butterflies that inspired Bates’ classic paper [10]. Lepidoptera are both diverse and well-studied; around 10% of all described living organisms are Lepidoptera (<http://www.ucl.ac.uk/taxome/>), and there exist large abundance datasets (<http://www.naba.org/pubs/countpub.html>). The proximate mechanisms that underlie mimetic resemblance are relatively well understood in butterflies. For example, the multiple forms of polymorphic mimicry in *Heliconius numata* are known to be controlled by a single ‘supergene’ [89]. Mimicry studies of Lepidoptera generally investigate systems in which both mimics and models are Lepidoptera, so they belong to the same taxonomic order, hence proximate signalling mechanisms are similar or the same in both [90], and mimics and models are morphologically very similar. Variation in butterfly mimicry covers several levels, including between individuals,

between sexes (sexual dimorphism [91]), between different forms of a species (polymorphic mimicry [91]), between populations [10] and between species [10]. Caterpillars can exhibit transformational mimicry [92], although adults do not. Predators are generally assumed to be birds [93].

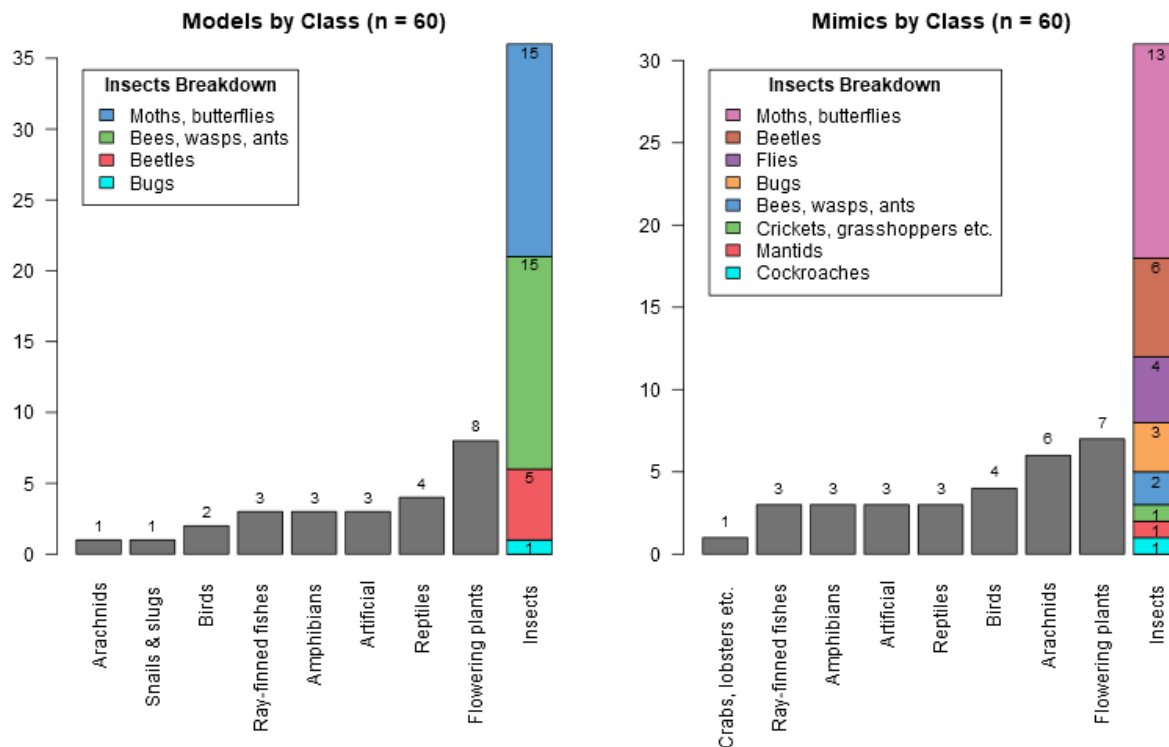


Figure 2 – Taxonomic class of models and mimics in studies of mimicry, from 60 randomly selected articles covering the period 1988 - 2017.

Wasps and bees tied with angiosperms as the second most frequently studied models (Figure 2, Table S1). Bees and wasps are mimicked by a variety of organisms, with hoverflies (Syrphidae) being the most frequently studied mimic (Table S1). The Syrphidae family is widespread, abundant and well-studied [94, 95]. Amongst hoverflies that mimic stinging hymenopterans, mimetic accuracy varies from poor to excellent [15], however there is little variation in morphology, and mimics and models are all restricted to the single class Insecta, which limits the scope for variation both within mimics and between mimics and models.

Most mimics of angiosperms are flowers, although there are some known notable exceptions such as the orchid mantis which visually mimics flowers [62], and beetles which may chemically mimic trees [96]. There are approximately 8,000 species of angiosperms which lack floral rewards, and the majority are orchids pollinated by animals that are deceived into expecting food or other rewards [97, 98]. The mimetic accuracy of deceptive flowers covers a spectrum from a highly

accurate resemblance to a specific model species [99], through to a vague resemblance to a generalised model [100]. The latter case is considered to be “generalized food deception” rather than mimicry since the system is lacking a model [65, 97, 101]. Nonetheless, there may be an adaptive cost to the inaccurate resemblance, since pollinators are able to learn to discriminate between non-rewarding and rewarding flowers, so generalised food deceptive flowers often rely for pollination on naïve pollinators which have not yet learnt to discriminate [97].

Ant mimics were study subjects in 12.6% of the articles (Table S1). Ant mimics are extremely abundant, widespread, and diverse, with more than 2000 species in 200 genera and 54 families [72]. There are many reasons why ant mimics make excellent comparative study subjects. They exhibit variation from many sources, including all of the sources identified above, from within-individual variation through to differences between sub-phylla [72] (ant-mimicry may even extend across different kingdoms [102]). Ant mimicry exists across a range of sensory modalities including visual, behavioural and chemical [72]. Ant mimics are prey to a wide range of different predators [72]. This extreme diversity allows a wide range of hypotheses to be tested within a single mimetic system. However, ant mimics have not been studied as extensively as other mimics. Many ant-mimics (and, indeed, ants) are yet to be described [7, 103]. In many cases, the identity of the predators that acts as the selecting agents for ant-mimics are not well established [8, 83].

Conclusion

Mimicry, despite being a topic of interest and study for well over 100 years, is still not fully understood. Finding general principles governing the widespread existence and persistence of imperfect mimicry is a particular challenge that will require broad comparative studies to resolve. It is clear from our analysis that ant mimics possess most of the characteristics required for comparative analyses including multiple origins of variation, multiple types of mimicry across multiple sensory modalities, and a wide variety of models. Our knowledge of ant mimics will broaden over time, making ant mimics an ideal system to address the question of imperfect mimicry.

Chapter 1. Can developmental constraints, multiple models or relaxed selection explain imperfect mimicry?

Abstract

Batesian mimicry is a defence strategy in which undefended prey have evolved to resemble noxious or dangerous models thereby deceiving predators and avoiding attack. Currently, the high levels of variation in the accuracy of mimics are unexplained, since predators are expected to attack poor mimics, selecting for accurate mimicry. Here, we used ant mimicking spiders and insects to investigate reasons for the variation in mimetic accuracy. We tested the predictions of several hypotheses: that developmentally constrained taxa are poorer mimics and exhibit less phenotypic variation (developmental constraints); that poor mimics are morphologically part-way between two or more different models (multiple models); and that smaller mimics are less accurate because they are less profitable prey, so are avoided by predators thus limiting selection for greater accuracy (relaxed selection on body size). Mimetic accuracy was estimated by comparing the body shapes of ants, mimics, and non-mimics using geometric morphometric analysis. We found some evidence that lateral body shapes of spiders may be developmentally constrained, although developmental constraints are unlikely to explain most of the variation in ant mimics. We also rejected the multiple models and relaxed selection on body size hypotheses.

Introduction

Mimicry is an intriguing example of the power of evolution by natural selection [1]. Batesian mimicry was the first form of mimicry whose adaptive significance was recognised almost 150 years ago [10]. Batesian mimics have evolved a resemblance to unpalatable or well defended models, thereby deceiving predators that mistakenly avoid attacking the mimics. Intuition tells us that predators are most likely to be deceived by mimics that bear a close resemblance to their models, which is predicted to result in strong selection on mimic accuracy. However, the widespread existence of mimics that only crudely resemble an unpalatable model [82, 104] poses a challenge to contemporary theory [9]. A substantial number of hypotheses have been proposed to explain this puzzle, although their relative importance is as yet unknown [9], and comparative studies are needed to evaluate them.

A suitable system in which to investigate imperfect mimicry are ant mimics. Ants are a major influence on the structure and function of terrestrial world. They are very abundant, they are the major herbivores in some environments, and they are among the major predators of other insects and small invertebrates [105]. Ants are also conspicuous, well-armed, often aggressive, distasteful, and attack in concert, all of which makes them suitable models for Batesian mimicry [72].

Unsurprisingly, then, ant mimicry is common, comprising more than 1% of spiders in some locations, or as much as 2% of some arthropod fauna [72]. Ant mimicry has evolved independently more than 70 times across 54 families, at least 15 times in spiders [72], and 12 or 13 times in jumping spiders (Genus: Salticidae) alone [106]. The abundance and taxonomic and morphological diversity of ant mimics makes them ideal subjects for comparative studies of mimicry.

Developmental constraints play an important role in evolution [37, 107], and imperfect mimics allow us to distinguish between developmental and selective constraints because imperfect mimics do not conform to *a priori* adaptive predictions [37]. In spite of this, the role that developmental constraints play in maintaining imperfect mimicry has only rarely been tested [38], and they are considered an unlikely mechanism as they are assumed to be overcome given adequate time and selection [9, 14]. Exactly what constitutes a developmental constraint has been the subject of much debate [108], however a commonly used definition is ‘a bias on the production of variant phenotypes or a limitation on phenotypic variability caused by the structure, character, composition or dynamics of the developmental system’ [37]. If variation in mimic quality in some taxa results from developmental constraints, we can make the following predictions. If two taxa have both been exposed to the same selective regime, yet respond differently, then developmental constraint is implied [37], resulting in poorer mimics in constrained taxa. Additionally, since directional selection for greater mimetic accuracy will eliminate the poorer mimetic phenotypes within a taxon, and developmental constraints prevent the generation of more accurate mimetic phenotypes, constrained taxa are predicted to exhibit lower levels of phenotypic variation than unconstrained taxa [38].

A number of imperfect mimicry hypotheses describe specific circumstances under which the selective pressure to accurately resemble a model is relaxed. Predators are expected to be more conservative in their judgments and avoid poor mimics when models are particularly noxious or well-defended [44], mimics occur at very low frequencies [109], abundant alternative prey exists [46], or if predators sacrifice accuracy for speed of decision-making or make decisions based on generalisations of prey categories [48]. Equally, predators may avoid certain types of prey for

other reasons. For example, in hoverflies (Diptera: Syrphidae), mimicry of bees and wasps is more accurate in larger species, which has been attributed to a preference in predators for larger, more profitable prey, hence selection for accurate mimicry is relaxed in smaller prey [15].

The aim of this study is to test for the roles of developmental constraints and relaxed selection in generating and maintaining imperfect mimics. We chose to compare two broad types of ant-mimics with widely differing developmental systems: ant-mimicking spiders and ant-mimicking insects. These groups were chosen because the developmental distance from spiders to ants is greater than from ant-mimicking insects to ants. Consequently, there is a high likelihood that there are significant differences in the developmental constraints applying to each group. The evolutionary divergence between spiders and insects was at least 500 Myr ago [110, 111]. The evolutionary pathway of spiders has resulted in two body parts, four pairs of legs, pedipalps, chelicerae, and typically eight lens eyes [112]. Ants, by contrast, have three body parts, three pairs of legs, antennae, mandibles, and typically two large compound eyes. Most of these ant morphologies are shared by insects that mimic ants, but not by ant-mimicking spiders. Thus, we predict that spiders will be more developmentally constrained than insects, resulting in poorer mimicry and less variation in mimicry. Our study differs from previous work on mimic variation [24, 38] by testing for the effects of developmental constraints between taxa with substantially different developmental histories, where constraints, if they exist, are most likely to have an effect. To test for relaxed selection, we assess the prediction that body size and morphological accuracy are positively related, as selection for accuracy is relaxed in small prey.

Material and Methods

Specimen Collection and Identification

We collected ant-mimicking spiders and insects, as well as ants and non-mimetic arthropods, from locations in NSW and Queensland between September 2016 and June 2017 (for a list of specimens with collection locations and times, see Table S2). Specimens were collected during daylight hours by visually searching trees and bushes, by inspecting loose bark on tree trunks, or by beating vegetation with a stick into nets or sorting trays. Collected specimens were stored alive in individual plastic vials and transported to the laboratory for later photography. Spiders in the laboratory were kept in vials (50 ml), provided with damp cotton wool as a source of moisture and fed on *Drosophila* twice weekly. In addition, a number of preserved mimetic spiders were

borrowed from the Australian Museum (Sydney, Australia) (identified with source “Borrowed from AM” in Table S2).

Spiders were identified to species where possible using Whyte and Anderson [113], otherwise into family or genus and morphospecies (many juvenile spiders could not be identified to species or even genus). Adult spiders from the genus *Myrmarachne* were classified according to Pekár, Petráková [28], and bugs of the genus *Daerlac* were identified using Cassis and Symonds [114]. Ants, non-mimics and remaining insect mimics were classified to order or family using Zborowski and Storey [115], and then sorted into morphospecies. Deciding whether a species is a visual mimic is still largely based on human visual assessment, and this is especially problematic with imperfect mimics. Ideally, mimics should be subject to predator trials to confirm their status as mimics, but few such studies have been performed to date. For this study, I have used published assessments where possible, although personal judgement was used in a number of insect mimics. Criteria used for the decision to classify as mimics are included in Table S3, consisting of either a reference or “pers. obs.”.

Photography

Specimens were photographed using a Canon 7D with an MP-E 65mm macro lens and an MT-24EX flash, at a magnification of 1-5x chosen to ensure that the specimen was suitably sized within the frame. Photographs were taken from both dorsal and lateral viewpoints, as body shapes clearly differ, and adaptive and developmental constraints may also differ between the viewpoints. Specimens were photographed alive when possible, otherwise they were positioned as naturally as possible and then photographed. The camera was hand-held for live specimens, and mounted on a Cognisys Stackshot automated macro rail for preserved specimens. Each specimen was photographed multiple times from both dorsal and lateral viewpoints, and the best two photographs from each viewpoint were selected for analysis. The photographs were selected based on four main criteria: the body outline was minimally obscured by appendages, the angle of the photograph was as close as possible to true dorsal or lateral, the body parts were parallel, and the specimen was sufficiently in focus. The specimens borrowed from the Australian Museum were photographed in a blob of alcohol based “hand-sanitiser” placed in a hollow acrylic well to prevent drying out [116]. Due to their fragility, the preserved specimens were only photographed once, from a dorsal aspect, minimizing handling and risk of damage.

Photos taken at high magnifications suffer from a very shallow depth of field, so focus stacking was used to increase the depth of field in images of preserved specimens [117, 118]. Focus

stacking works by combining multiple photos of the same object, each focused at a slightly different depth. The photos were composed in software (Zerene Stacker), resulting in a single composite image. Focus stacking could not be used on live specimens as they were constantly in motion. Body lengths were measured from the foremost point visible on the prosoma or head (excluding eyes, appendages and jaws) to the hindmost point visible on the abdomen or opisthosoma (excluding spinnerets and stings). Lengths were measured in the open source image analysis program Fiji [119], from dorsal photos. The photo scale was first set in Fiji, and was calculated as *magnification / pixel width*, where *magnification* was read from the EXIF data for the photo using ExifTool [120] (EXIF tag 'MacroMagnification'), and *pixel width* is the width (in mm) of a single physical pixel on the sensor as specified by the camera manufacturer.

Morphometric Analysis

We applied geometric morphometric analysis to quantify body shapes. Morphometrics is a quantitative method of addressing shape variation and comparisons [121] that has traditionally involved the use of "landmarks", a set of structurally homologous points on each specimen. Since our specimens lack consistent structural homologies (e.g. spiders have no petiole or neck), we instead used elliptical Fourier analysis to quantify whole outlines [122]. Photographs were prepared for morphometric analysis by converting them to a monochrome image with a solid black body shape on a white background. This was achieved by manually tracing the body outline (excluding appendages) in Adobe Photoshop CS2, then filling the traced outline with black and the background with white. Body outlines were rotated so that the body was horizontal with the head to the left, and cropped to leave a small margin. Since non-parallel body parts tend to overwhelm other aspects of shape in the morphometric analysis, body parts were manually aligned, and any obscured portions of the outlines were manually interpolated. Outline images were resized to 1200 x 800 pixels then converted to monochrome.

To perform the morphometric analysis, outline image files were read into memory and converted into outline coordinates which were first subsampled so that all outlines had an equal number of coordinates, then smoothed to remove noise. The resulting shapes were aligned using a Procrustes superimposition, followed by an elliptical Fourier analysis and a principal components analysis [122]. The result of the principal components analysis is a set of n-dimensional points, each of which represents a single outline. Multiple points for each specimen (which result from multiple photographs of the same specimen) were averaged to obtain the average shape for the specimen. The Euclidean distance between any pair of these points may be used to quantify the

difference in shape of the corresponding two outlines. To assess interspecific variation, representative points for species (or morphospecies) were generated by taking the centroid of the points for all individuals within the species. A two-dimensional scatter plot was used to visualise the variations between the species' average outlines, with the two most significant principal components plotted on the x- and y-axes. Dorsal and lateral photographs were processed independently.

Mimetic Accuracy

We constructed an index of mimetic accuracy based on a linear discriminant analysis [123], which is a dimension reduction technique that maximises the ratio of between-class variance to within-class variance in a dataset, and is used to identify the variation that can discriminate between different classes of objects in a set of multi-dimensional points [123, 124]. Inputs to the algorithm are a set of n-dimensional points together with the classification of each point; in our case, the inputs were the n-dimensional points resulting from morphometric analysis of species' average body shapes, and each point was classified as either an ant or a non-ant. To avoid constant valued variables (which cannot be processed by the algorithm), and to reduce the number of dimensions to be processed, points were first transformed by principal components analysis, then linear discriminant analysis was applied to the first principal components which accounted for at least 99% of the total variation in the data set (PCA+LDA [124, 125]). The linear discriminant value that is calculated by the algorithm quantifies how “ant-like” an outline is. Accordingly, it was used as an index of mimetic accuracy. Lateral and dorsal shapes were processed independently, resulting in separate mimetic accuracy values for each aspect (for a complete list of dorsal and lateral accuracy values for individuals, see supplementary Table S2, and for species, Table S3). Morphometric analysis was implemented in R (version 3.3.2) [126] using the Momocs package (version 1.0.0), including extraction of outline coordinates from photos, Procrustes alignment, Fourier analysis, principal components analysis, linear discrimination analysis and some plotting [127].

Image Analysis through Machine Learning

We assumed that body shape is an important component of visual ant mimicry, consequently we expected it to be positively correlated with other measures of visual accuracy. To test this relationship, we constructed an alternative index of mimetic accuracy derived from a machine learning algorithm. This was achieved by using the commercial Cloud Vision API service offered by Google (<https://cloud.google.com/vision/>). The service analyses an image, returning a list of the

objects it is considered likely to be. For example, in one case when analysing a dorsal photo of an ant-mimicking spider, it calculated a 72% probability that it was a photo of an ant, 51% that it was a beetle, 96% that it was an insect, 84% that it was a membrane winged insect, 87% that it was a pest, and so on. The algorithm does not return any probabilities lower than 50%. The same photos used for morphometric analysis were supplied to the machine learning algorithm, and the probability that the subject of the photo was an ant was used as a mimetic accuracy index. Probabilities for multiple photos were averaged to obtain scores for individuals and species. Photos of preserved specimens were excluded from this analysis as the machine learning algorithm performed very poorly on these photos, presumably because the algorithm had not been trained to analyse preserved specimens. A linear least squares regression was used to test for a correlation between machine learning and morphometric assessments of mimetic accuracy. Adjusted r^2 was used to assess the proportion of machine learning accuracy explained by body shape.

Hypothesis Testing

To test the developmental constraints hypothesis, the distribution of mimetic accuracy for spiders and insects was visualised using a kernel density plot (a kernel density plot can be intuitively thought of as a smooth histogram). A one-tailed Welch Two Sample t-test was used to determine whether the mean accuracy of mimetic spiders was lower than that for mimetic insects, and a one-tailed F-test was used to test whether the accuracy of mimetic spiders was less variable than that for mimetic insects.

The relaxed-selection hypothesis was tested with a linear regression comparing average body length of species to morphological accuracy (as determined by morphometric analysis). Within-group centring was used to eliminate potential confounding effects of differences in the sizes of mimetic insects and mimetic spiders, which involves subtracting the mean body length of the appropriate group (mimetic insects or spiders) from the mean for each species [128]. Tests were performed independently for dorsal and lateral aspects. Statistical analysis was performed in R (version 3.3.2) [126] with $\alpha = 0.05$ to test for significance for two-tailed tests and 0.025 for one-tailed tests.

Results

In total, we used 635 outlines to obtain shapes for 248 individuals and 107 species (Table 4). Body lengths of all specimens ranged from 1.7 to 15.3 mm, mimetic spiders ranged from 1.9 to 8 mm and mimetic insects ranged from 1.8 to 14 mm (Table 5). The plots of species shapes in principal component reduced-morphospace revealed clusters for ant models, spider mimics and insect mimics, with lateral body shapes less tightly clustered than dorsal shapes (Figure 3). The dorsal shapes of mimetic spiders and mimetic insects appear to lie between those for ants and non-mimics, whereas the lateral shapes for mimetic insects lie between the shapes of ants and mimetic spiders.

Table 4 Numbers of species shapes by type and viewpoint

	Models	Mimetic spiders	Mimetic insects	Non-mimics
Dorsal	22	27	20	28
Lateral	21	21	17	17

Table 5 Body lengths, mean and standard deviation by type

	Mean (mm)	σ
Models	6.8	3.0
Mimetic spiders	4.1	1.1
Mimetic insects	7.1	3.7
Non-mimics	6.1	3.1

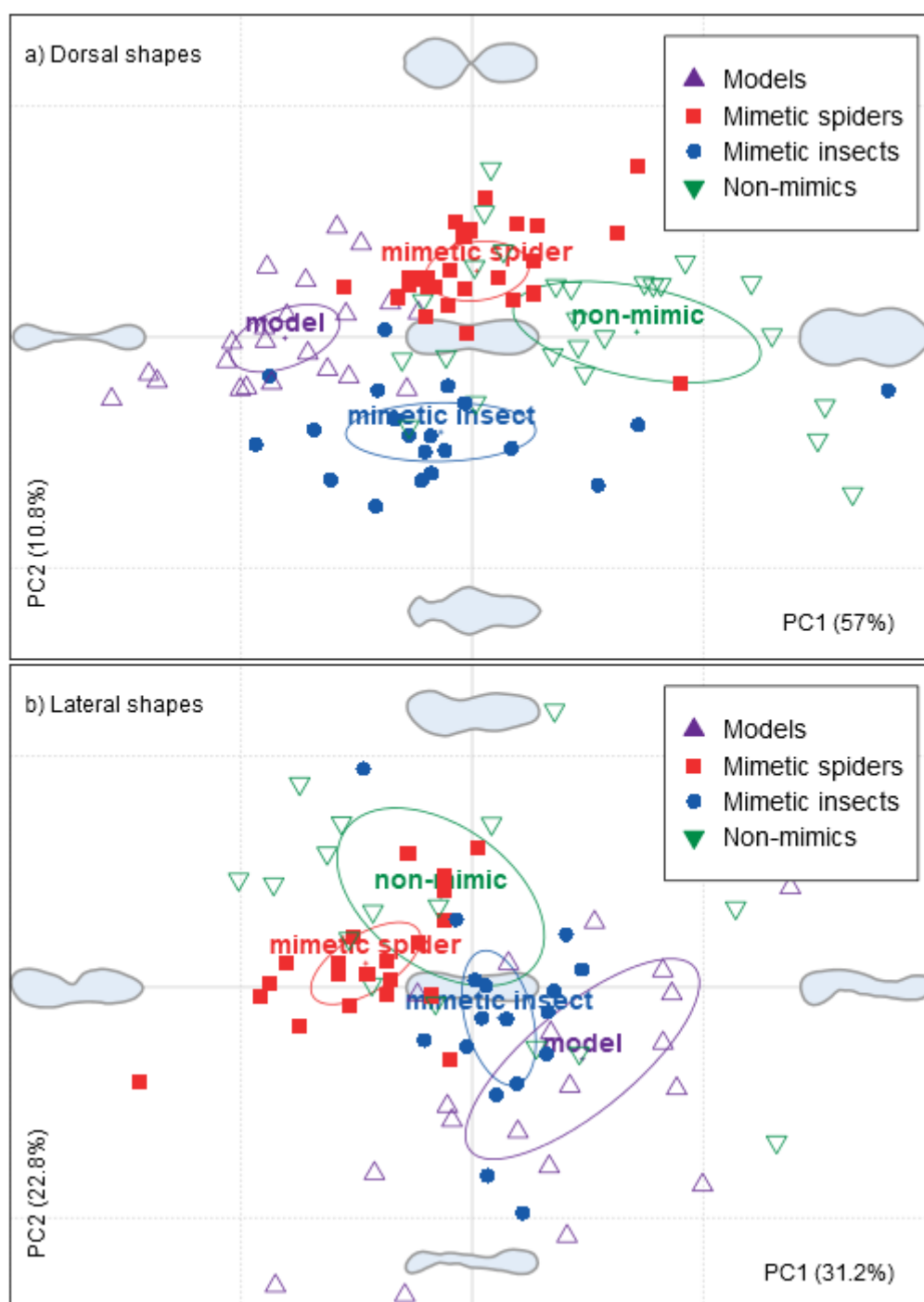


Figure 3. Dorsal (a) and lateral (b) species average shapes plotted in morphospace. X and y axes are the first two principal components after principal components analysis, which together account for 68% and 54% of the total variation in the dorsal and lateral data sets respectively. Ellipses show 95% confidence regions for ant models, spider mimics, insect mimics and non-mimics. The amorphous shapes graphically indicate the morphospace dimensions of the axes.

Developmental Constraints

If mimetic accuracy is limited by developmental constraints, we predicted firstly that the mean mimetic accuracy of spiders would be lower than that of mimetic insects; and secondly that spiders would have less phenotypic variation than insects. For dorsal species shapes, the mean accuracy for spiders was not significantly lower than mean accuracy for insects (one-tailed t-test, $t = -1.02$, $df = 35.8$, $p = 0.157$). Variance was smaller, but not significantly so (one-tailed F-test, $F_{26, 19} = 0.657$, $p = 0.158$) (Figure 4a). The situation with lateral shapes was somewhat different; spiders were significantly poorer mimics (one-tailed t-test, $t = -4.0$, $df = 25.1$, $p = 0.0002$), and showed significantly less variance in mimetic accuracy (one-tailed F-test, $F_{20, 16} = 0.36$, $p = 0.017$) than ant mimicking insects (Figure 4b).

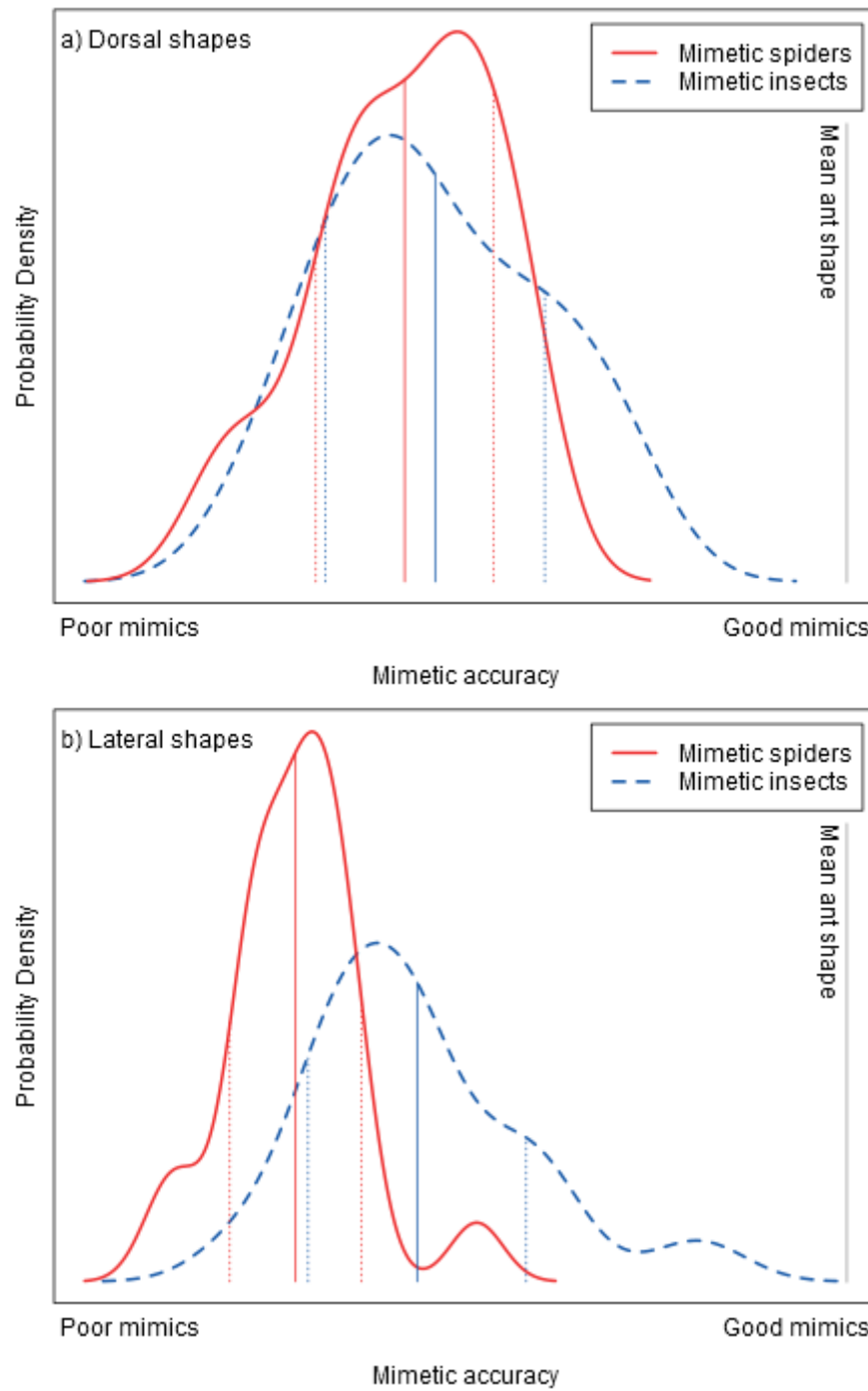


Figure 4. Distributions of mimetic accuracy for dorsal (a) and lateral (b) species average shapes. Mimetic accuracy increases along the x-axis. Vertical lines are situated at the means (solid) and the mean $\pm \sigma$ (dashed) for each distribution. The grey vertical line at the rightmost extent of the x-axis indicates the mean value for ants. Means and variances are not significantly different between the dorsal shapes of spiders and insects, but they are significantly different for lateral shapes.

Machine Learning

While machine learning assessment of mimetic accuracy correlated positively with morphometric accuracy for both dorsal and lateral aspects of spiders and insects (Figure 5), the correlation was only significant for spiders (linear least squares, dorsal shapes: $n = 21$, $F_{1,19} = 20.2$, $p = 0.0002$, adjusted $r^2 = 0.49$; lateral shapes: $n = 20$, $F_{1,18} = 12.8$, $p = 0.002$, adjusted $r^2 = 0.38$), not for insects (dorsal shapes: $n = 20$, $F_{1,18} = 2.1$, $p = 0.16$, adjusted $r^2 = 0.06$; lateral shapes: $n = 17$, $F_{1,15} = 1.6$, $p = 0.23$, adjusted $r^2 = 0.04$).

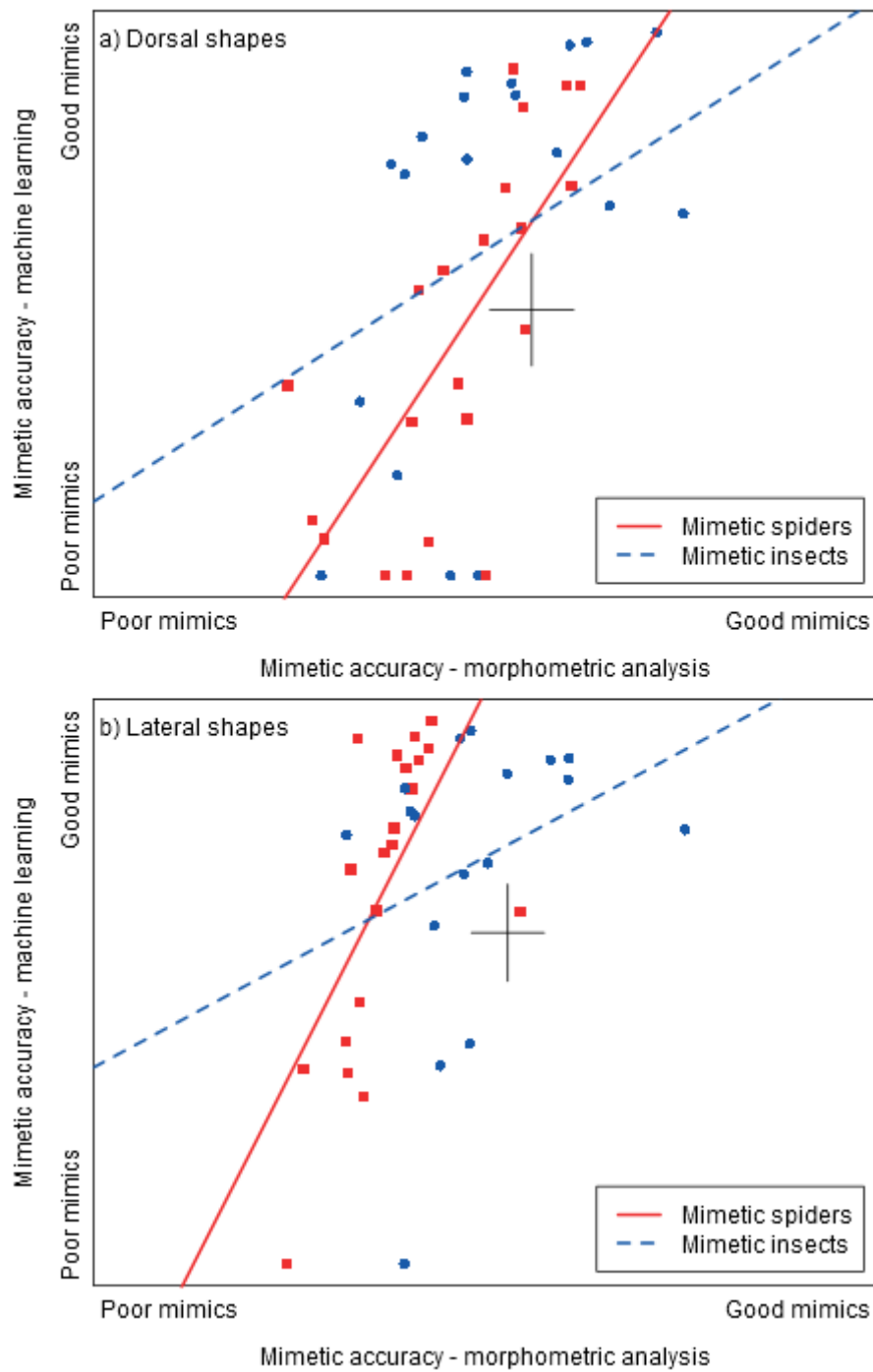


Figure 5. Relationship between the mimetic accuracy of species as measured by morphometric analysis and by machine learning, for dorsal body shapes (a) and lateral body shapes (b). Each point represents the average body shape of a species, lines are linear regressions. Both axes are z-transformed and the plot has an aspect ratio of 1. The black cross marks the origin. The two axes are closely correlated for spiders, and not for insects.

Relaxed Selection

The hypothesis that selection is relaxed for small mimics predicts that accuracy will increase with body size, yet our analysis found no relationship between mimetic accuracy and within-group centred body length (linear regression, dorsal shapes, $n = 47$, $F_{1,45} = 1.1$, $p = 0.3$, adjusted $r^2 = 0.001$; lateral shapes $n = 38$, $F_{1,36} = 0.8$, $p = 0.4$, adjusted $r^2 = -0.005$, Figure 6).

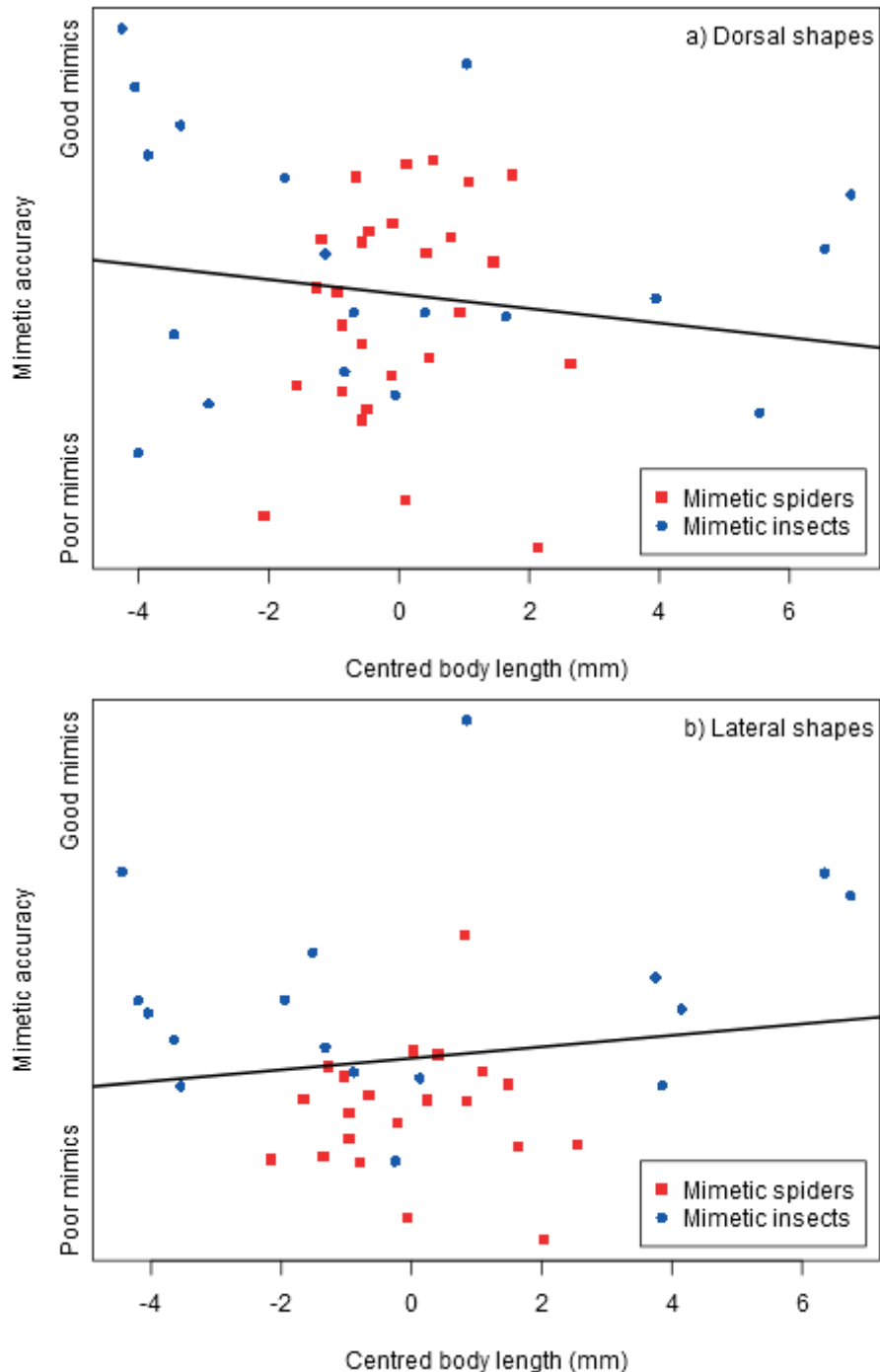


Figure 6. The relationship between within-group centred body size and mimetic accuracy. Each point represents the average dorsal (a) or lateral (b) body shape of a species. Plotted lines have been fitted to all points with a linear regression. Neither slope is statistically significant.

Discussion

Overall, we found mixed evidence for the hypothesis that developmental constraints result in less accurate mimics: only when considering the lateral view of the body, did we find spiders less accurate than insects. Similarly, we found no evidence that smaller species of ant mimics are under relaxed selection from predators and consequently express less accurate mimicry.

Mimic Variation

Our analysis revealed substantial variation in mimetic quality, from very poor to good, amongst both ant-mimicking spiders and insects, which is consistent with results in other systems including mimetic hoverflies [15, 129], mimetic snakes [130], and egg mimicry in avian brood parasites [131]. For both groups of mimics, accuracy is distributed across the range, with no clear groupings into good and poor mimics. Several of the poorest mimics, *Eilica* sp1, *Zodarid4* sp1, *Apricia jovialis* and *Colobathristid1* sp1, scored so poorly that they may not be morphological mimics, although all appeared to match their putative models in colour.

Developmental Constraints

The developmental constraints hypothesis predicts that developmentally constrained mimics will be less accurate and have lower phenotypic variation than non-constrained mimics. We expect that ant-mimicking spiders are more likely to experience developmental constraints compared with ant-mimicking insects because of their considerable phylogenetic distance from the model. The results for dorsal body shapes provide no evidence of a developmental constraint, which fits with current expectations that developmental constraints are unlikely to contribute to imperfect mimicry [9, 14]. By contrast, the results from analysing lateral body shapes supported our hypothesis that spiders were poorer mimics compared to insects and showed significantly less phenotypic variation.

Could an adaptive mechanism (rather than a developmental constraint) explain why lateral mimicry is worse in spiders than insects? Many imperfect mimicry hypotheses posit relaxed selection for a variety of reasons [9], however, when selection on a trait is relaxed, the result is wide phenotypic variation [24, 38]. Laterally, spiders exhibit significantly less variation in accuracy, therefore it is unlikely to result from any form of relaxed selection. However, experimental confirmation that poor lateral mimics have lower fitness than more accurate mimics would provide a more definitive confirmation that the poor mimicry results from developmental

constraints rather than relaxed selection. Yet another hypothesis, multiple models, argues that poor mimics benefit from a partial resemblance to two or more models [41], however our analysis shows no mimetic phenotypes positioned partway between two model phenotypes in morphospace (Figure 3).

Why would mimicry differ between dorsal and lateral body shapes? There may be developmental constraints on lateral body shapes that do not apply to dorsal body shapes. Visual inspection of the axes in the principal components plot for lateral shapes (Figure 3) suggests that spiders are somehow prevented from moving to the right and downwards in morphospace. From the shapes that illustrate the axis extents, it appears that moving to the right and downwards both involve dropping the head relative to the body. Perhaps the placement of eyes on the spider's cephalothorax, or else their lack of an articulated neck, prevents them from being able to evolve or adopt an appropriately ant-like head position. Alternatively, developmental constraints in dorsal body shapes may have been overcome, possibly as a result of greater selective pressure. Selective pressure may differ between the two viewpoints as the predators selecting for accuracy may differ – flying predators such as birds and wasps are more likely to select for dorsal body shapes, while cursorial predators such as lizards and spiders likely select for lateral body shapes. Alternatively, there may be less selective pressure on lateral body shapes as they are often obscured by appendages, so body shape may play only a small part in lateral mimetic resemblance.

Methodology factors may have contributed to the difference in the dorsal and lateral results. The sample size was smaller for lateral shapes (Table 4), as museum specimens were not photographed laterally, and some live specimens were too active to obtain satisfactory lateral photographs. Artefacts may have arisen from photos not taken from the correct angles, or difficulties in conversion of photos to outlines due to obscured sections of outline (66% of lateral outlines were partially obscured, as opposed to only 11% of dorsal outlines). However, these factors are unlikely to have resulted in a systematic bias in either insects or spider outlines, so are unlikely to produce our result of poorer and less variable mimicry in spiders.

These results suggest that developmental constraints do impose an upper limit on the morphological mimetic accuracy of the lateral body shape of spiders, although not on dorsal body shapes. Developmental constraints are unlikely to explain variation in accuracy between species that share the same developmental system, so cannot explain the variation within lateral body shapes. So, while it appears that developmental constraints do exist in some situations, they

cannot explain most of the overall variation in mimicry (both dorsal and lateral), even within the group (spiders) where we most expect them to apply, and consequently they probably do not offer a universal explanation for imperfect mimicry.

Machine Learning

We expected that mimetic accuracy based on morphology would be positively correlated with accuracy as measured by machine learning, and while that was the case for spiders, for insects the correlation surprisingly was not significant. This suggests that while body shape is likely to be an important component of visual mimicry in spiders, there may be additional visual features, such as wings, antenna or appendages, that can be used to discriminate between ants and other insects.

Machine learning is potentially a very powerful technique that is gaining increased use within biological research [132]. As a tool for quantifying mimetic accuracy, it has several desirable properties: it is flexible, fast (once training is complete), and powerful, as it potentially makes use of all of the information in a photograph, taking into account colours, patterns, appendages etc. Consequently, it has the potential to assess visual mimetic accuracy more completely and accurately than previously used methods [25, 27, 133]. However, machine learning models are complex and difficult to set-up and use. In this project, we used Google cloud vision which provides access to a pre-built and pre-trained model, so we avoided the complexity. This simplicity came at the cost of performance in results - the service is trained on common commercial use cases, whereas training on domain specific datasets would yield superior image classification. Whether the benefits of machine learning methods outweigh their costs for mimicry research remains to be seen.

Relaxed Selection

Our analysis did not reveal any correlation between size and mimetic accuracy, hence it did not support the hypothesis that selection is relaxed for smaller mimics [53]. A correlation between size and accuracy has previously been found in hoverflies, which are mimics of stinging hymenopterans [15], although a later study—using intra-specific phenotypic variation as an indicator for relaxed selection—failed to find evidence that selection for better mimicry is relaxed in smaller hoverflies [24]. Birds, which are major predators of hoverflies, have a preference for larger prey [134, 135], which could explain why selection may be relaxed for smaller hoverflies. In general, however, the preferred size of prey depends on the size and species of predator, with both upper and lower size

limits [136, 137]. Consequently, the relationship between selective pressure for mimicry and size depends upon the predators that are driving selection for mimicry. There are multiple predators of ant mimics, such as spiders (e.g. Salticidae and Thomisidae) [19, 69], assassin bugs (Reduviidae) [138], mantids [18], wasps [71, 139], skinks and birds [28]. Therefore, the combined size preferences of predators are likely to be highly variable, possibly encompassing the entire range of mimic sizes, with the result that selection is not relaxed based on body size.

Conclusion

Variation in mimetic accuracy is a problem attracting much interest, and our study was the first to apply geometric morphometric analysis to evaluate one aspect of visual mimetic accuracy—body shape—in a quantitative, repeatable and objective measure. We selected ant mimics as an appropriate system for hypothesis testing as developmental constraints, if present, were most likely to be discovered due to the vast phylogenetic distance between mimics. We developed and trialled the suitability of several methods for assessing mimetic accuracy, including machine learning, however these measures are not intended to represent the cognitive processes of predators, but rather to identify the information that is available to predators during decision making. These measures need to be validated by comparing them to the ultimate measure of mimetic accuracy - the responses of predators.

Chapter 2. Mimicry in Motion: can behaviour compensate for poor morphological mimicry?

Abstract

Batesian mimicry is the result of natural selection from predators avoiding palatable mimics which are mistakenly identified as unprofitable models. Selective pressure should result in constant improvement in the resemblance of mimics to their models. However, the presence of many imperfect mimics seems to contradict this intuitive mechanism. One possibility is that mimicry is comprised of multiple components that reinforce each other. The net effect is a resemblance based on multiple signals that is consistently mistaken for a model. We assessed two components of mimicry: morphology and behaviour, predicting a negative relationship between the two. Mimics that are inaccurate in one component compensate by greater accuracy in the other, thereby explaining the persistence of mimicry inaccuracy in either morphology or behaviour. We tested this hypothesis using ant mimics with species from several different families. We measured behaviour by analysing the trajectories of walking animals, and used morphometric analysis to measure body shapes. Contrary to our prediction, we found no clear relationship between behaviour and morphology, and we conclude that the two aspects of mimicry are likely to be independent, possibly resulting from selection by different sets of predators.

Introduction

Batesian mimicry serves as a primary defence mechanism—palatable prey resemble unpalatable models, deceiving predators and thereby avoiding attack [1, 10]. Mimicry may be communicated through different sensory modalities such as visual, aural, olfaction and tactile. Mimetic species may utilise multiple types of mimicry (multicomponent mimicry [32, 84]), although little is known about the relationships between the different modalities. Studies of complex signals have generated multiple hypotheses regarding the interactions between different signals [54], several of which may apply to the relationship between the components of multicomponent mimicry [53]. If mimicry is the net sum of signals via several modalities, then convincing signals in one modality may compensate for poor mimicry in a different modality (the ‘increased deception’ hypothesis [27, 32, 53, 54]), explaining the otherwise puzzling existence of apparently poor mimics [9]. Two additional hypotheses may apply to multicomponent mimicry, although they have no power to explain the presence of imperfect mimicry. The ‘backup signals’ hypothesis states that the factors

that select for high mimetic accuracy in one modality may also select for high accuracy in other modalities [53, 54, 140]; and according to the 'receiver variability' hypothesis, the different signals are independent, driven by different sets of predators [54, 140, 141]. An additional behaviour-based explanation for imperfect static mimicry argues that rapid motion of potential prey is not a signal, rather that it functions to limit the ability of predators to perceive differences between mimics and models, thus relaxing selection for accurate mimicry in fast-moving species [27].

Behaviour is considered an important component of mimicry and is often the most conspicuous feature of mimics [72, 142]. Despite this, it is only recently that attempts have been made to analyse and quantify behavioural mimicry [26, 27, 53, 143-148], and mimetic behaviour has been identified in butterflies [148], wasp-mimicking hoverflies [146, 147], mimetic octopuses [149], and ant mimics [3, 26, 27]. To date, no studies have attempted to quantify the accuracy of mimetic behaviours, rather assessing whether or not particular behaviours can be classified as mimetic.

We chose to use ant mimics to test the relationships between behavioural mimicry and static visual mimicry. Ants possess many characteristics that make them undesirable prey: they are aggressive, and bite and sting, they produce noxious secretions and they collectively attack intruders [105]. As a result, there is a remarkable diversity of arthropods that mimic ants, with more than 2000 species in 54 families, including spiders, mantids, katydids, stick insects, treehoppers, bugs, thrips, beetles, flies, moths and wasps [72]. Among these, spiders are most likely to differ from ants in shape and behaviour due to their taxonomic distance from ants [110, 111]. Spiders have two body parts, four pairs of robust legs, multiple lens eyes, chelicerae, pedipalps and spinnerets, whereas ants have three body parts, three pairs of thin legs, usually two compound eyes, mandibles, antennae and stings. Behaviourally, spiders exhibit a wide range of foraging and hunting behaviours [112], with the jumping spiders being mostly day active ambushers which leap on their prey from several body lengths away [150]. By contrast, ants are generally social foragers which typically rely on chemical cues [105]. Despite these differences, ant mimicry has evolved independently in spiders more than 15 times [72] and 12 or 13 times in the jumping spiders alone [106]. Given the conspicuous behaviour of ants, behavioural mimicry is likely to be an important component of ant mimicry, and may have evolved in spiders before morphological mimicry [139]. Behavioural mimicry of ants may encompass such behaviours as opisthosoma "bobbing", waving the first or second pair of legs in the air to simulate antennae, posture, gait, speed of movement, and overall motion [3, 26, 27, 143].

In this project, we test whether behavioural mimicry compensates for poor morphological mimicry, thereby providing an explanation for the persistence of imperfect mimics. We predict that behavioural accuracy will be negatively correlated with morphological accuracy [53], in accordance with the increased deception hypothesis. Additionally, we test for a negative correlation between walking speed and morphological accuracy, as predicted if high speed motion reduces discriminability [27, 151]. Finally, we test the predictions of the two additional multi-component signal hypotheses which are mutually exclusive with each other and the increased deception hypothesis. Firstly, that behavioural and morphological accuracy will be positively correlated (the ‘backup signals’ hypothesis) [53, 54]; and secondly, there is no correlation between behaviour and morphology (the ‘receiver variability’ hypothesis [54, 141]).

Materials and Methods

Spiders and ants were collected in eastern New South Wales and south-east Queensland, Australia, between April and August 2017 (Figure 7, Table S4). Animals were collected from trees and bushes during daylight hours by searching under loose bark and by beating vegetation into sorting trays. The spiders and ants were housed individually in 50 ml plastic jars containing damp cotton wool for moisture, and spiders were fed twice per week on fruit flies. Spiders were identified to species when possible using Whyte and Anderson [113]. Spiders from the genus *Myrmarachne* were identified from Pekár, Petráková [28]. Immature *Myrmarachne* could not be identified beyond genus, and since they differ from adults in colour and habitus, and possibly in behaviour, they were assigned to a separate category so that they could be analysed independently from the adults. Ants were sorted into morphospecies based on appearance. In order to measure body lengths, live animals were photographed using a Canon 7D with an MPE-65mm macro lens and a MT-24EX macro twin lite. The scale of each photo was calculated from the magnification obtained from the photo file’s EXIF data. The Fiji image processing program [119] was then used to enter the scale and measure the body length from the front of the head to the rear of the abdomen, excluding spinnerets or stings, in millimetres, to an accuracy of one decimal place.

An index of morphological mimetic accuracy was constructed by photographing specimens, characterising body outlines using geometric morphometric analysis, then applying a linear discriminant analysis to calculate a single index value for each shape. Dorsal and lateral shapes

were analysed separately, and individual shapes were averaged to obtain shapes and mimetic accuracy index values for species (chapter one).

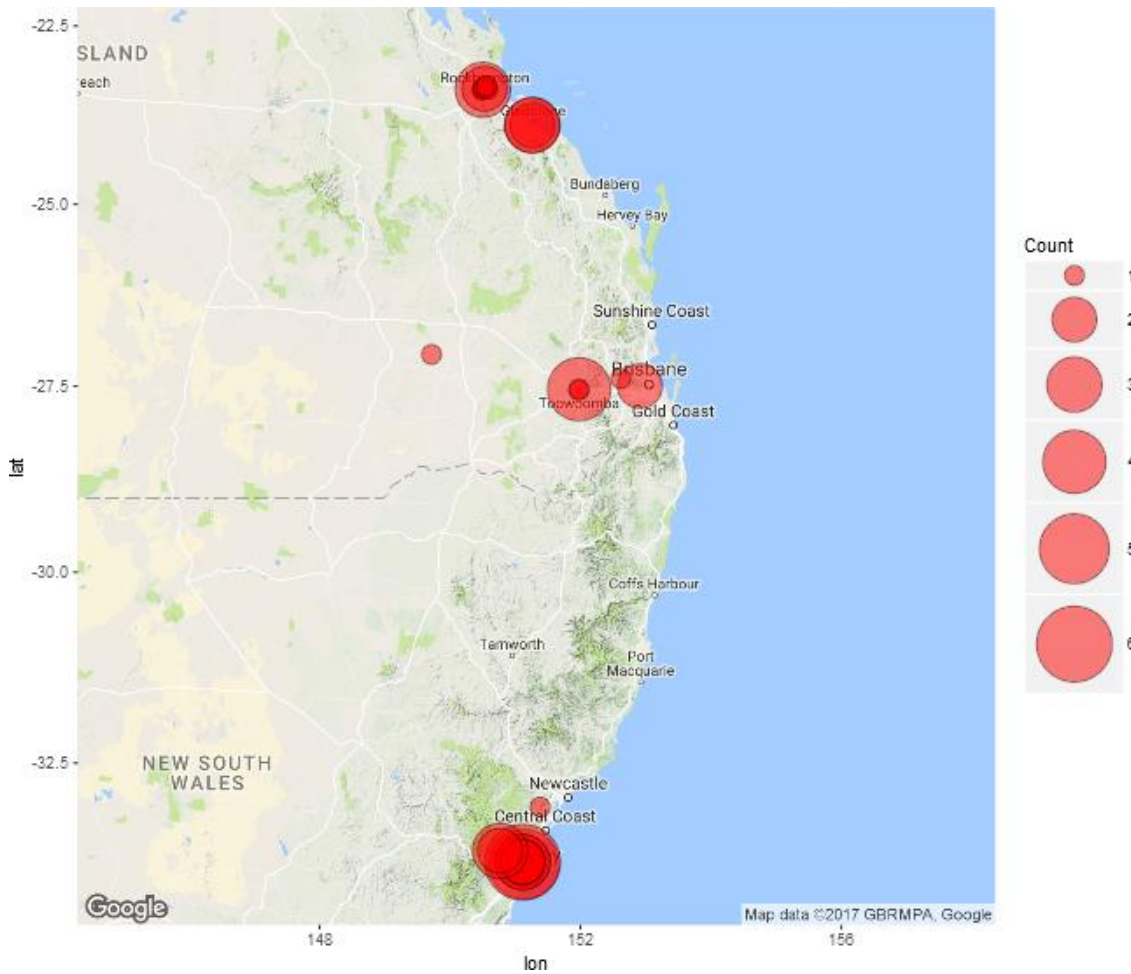


Figure 7 Map of specimen collection sites. Circle sizes represent the number of specimens collected at each site.

Videoing

The walking behaviour of ants and ant mimics was videoed on a featureless MDF board (600mm x 900mm). Since the majority of mimics were collected from the trunks of trees, the board was positioned vertically. The board was lit with two LED lights (Liteworks Electra-desk lamp) and one 150-Watt incandescent light (HPM PAR38). The laboratory lights were turned off to reduce flicker. To reduce distractions for the animals, the experimenters remained in the dark behind the lights for the duration of each trial. The board was cleaned before every trial with 70% ethanol, and allowed to dry. Videos were filmed using a Panasonic 3MOS HD Camcorder mounted on a tripod, with resolution 1920 x 1080, at a frame rate of 50 fps. In order to maximise the effective

resolution of the videos, we had previously observed that the trajectories of most animals were roughly upwards, so the camera was rotated 90° so that the long axis was vertical (i.e. portrait orientation) and the entire height of the board was visible, but the two sides of the board were just beyond the edges of the frame.

For each trial, the 50 ml vial containing the animal was positioned approximately 300 mm above the base of the board, horizontally centred, with the rim touching the board. The lid of the vial was removed, and a small strip of paper was inserted to encourage the animal to exit the vial and walk onto the board. The animal was left undisturbed for 2 minutes, after which time it was gently encouraged to leave the vial using a paintbrush if it had not already done so. Several spiders refused to leave the vial, in which case the trial was terminated and excluded from the analysis, and another attempt was made on a later day. The trial ended when the animal reached an edge of the board or else remained motionless for two minutes.

Video Analysis

Trajectories were extracted from video files by converting frames to greyscale, reducing the video resolution and applying gaussian blur to reduce noise, then subtracting the background.

Background subtraction removes features that are unchanging throughout the video (such as the vial and the clamp holding the vial). The first 10 frames of the video were averaged to create the background; 10 frames were used to reduce the effects of noise or movement. Adaptive mean thresholding was used to segment the foreground animal from the background, and the centroid of the resultant foreground area was used as the centre of mass of the specimen. The trajectory was composed from the coordinates of the centre of mass from each frame, together with the time of the frame, resulting in a series of (x, y, t) values, where x and y were in mm and t was seconds since the start of the video. Trajectories were smoothed over time using Kalman filtering, and the resulting path saved in CSV format for subsequent statistical analysis. Trajectory extraction was implemented in Java using the OpenCV library [152].

Trajectory characterisation

Animal trajectories were read from the CSV files and characterised by calculating the values of a number of test statistics for each trajectory (Table 6). The statistics were selected based on expert opinion (A. Narendra, pers. comm.) as being descriptive of ant behaviour. The selected statistics may be loosely classified into measures of speed (including the frequency and timing of starting and stopping), and measures of path straightness. The following measures of speed were used: maximum speed (*body lengths sec⁻¹*); mean speed while moving (*body lengths sec⁻¹*);

coefficient of variation of speed while moving; mean duration of periods of motion (*sec*), which we considered to be relevant as most of the animals trialled interspersed periods of motion with periods of immobility; coefficient of variation of duration of periods of motion; mean duration of stopped periods (*sec*); coefficient of variation of stopped period duration; and proportion of time moving (the ratio of total time moving to total time stopped over the trial period). Coefficient of variation was used as a measure of variance in preference to standard deviation, as it has a lower correlation with the value it describes. Speed statistics were calculated from a sequence of (*displacement, time*) points, where *displacement* is the distance between two consecutive points along the trajectory. The (*displacement, time*) sequence was smoothed by fitting it to a polynomial of order three (using the R function `sm.spline`). This smoothing step was considered necessary since noise in position measurements is effectively amplified when converting to speed. Speed was simply calculated as *displacement* / *time*. An animal was considered to be stopped if its speed dropped below 5 mm sec^{-1} .

Several measures of path straightness or tortuosity were selected. Straightness measures were applied to paths that were first resampled to a constant step length (called *rediscretization* [153]). A *straightness index* [154] was calculated as the ratio of the net distance travelled to the path length. This value varies from 0 for a path that starts and finishes in the same location, to 1 for a perfectly straight path. *Sinuosity* is defined as $1.18\sigma_R^*/\sqrt{R}$, where R is the rediscretization step length and σ_R^* is the standard deviation of the step turning angles in the path [153]. E_{max} is defined as $\frac{\beta}{1-\beta}$ where β is $\langle \cos\Delta \rangle$, and Δ represents the turning angles in a fixed length path [155]. Finally, Shamble, Hoy [143] define a *direction autocorrelation* function which captures regular oscillations in direction. The correlation $C(\Delta_s)$ for a given relative step length s is defined as $\langle \cos[\theta(s) - \cos(s + \Delta_s)]_s \rangle$ where θ_s is the tangent to the path at segment s . The correlation function C is evaluated for values of Δ_s ranging upwards from 1. Shamble, Hoy [143] use the location (Δ_s, C) of the first local minimum in C to characterise the path. Accordingly, we used both Δ_s and C of the first local minimum as statistics of straightness/tortuosity.

Table 6 Summary of statistics used to characterise trajectories

Category	Statistic	Description
Speed	Maximum speed	Maximum speed
	Mean speed	Mean speed while moving
	Speed CV	Coefficient of variation of speed while moving

	Moving duration mean	Mean duration of periods of motion
	Moving duration CV	Coefficient of variation of duration of periods of motion
	Stopped duration mean	Mean duration of periods of no motion
	Stopped duration CV	Coefficient of variation of duration of periods of no motion
	Proportion time moving	Ratio of total time moving to total time stopped over trial period
Straightness	Straightness	Ratio of distance travelled to trajectory length
	Sinuosity	A function of the standard deviation in path turning angles
	E_{\max}	A function of the cosine of the path turning angles
	First minimum Δs	A measure of the magnitude of the highest frequency regular oscillations in direction
	First minimum C	A measure of the wavelength of the highest frequency regular oscillations in direction

Trajectory characterisations were obtained for species by averaging the measured values for each trajectory within the species.

Mimetic accuracy calculation

To quantify the mimetic accuracy of trajectories, it was necessary to determine the characteristics that differentiate ant trajectories from the trajectories of everything else (“non-ants”). For example, if ants always (or usually) walk faster than non-ants, then higher speed is more ant-like, so speed could be used as a component of a mimetic accuracy index. Conversely, if ants generally walk slower than non-ants, negative speed could be used. If ants and non-ants generally walk at the same speed, then speed should not contribute to a mimetic accuracy index. We used a linear discriminant analysis to determine how the various trajectory characteristics should be combined so as to maximise the difference between ant and non-ant trajectories [123]. The linear discriminant value produced by the analysis quantifies how ant-like the trajectory is, accordingly we used it as a measure of mimetic accuracy. Undefined values are generally excluded from linear discriminant analyses since they are considered uninformative. In the case of local minimum of direction autocorrelation, however, an undefined value indicates that the trajectory does not contain regular oscillations, which is potentially informative. To include this information in the analysis, a new statistic was created with value 1 if the local minimum was undefined, otherwise 0, and undefined values of Δ_s and C were set to the arbitrary value of 0. The linear discriminant analysis was applied to the average trajectories for each species. Whilst linear discriminant

analysis assumes normally distributed data, it is still robust even if this assumption is not met [156].

We also visualised the characterised trajectories by performing a principal components analysis (PCA) [157] and plotting the first two principal component values for each species, although this analysis was not used for any statistical calculations.

Statistical analysis

To test whether poor morphological mimicry is negatively correlated with behavioural accuracy (i.e. the prediction of the Increased deception hypothesis), we utilised the index of morphological mimetic accuracy from chapter one. We compared the results of trajectory assessment with the morphological mimetic accuracy index using a linear least squares regression. To test whether faster animals are less accurate morphological mimics, we used a linear least squares regression to test for a correlation between mean walking speed while moving (i.e. excluding times when the animal was stopped) and the morphological accuracy index from chapter one. A one-tailed t-test was used to test whether mimics were more ant-like than non-mimics. Tukey's 'Honest Significant Difference' method was used to test for differences between the means of ants, mimics and non-mimics for each statistic. In all cases, significance was tested with $\alpha = 0.05$, or $\alpha = 0.025$ for one-tailed tests. All analysis was performed in R [126]. Maps were plotted using ggmap [158]. Linear discriminate analysis was performed using the MASS package [159].

Results

We videoed 68 individual animal trajectories from 25 species: five ant species, eight mimetic spider species and 12 non-mimetic spider species (Table S5). Subjectively, trajectories appeared to differ between ants, mimics and non-mimics (Figure 8), with ants following meandering paths with lots of turns and occasional loops, non-mimics walking in generally straight lines, and mimics following highly periodic, low-amplitude meandering paths which appear part-way between those of models and non-mimics. It is also apparent that there was variation within each group. The autocorrelation function confirmed the presence of periodic oscillations in mimic trajectories, while plots of speed over time revealed that most spiders were not moving continuously, but stop and start repeatedly (Figure 9).

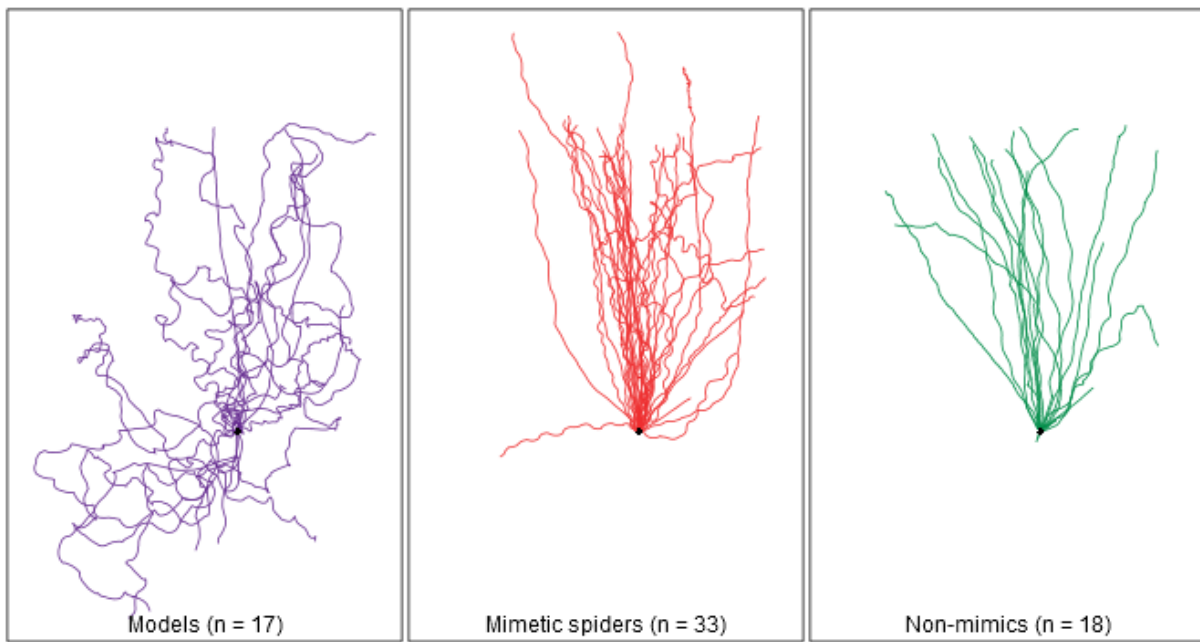


Figure 8 Plots of trajectories for models (i.e. ants), mimics and non-mimics. Each line is a single trajectory of one animal, n = the number of trajectories. Trajectories are translated so that they all share a common starting location (indicated with a black dot).

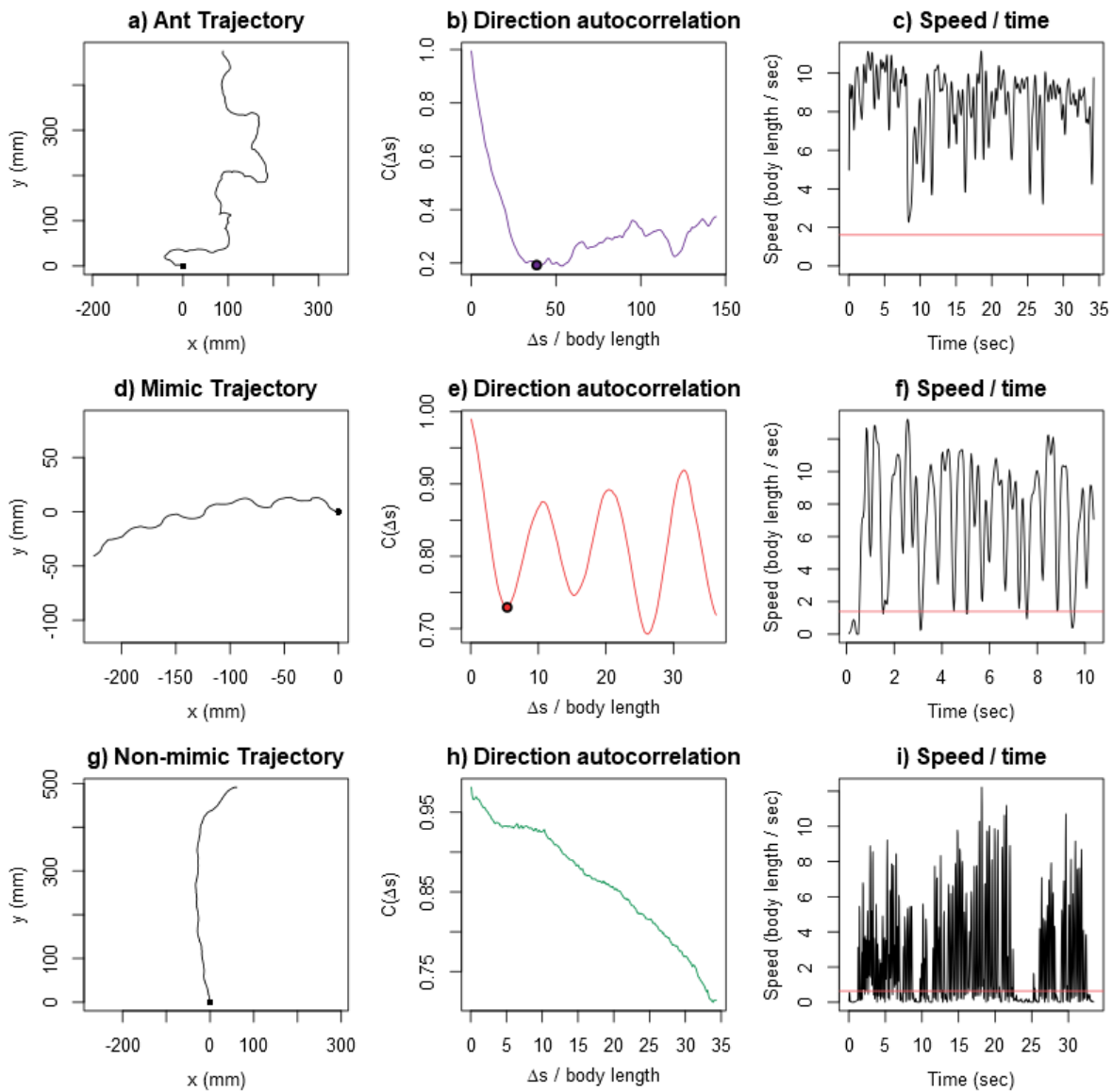


Figure 9 Details of three trajectories: a) model, *Froggattella* sp. (a, b, c); a mimetic spider, *Myrmarachne erythrocephala* (d, e, f); and a non-mimetic spider, *Servaea incana* (g, h, i). The left column (a, d, g) shows the trajectory of the walking animal as viewed from overhead. The centre column (b, e, h) is a plot of the direction autocorrelation function, with a dot indicating the location of the first local minimum (which does not exist for the non-mimic). The right column (c, f, i) shows speed over time. The horizontal line indicates the speed below which animals are considered to be stopped (5 mm / sec). Note that axis scales are different for each plot to allow the complete data set for each trajectory to be shown.

Visually, the principal components analysis of the characterised trajectories plotted in two dimensions showed evidence of three clusters: models, mimics and non-mimics, with the cluster of mimics lying roughly in the intersection between models and non-mimics (Figure 10), however the first two principal components together explain only 42% of total variance in individual

trajectories, and 46% of the total variance in species averaged trajectories (Table 7). For species, multiple statistics contribute almost equally to PC1, including measures of straightness, variability in speed and stop/start behaviour. PC2 is dominated by mean speed.

Table 7 Contributions of statistics to principal components for species and individuals. Rows are ordered by their relative contributions to the first component for species trajectories.

Statistic	Species PC1	Species PC2	Individuals PC1	Individuals PC2
Moving duration mean (sec)	10%	0.4%	10%	4%
First minimum Δs	10%	3%	7%	10%
Straightness	10%	4%	6%	20%
Proportion time moving	10%	10%	20%	4%
Speed CV	10%	2%	10%	4%
Stopped duration CV	9%	10%	10%	6%
Maximum speed (bl/sec)	7%	10%	5%	0.2%
Sinuosity	6%	9%	0.7%	10%
First minimum C	4%	3%	4%	20%
Stopped duration mean (sec)	4%	10%	9%	4%
No local minimum in C	3%	5%	4%	4%
E_{\max}	3%	0.9%	0.9%	6%
Mean speed (bl/sec)	2%	20%	2%	4%
Moving duration CV	0.2%	10%	7%	5%

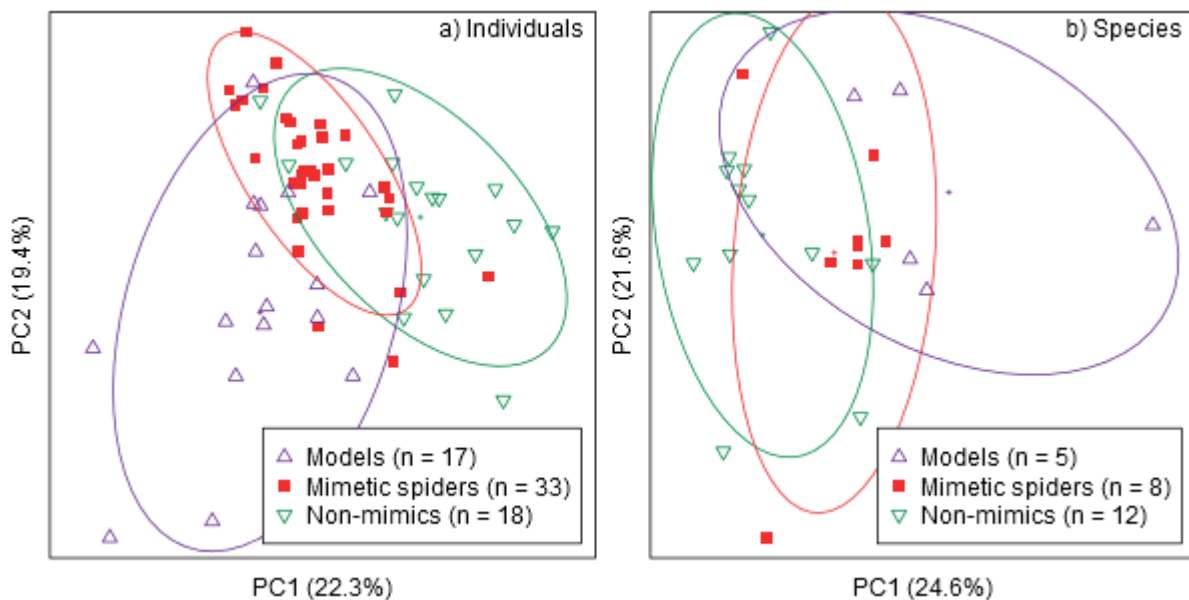


Figure 10 Trajectories visualised using a principal components analysis. Plots are for (a) individuals and (b) species. Each trajectory is characterised by the values of multiple statistics (Table 6), which are projected onto 2 dimensions (PC1 and PC2) using a principal components analysis (Table 7).

The linear discriminant analysis was able to effectively discriminate between the averaged species trajectories of ants and non-ants, correctly classifying the trajectories of all ants, all non-mimics, and all mimics except for the spider *Myrmarachne macleayana*, which was classified as an ant. Comparing the means of the linear discriminant values for mimics with those for non-mimics revealed that ant-mimics were significantly more ant-like than non-mimics (one-tailed t-test, $t = 3.7$, $df = 14.6$, $p = 0.001$). The statistic with the greatest contribution to the discrimination function was the proportion of time moving—ants spent a higher proportion of time moving. The next two most important measures were indicators of trajectory shape: straightness (non-ant trajectories were straighter), and first minimum C, which is a function of the amplitude of regular oscillations, and was lower for ants. The fourth most important statistic was coefficient of variation in speed – ants were more consistent in their walking speeds (Table 8). There were significant differences between the means of ants, mimics and non-mimics for four statistics: straightness, speed coefficient of variation, moving duration mean and first minimum Δs (Tukey's HSD method, $p < .05$, Table 8).

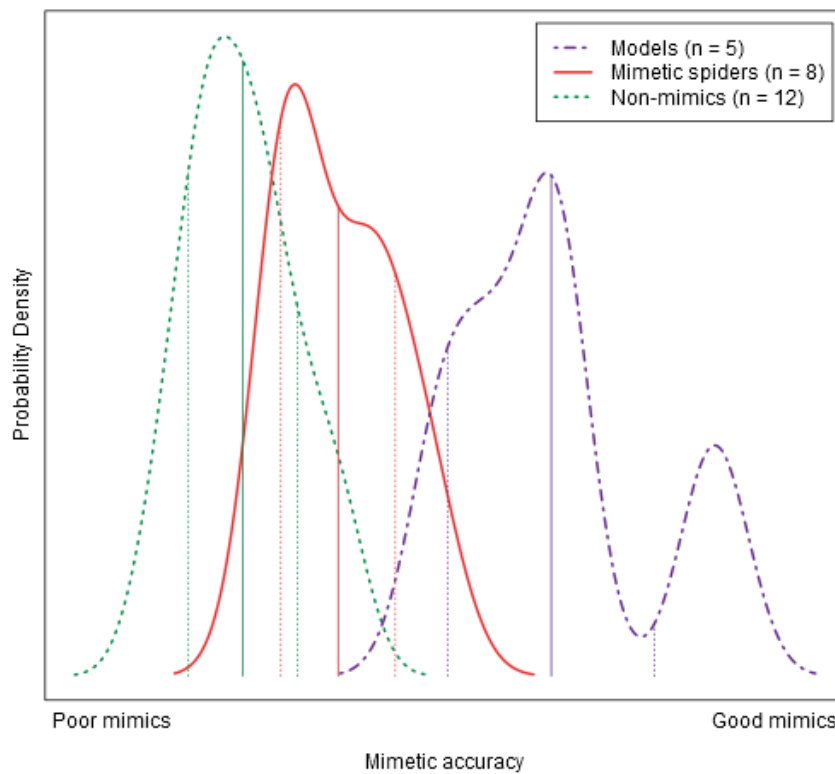


Figure 11 Distribution of behavioural mimetic accuracy by type. Vertical lines indicate the mean (solid) and the mean $\pm \sigma$ (dashed) for each distribution.

Table 8 Statistics used to characterise trajectories, with means and standard deviations for ants, mimics and non-mimics. Rows are ordered according to their contributions to the mimetic index. Units for measures of speed are in body lengths / sec (abbreviated as bl/sec). The C value indicates the magnitude of regular oscillations in direction. CV is coefficient of variation. Mean values that differ significantly from the means of the other groups are in bold, subscripts indicate the groups they differ from (a = ants, m = mimics, n = non-mimics).

Statistic	Ants	σ	Mimics	σ	Non-mimics	σ
Proportion time moving	0.79	0.25	0.75	0.28	0.49	0.28
First minimum C	0.29	0.41	0.66	0.3	0.6	0.44
Straightness	0.62_{m,n}	0.23	0.87_a	0.087	0.92_a	0.059
Speed CV	0.3_n	0.092	0.38_n	0.079	0.59_{a,m}	0.2
Stopped duration CV	1.1	0.78	1.1	0.87	1.7	1.1
Moving duration CV	1	0.38	0.94	0.39	0.71	0.35
Sinuosity	0.62	0.34	0.32	0.11	0.38	0.3
Moving duration mean (sec)	8.5_n	8.7	2.9	1.8	1.5_a	1.9
First minimum Δs	21_n	22	8.7	9.6	4.6_a	4
No local minimum in C	0.2	0.45	0.12	0.35	0.33	0.49
Mean speed (bl/sec)	5.4	3.5	8.5	9.6	6.1	5
Maximum speed (bl/sec)	12	6.9	15	13	20	18
Stopped duration mean (sec)	2	3.8	1.3	2	2.3	3.7
E_{\max}	150000	120000	520000	760000	840000	2500000

Behavioural and morphological mimicry

Based on the increased deception hypothesis, we predicted a negative relationship between species average values of behavioural and morphological mimetic accuracy, however we found no significant relationship (linear least squares regression, dorsal body shapes: slope = 0.2, $n = 8$, $F_{1,6} = 0.05$, $p = 0.8$, adjusted $r^2 = -0.16$; lateral body shapes: slope = 1.1, $n = 8$, $F_{1,6} = 0.7$, $p = 0.4$, adjusted $r^2 = -0.04$) (Figure 12).

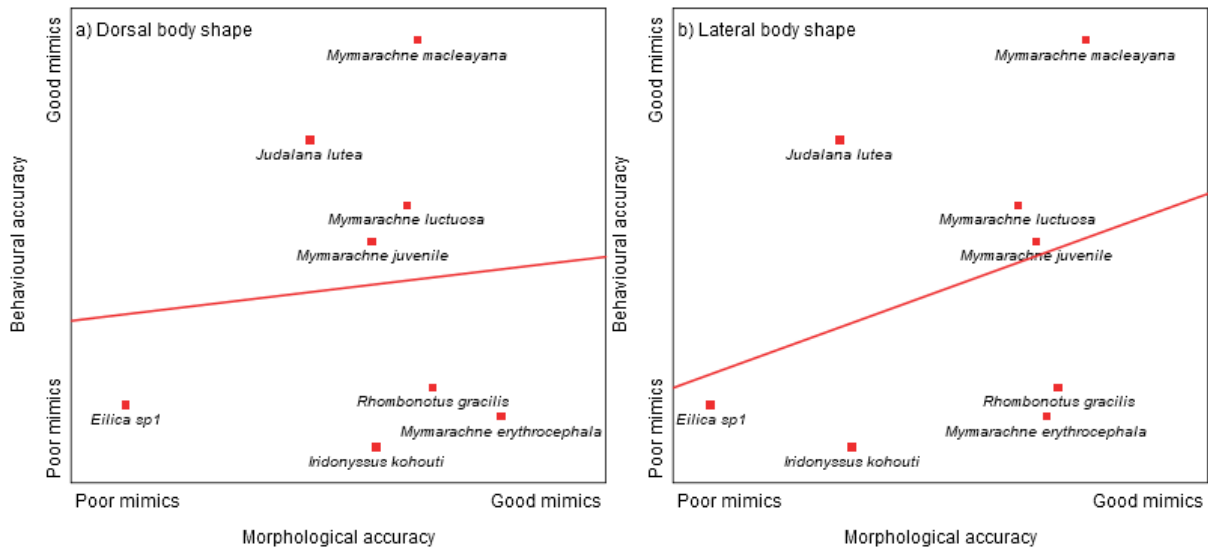


Figure 12 Relationship between morphological mimetic accuracy and behavioural mimetic accuracy for dorsal (a) and lateral (b) body shapes of ant-mimicking spiders. Each point represents a species of mimetic spider, with accuracy in body shape mimicry along the x-axis, and ant-like walking along the y-axis. The lines show a linear regression fitted to the points. We did not find a significant relationship between body shape and behavioural mimicry.

Speed and morphological mimicry

The hypothesis that fast walkers need not be accurate mimics predicts a negative relationship between walking speed and morphological accuracy, and we found such a relationship (dorsal shapes: slope = -0.03, $n = 8$, $F_{1,6} = 18.0$, $p = 0.005$, adjusted $r^2 = 0.7$; lateral shapes: slope = -0.02, $n = 8$, $F_{1,6} = 7.8$, $p = 0.03$, adjusted $r^2 = 0.5$) (Figure 13). However, this result is almost entirely due to a single species (*Elilica*), which is both a very fast walker and a very poor mimic when compared to all other mimetic spiders. All other mimics had very similar walking speeds (mean = 5.1 body lengths/sec, $\sigma = 1.1$, *Elilica* mean walking speed = 32 body lengths/sec). If *Elilica* was excluded from the analysis, there was no longer a significant relationship between mean speed and mimetic accuracy (dorsal shapes: slope = 0.004, $n = 7$, $F_{1,5} = 0.003$, $p = 0.9$, adjusted $r^2 = -0.2$; lateral shapes: slope = -0.06, $n = 7$, $F_{1,5} = 1.0$, $p = 0.4$, adjusted $r^2 = 0.003$).

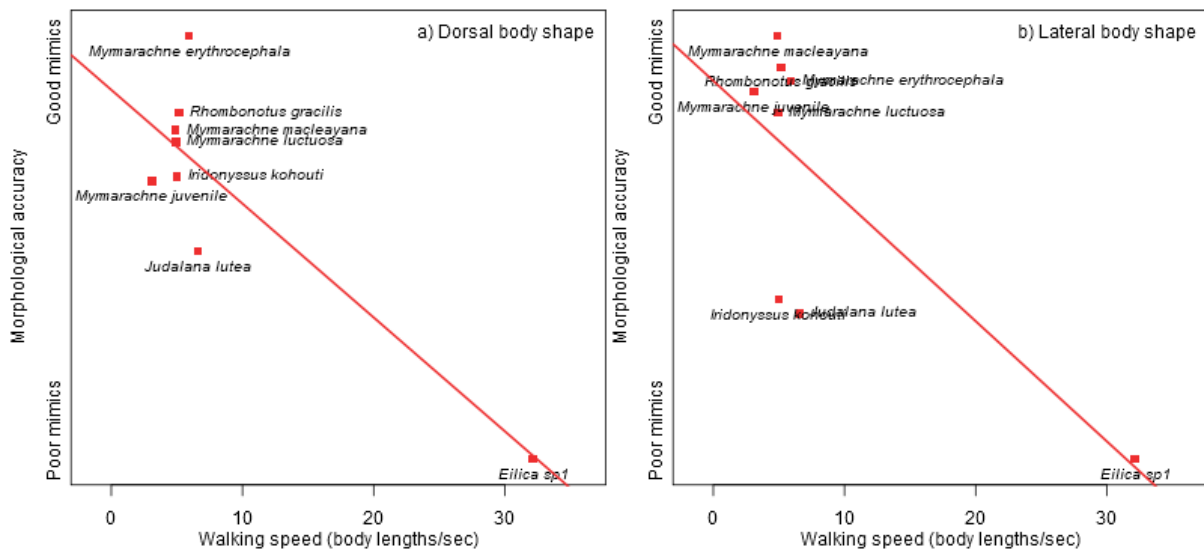


Figure 13 Relationship between mean walking speed and body shape mimetic accuracy. Each point is a single species, lines are linear regressions fitted to the points. There is a strong negative correlation between mean walking speed and mimetic accuracy, however it results almost entirely from a single species which is a poor mimic and a very fast walker.

Discussion

The linear discriminant analysis identified significant differences between the trajectories of ants and those of everything else. Even though mimic trajectories were mostly distinguishable from ants, they were still significantly more ant-like than the non-mimics. Mimic trajectories varied considerably in mimetic accuracy, from good (indistinguishable from ants) to poor (Figure 11). Mimics trajectories were more ant-like in all 14 of the measurements used to characterise trajectories except for mean speed, sinuosity, and first minimum C (i.e. amplitude of regular oscillations in direction), although the differences in mean values between mimics and non-mimics were mostly not significant (Table 8).

Behavioural and morphological mimicry

We predicted that the accuracy of behavioural mimicry would be negatively related to the accuracy of morphological mimicry, as we expected that behaviour could compensate for poor visual mimicry [53]. However, our results do not support this hypothesis as we failed to find any significant relationship between behavioural and visual mimicry. We can also reject the backup signals hypothesis which predicts a positive correlation between behavioural and morphological mimicry [53]. Our results suggest that the two modalities may be independent of each other (the receiver variability hypothesis [141]). A study into the relationship between behavioural and visual

mimicry in hoverflies similarly rejected the increased deception hypothesis, although some evidence in support of the backup signals hypothesis was found, albeit the relationship was only significant for mimics of certain types of models [53].

Morphology and behaviour are both visual signals, so is it plausible that they are subject to different selective pressures? A moving animal must be observed from a sufficient distance and over an adequate period of time to determine its trajectory. By contrast, morphology can be observed almost instantaneously, limited only by the visual and cognitive systems of the predator. Morphology is likely to be assessed more accurately from short distances because perceived resolution decreases with viewing distance [151]. Consequently, it seems likely that different predators may hone in on, and hence select for the different mimetic signals, resulting in independent selection pressure. Since prey detection distances are a positive function of body size [160], larger predators such as birds and lizards may observe – and select for – behavioural mimicry, while smaller predators such as wasps and other spiders may select for morphological mimicry.

The trajectories of mimics are mostly distinguishable from those of ants, and they vary considerably in mimetic accuracy. As this variation is not explained by a relationship with morphological accuracy, it remains to explain why poor behavioural mimicry exists. A range of hypotheses have been proposed to explain imperfect mimicry in general [9], and they may equally apply to behavioural mimicry. Since morphological and behavioural mimicry appear to be independent, different imperfect mimicry hypotheses may apply to each. The significant morphological differences between spiders and ants suggest that developmental constraints or trade-offs may contribute to behavioural differences. For example, it has long been thought that many ant-mimicking spiders walk on six legs, holding the remaining pair aloft to emulate antennae [139]. However, it has recently been discovered that at least one species, *Myrmarachne formicaria*, walks on all eight legs and regularly pauses to lift its front pair [143]. Pausing in this way may constrain other aspects of its walking behaviour, such as maximum—or consistency of—speed, which could lead to a trade-off between different elements of behavioural mimicry.

It is also possible that this study failed to detect a real effect, and there are several possible changes which, if implemented, might affect our results. Firstly, increasing the number of individuals and species sampled would increase the power of the study. Secondly, ensure that we are recording ecologically relevant behaviour, possibly by recording the behaviour of animals, particularly ants, *in situ*. Ants exhibit a huge variety of foraging behaviours, and mimics may be

emulating the behaviour of ants that are following pheromone trails rather than the searching behaviour measured in our study [143]. Lastly, taking species-specific models into account when constructing our indices of mimetic accuracy may yield more accurate results, although predator preference trials are required to determine which approach is more biologically meaningful.

Speed and morphological mimicry

Retinal images are subject to motion blur when the eye and its subject move relative to each other [151], consequently, fast moving animals may be subject to relaxed selection as predators are unable to discriminate between poor mimics and models [27]. Accordingly, we tested for, and found, a negative correlation between walking speed and morphological mimetic accuracy. However, this relationship is due to a single species (an undescribed Gnaphosid, the Yellow-tailed *Eilica* [113]) that is considered to be a member of an extensive mimicry complex which includes various golden coloured ants, wasps, bugs and spiders [29]. Other members of its genus are known to associate closely with ants [161]. Since it scores very poorly for both morphological and behavioural mimicry, its classification as an ant mimic should be considered dubious, and requires confirmation through predator preference trials. If we exclude *Eilica* from the analysis, there is no longer a significant relationship between morphological and behavioural accuracy, so we conclude that we found no effect of speed on mimetic accuracy, and to our knowledge no other studies have found such a relationship [27].

Conclusion

Our study tested an explicit hypothesis that may explain some of the unexpected variation in the accuracy of mimetic resemblances. We predicted that poor morphological mimicry could be at least partially explained by compensatory behavioural mimicry. Instead, we found that behavioural mimicry is yet another source of poor mimicry in need of an explanation. Poor behavioural mimicry might be explained by any of the many hypotheses that have been proposed to explain imperfect mimicry in general [9]. To evaluate and compare the hypotheses, comparative studies and tests of predator responses to varying mimicry are needed, and the multi-faceted nature of behavioural mimicry may make it a productive and fascinating system within which to conduct these studies.

Supplementary Material

Supplementary material (tables S1 to S5) and R source code is available online at

<https://github.com/JimMcL/MRes>. Motion tracking source code is available online at

<https://github.com/JimMcL/SpiderTracker>.

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