Taking a Hit: Resistance to Handling in Model-Mimic Insect Complexes

by

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Abstract

Aposematism, in which an organism's unprofitability to predators is advertised with a conspicuous signal, has been hypothesised to co-occur with an improved ability to survive being handled by a predator with negligible damage.

In Chapter 1, the current literature on this "resistance to handling" and its association with aposematism is reviewed, including anecdotal accounts, experimental evidence and bio-physical considerations. Finally, future directions for this emerging research area are discussed.

In Chapter 2, the relationship between resistance to handling and Batesian mimicry is tested through two experiments on field-caught specimens. The first evaluates the ability of insect species to survive a given amount of compressive force using the successive application of increasing weights, comparing Batesian mimics (Diptera: Syrphidae) to non-mimics (non-syrphid Diptera) and their models (Hymenoptera). The second experiment measures the force required to deform an insect by a given proportion. The relationship between kill weight titration and deformation metrics is also elucidated.

Hymenopterans were the most resistant group, syrphid flies the least, and non-syrphid dipterans intermediate between the two. In all groups, both larger body size and a greater resistance to deformation were correlated with higher kill weights, while mimicry status and mimetic fidelity (once one controls for body size and phylogeny) were not. The implications and possible explanations for these findings are discussed.

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Chapter 1: Literature Review: Aposematism, Mimicry, and Resistance to Handling

This review chapter is intended as an overview of the current literature on resistance to handling as it relates to aposematism and mimicry. First, basic background information will be given on aposematism as a defence and its evolution, followed by an explanation for its purported association with resistance to handling. The terminology of resistance to handling will be stated, followed by a review of the literature's anecdotal accounts and observational evidence of aposematic species being "tougher" than other species. After this, the experimental and quantitative research for this association will be systematically reviewed, starting with Carpenter's beak mark papers, experiments measuring the survival of aposematic species following sampling events, and experiments evaluating the physical and material properties of organisms in the context of predation. Finally, the role of predator cognition, and in particular the possibility of resistance to handling functioning as a tactile cue of unpalatability in addition to its protective effect, will be discussed, before gaps in the literature and future directions are suggested and explored.

1.1 Aposematism

While avoiding detection is among the most common ways in which organisms have evolved to avoid contact with a predator (Thayer 1908; Rojas & Burdfield-Steel 2018), several other defensive strategies have arisen across the animal kingdom and beyond (Ruxton *et al.* 2018). In contrast to easily detectable defences (such as large claws, spines, or offensive orders), some defences are effectively invisible up until the point in the predation sequence when direct contact is made (Rojas & Burdfield-Steel

2018; Caro & Ruxton 2019). In such cases, it is advantageous to both would-be predators and prey for these defences to be signalled, preventing the defended individual from being attacked and the predator from incurring the costs associated with attacking such prey (Rothschild 1960; Ruxton *et al.* 2019). Unpalatable species that signal such defences through conspicuous visual, olfactory, or other sensory indicators are described as being *aposematic* (Poulton 1908; Fisher 1930; Mappes *et al.* 2005; Ruxton *et al.* 2018).

1.1.1 Selecting for aposematism: collective or individual?

While some research suggests that organisms may show a certain degree of innate avoidance towards conspicuous or novel prey items (Lindström et al. 1999a; Thomas *et al.* 2003; Exnerová *et al*. 2006; Nelson *et al.* 2006; Svádová *et al.* 2009) – implying a degree of coevolution (Sherratt 2002) or sensory biases – most studies exploring aposematism have highlighted the importance of predator education in recognizing these signals and learning to respond accordingly (Carle *et al.* 2018; Garcia *et al.* 1985; Speed 2000; Darst *et al.* 2006; Gittleman & Harvey 1980; Exnerová *et al.* 2006; Svádová *et al.* 2009). In order for the conspicuous signal to impart a protective effect to the defended species, the predator must learn to consistently recognise and associate the conspicuous signal with the defence. This learning process requires a number of individuals to be sampled by the predator, which can result in injury and/or death in the sampled prey. For this reason, the initial evolution of aposematism from rarity has long been regarded as something of a puzzle, because emergent aposematic individuals will face something of a “double-whammy”: predators do not recognise their signals without sampling, and their rarity implies that many if not all of these individuals

will be sampled before their unprofitability is learned (Speed & Ruxton 2005b). How, then, could aposematism eventually become fixed in a population?

The earliest explanations for the evolution of aposematism have centred around kin selection, in which a gene (such as one for a conspicuous signal) spreads in a population, not because it benefits the *individual*, but because it benefits *others* who carry the same gene by common descent (Fisher 1930; Gittleman & Harvey 1980; Malcolm 1986). Only later did it become more widely appreciated that predator attacks do not always end in the death of the signalling organism (Wiklund and Järvi 1982; Sillen-Tullberg 1985; Leimar et al. 1986; Lindström 1999b). This survivorship allows aposematism to spread in a population by individual selection, and is further enhanced by a series of traits collectively known as "resistance to handling", which we now introduce.

1.2 Aposematism and resistance to handling

"*This resistance is part and parcel of the process whereby an aposematic insect teaches an enemy that it is harmful or unpalatable*." - Carpenter (1929, pg. 622).

1.2.1 Terminology

At the time when the earliest works on aposematism were being published, an organism's resistance to being killed by a sampling predator was known as "tenacity of life" (Trimen & Bowker 1887; Hasse 1896; Parker & Haswell 1899; Cott 1940; Rothschild & Kellet 1972). Here we simply use the term "resistance to handling", which is more direct and at the same time avoids physical terms such as "hardness" and "toughness" which have specific (and various) meanings, an issue which is expanded upon in section 1.2.5 of this thesis, "Testing the physical properties of insects".

In this paper, we define “resistance to handling” simply as an organism’s ability to survive being handled by a predator with little to no permanent damage, thus allowing them to remain a part of the reproductive population.

1.2.2 Anecdotal evidence: pinching butterflies

Among lepidopterists, a common technique for immobilising butterflies without damaging their wings is known as “pinching”, in which the insect’s thorax is held between the thumb and forefinger and squeezed until a soft “pop” is felt. This is considered by many collectors to be the best way to kill a butterfly and has been used in numerous scientific studies for over a hundred and fifty years (e.g., Trimen 1868; Swynnerton 1926; Steppan 1996; DeVries 2003). Being so widely used, it is unsurprising that Trimen (1868) took notice when pinching the thorax of several species of African *Danais* and *Acraea* butterflies failed to have any noticeable effect, up until a very high amount of force was used. Even after the butterflies’ apparent death, Trimen describes, with surprise, how several of the seemingly dead specimens flew off “with perfect ease and apparent *nonchalance*” (emphasis not added; Trimen 1868, pg. 499).

Trimen credits this “tenacity for life” to the butterflies’ “remarkable elasticity”, and quickly makes the connection to the noted unpalatability of these genera, stating that naïve birds and other insectivores may sample the butterflies only to release them upon discovering their noxious taste, leaving the butterflies largely unharmed where other species may have been killed. This observation is one that would be oft-repeated in the literature for years to come.

Anecdotal accounts similar to Trimen’s are quite common in the literature regarding aposematism; many researchers and naturalists have noted that aposematic

species appear to be “tougher” than palatable, cryptic species (see Appendix A, Table A.1; Trimen 1868; Haase 1896; Poulton 1908; Cott 1940 pp. 259-260; Carpenter 1929; Carpenter 1941; Blest 1963; Rettenmeyer 1970; Rothschild 1971; Järvi et al. 1981; Wiklund & Järvi 1982; Chai 1986; Evans 1987; Evans & Schmidt 1990; DeVries 2002, 2003; Gilbert 2005; Halpin et al. 2008; Pinheiro & Campos 2013). Insects in particular are commonly noted for their variable resilience, likely because the tactile experiences of butterfly-pinching and insect-pinning have led many entomologists to incidentally note such variation.

In many ways, an association between resistance to handling and aposematism seems intuitive. Palatable prey with effective crypsis may rarely come into close contact with predators, while prey that are conspicuous may be more frequently sampled by naïve or hungry predators (Brodie *et al.* 1991; Carroll & Sherratt 2013). Under these conditions, the aposematic prey would face strong selective pressure to evolve sufficient resistance to this handling so that they may employ their post-capture defences and still survive, should they escape or be rejected. Essentially, if you must be in a predator’s mouth before the predator knows you are unprofitable, it would serve you well to be able to withstand the mechanical stresses of such an experience without significant harm.

1.2.3 Beak marks and Carpenter

The numerous anecdotal observations of resistance to handling in the literature are punctuated by a number of experiments which have attempted to quantify this relationship empirically. One of the earliest attempts to measure the relationship between resistance to handling and palatability was Carpenter’s 1941 paper, in which

museum specimens were scoured for butterflies with beak marks on their wings, focusing on aposematic taxa in the family Nymphalidae (*Euploea* spp., *Danaus chrysippus*, *Tirumala* spp., and *Amauris* spp.), and one genus in the family Pieridae, *Colotis*, which is known to be palatable. It was found that the aposematic species had beak marks on their wings 10 times more often than the cryptic species (1.5% vs 0.15%, respectively), and that, when comparing aposematic models and Batesian mimics of different African mimicry complexes, the models seemed to have a higher frequency of beak-marks than their mimics in all cases.

At first glance, these results may seem to indicate that aposematic species are attacked by predators 10 times more often than palatable, cryptic species, perhaps by virtue of their conspicuousness. However, it is important to remember that beak marks represent *unsuccessful* predation attempts which the individual specimens escaped from and survived. Indeed, the distribution of beak marks is reminiscent of the classic

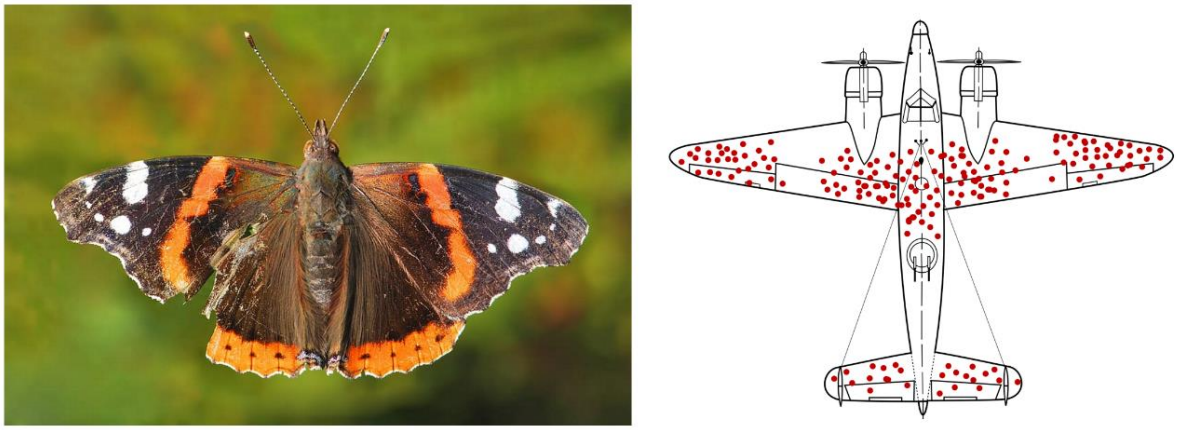

Figure 1.1: Red Admiral butterfly (*Vanessa atlanta*) with wing damage. b) illustration of hypothetical damage on a WWII bomber based on the report by Wald (1943); red dots indicate where damage was found on surviving planes. Plane diagram by McGeddon et al. (2016).

work by Abraham Wald on the “survival bias” that arises when evaluating the distribution of damage in WWII bombers (Wald 1943; Mangel & Samaniego 1984). It is only the individuals which somehow *survive* sampling that are available to be captured and added to the museum collection, allowing their beak-marked wings to be evaluated.

This method is not without its faults; Carpenter himself stated that the common practice of discarding particularly damaged individuals for aesthetic reasons has likely led to a bias in museum collections, and thus the proportion of damaged to non-damaged individuals in the wild is likely greater than that which is found in his work. Subsequent field studies of beak marks on butterflies, such as Kiritani *et al.* (2013), further highlights this museum bias, finding that, among six Lepidopteran families in Japan, beak marks were found on 12.3-52.0% of individuals captured over the course of a year. While palatability was not examined in this study, it does suggest that the numbers uncovered by Carpenter may severely underestimate the true occurrence rates of beak markings.

1.2.4 Surviving sampling: a brush with death

Of course, an organism does not necessarily need to be consumed in order to sustain mortal injuries, but it would still be incorrect to assume that sampling *always* results in death. Several studies have observed that aposematic prey can often survive being handled by a predator, especially when the predators are in the field or wild-caught (Wiklund and Järvi 1982; Sillén-Tullberg *et al.* 1985; Evans 1987; Halpin *et al.* 2008; Halpin & Rowe 2017; Wang *et al* 2018a,b; Winters *et al.* 2021). For example, Järvi et al. (1981) experimentally demonstrated that unpalatable Old World swallowtail butterfly caterpillars (*Papilio machaon*) survived being sampled by wild-caught great tits

(*Parus major* L.) in 100% of laboratory trials. Similarly, aposematic velvet ants (Hymenoptera: Mutillidae), which are the models of several well-known mimicry rings, have also been shown to have remarkable survivorship after predator encounters. Gall *et al.* (2018) staged interactions between seven species of live velvet ants and ten different species of potential predators (lizard, n=3; toad, n=2; bird, n=2; and mammal, n=3). After over one hundred trials, only one velvet ant was killed and consumed by a toad, with further experiments finding that two-thirds of the velvet ants that were swallowed by toads were regurgitated, unharmed, after spending up to 20 minutes in the toad's stomach (Mergler & Gall 2020). This impressive rate of survival replicated the findings of Schmidt & Blum (1977) who conducted similar trials with the species *Dasymutilla occidentalis* (Hymenoptera: Mutillidae); out of 122 trials, only two specimens were killed, one by a gerbil and one by a tarantula.

1.2.5 Resistance to handling as a signalled defence

While these cases involve chemically defended species, it appears that a suitably "tough" integument alone can serve as an effective defence, enough so that predators may learn to avoid such prey (Matthews & Matthews 2010; Swynnerton 1926; Wang *et al* 2018b). Even in cases where it *is* possible to consume such intractable prey items, the energy economy of the increased handling time may make them undesirable, and thus softer prey may be preferentially selected (Fisher & Dickman 1993b; Herrel *et al.* 2001).

An excellent example of hardness as a signalled defence is seen in the hard-bodied weevils *Pachyrhynchus sarcitis*, which were found to always survive being sampled by lizard predators (Wang *et al.* 2018a,b). Despite finding no evidence of

chemical defences, as demonstrated by the ease and enthusiasm with which naïve lizards consumed the soft-bodied, fresh-moulted weevils of the same species (Wang *et al.* 2018b), the lizards which were given hard-bodied weevils learned to reject subsequently offered weevils after only a single encounter (Tseng *et al.* 2014), suggesting that this is a fascinating example of aposematism which solely signals cuticle hardness.

Another example, which may be a combined defence of cuticle intractability and escape behaviours, are butterflies in the genus *Charaxes.* Swynnerton (1926) did many palatability experiments with these butterflies and various African bird species, finding that, although the *Charaxes* butterflies had no detectable chemical defences, they appeared to be prohibitively difficult to eat. He details numerous accounts of hungry birds repeatedly failing to capture or consume the live *Charaxes* butterflies that were released into their small cages, after which they rejected subsequently offered conspecifics. Swynnerton attributed the difficulty to the butterflies' "hard" bodies and the violence with which they struggled upon being seized, noting that one bulbul (*Pycnonotus barbatus*), who had rejected the *Charaxes* repeatedly, readily consumed the "slightly nauseous" *Neptis agatha* butterfly it was offered. This case is especially interesting due to the fact that many *Charaxes* species are considered to be Batesian mimics of chemically defended models. That is, they have been categorised as palatable species that mimic an undesirable model in order to gain the protection of the aposematic signal without investing any resources into the defence (Bates 1862; Mostler 1935; Ruxton *et al.* 2018). If their "tough" integument and violent escape behaviours make them unprofitable enough for predators to learn to reject them on

sight, then they could potentially be considered *Mullerian* mimics, a defended species using the same aposematic signal as other defended species, in a mimicry ring containing diverse defence modalities.

1.2.6 The physical properties of insects

Experiments using live predators and prey are a highly effective way to examine resistance to handling in the context of predator-prey interactions. However, identifying and measuring the quantitative *physical* traits associated with such resistance to handling would allow for easier comparisons between a diversity of taxa, including those which are not closely related. These can be challenging data to obtain, in part due to the irregularity of biological materials and associated technical constraints, and in part due to human limitations; accurate and objective measurements require specific variables to be controlled, special tools utilised, and, perhaps most daunting for many entomological collectors, specimens to be damaged or even destroyed. All the while, scientists must agree on the vocabulary they use to describe such properties, which has been a noted barrier in the past (Evans & Sanson 2005; Freeman & Lemen 2007).

Although it is tempting to use such terms as “hardness”, “strength”, “toughness”, and “resilience” synonymously when describing an organism’s ability to resist permanent damage, an examination of the literature quickly makes it apparent that these terms have very specific definitions which can change depending on the field in which they are being discussed, and thus cannot be interchanged as readily as they are in everyday usage. The term “hardness”, for example, is notoriously inconsistent, with different, sometimes non-overlapping definitions given by engineers, materials

scientists, and even potters (Boyer 1987; Wright & Vincent 1996; Evans & Sanson 2005; Vincent 2012; Parle *et al.* 2017).

When it comes to how well an insect can survive a sampling event, the trait we are interested in is how much mechanical stress (force per unit area) it can withstand without accruing deadly damage. Some measurable traits of interest, along with the definitions that will be used for the purpose of this thesis, include stiffness (resistance to non-permanent deformation), hardness (resistance to permanent deformation), elasticity (ability to return to its original shape after the removal of the deforming force), resilience (ability to store strain energy via elastic deformation without breaking), strength (*force* per unit area required to *initiate* a crack), toughness (*energy* per unit area required to *propagate* a crack), and viscoelasticity (properties of a material exhibiting both elastic and viscous properties upon deformation; typical of biological materials), to name a few (Vincent 2012; Wright & Vincent 1996; Miller *et al.* 2009). A table containing a list of these terms, their definitions, their associated equations, and the sources for the definitions can be found in Appendix A, Table A.2.

There has been considerable research on the physical properties of relatively homogenous biomaterials, such as an insect's endocuticle or a vertebrate's bone, with one of the most commonly used methodologies being nanoindentation (Chudoba *et al.* 2006; Klocke & Schmitz 2011; Labonte *et al.* 2017; Sun et al. 2018). By using probes of different shapes, measurements can be made of such material properties as elasticity, viscosity, plasticity, and fracture point (Vincent 2012). However, nanoindentation is, by definition, a microscale measurement and cannot be reliably used to determine the properties of an organism as a whole. Unlike a relatively homogenous, isotropic

material, such as a block of steel, the body of an insect may be more accurately thought of as a complex structure made up of many components of variable physical and chemical makeup – including cuticle, muscle, trachea, haemolymph, etc.— layered in various arrangements and combinations depending on the structural and biomechanical needs of the organism (Evans & Sanson 2005; Vincent 2012; Xing & Yang 2020). Nanoindentation can only examine a single component of that structure in isolation, and since a predator is unlikely to encounter a piece of endocuticle living independently from the insect as a whole, for example, this method is useful but limited when exploring the evolutionary context of resistance to handling.

1.2.7 Experiments with aposematism and physical properties

In the case of predation on insects, much of the existing work on cuticle "hardness" has been completed by researchers looking at the diets of insectivorous mammals and has utilised diverse methodologies to quantify this trait. This includes a qualitative scale of "hardness" (Freeman 1981), force at collapse (N) using an aluminium cone (Fisher & Dickman 1993a), force to penetrate (N) using different-shaped punches representing variations in bat tooth morphology (Evans & Sanson 1998), maximum force (N) to close scissors (Freeman & Lemen 2007), maximum force (N) to punch a hole using a leather punch tool on cuticle that had already passed through the digestive tract of the predator (Evans & Sanson 2005), and force at collapse (N) using a screw with a flattened end (Aguirre *et al.* 2003). This variation in methodology makes comparisons between these studies difficult, as does the higher-order taxonomic classification of the majority of insects tested. While these studies provide an excellent overview on the specific topic for which it was collected, it is

unfortunately of limited use for research into aposematism, mimicry, and resistance to handling in insects.

Luckily, a small amount of aposematism-focused research on physical properties has been undertaken through the years (Herrel *et al.* 2001). For example, in their explorations on the defences of velvet ants, Schmidt & Blum (1977) experimentally crushed the thoraxes of dry specimens of several insect species using a Hanson Cook-O-Meter and a "bar". They tested four species of stinging Hymenoptera (velvet ant, *Dasymutilla occidentalis* (Hymenoptera: Mutillidae); scoliid wasp, *Scolia dubia* (Hymenoptera: Scoliidae); honey bee, *Apis mellifera* (Hymenoptera: Apidae); and yellowjacket, *Vespula maculata* (Hymenoptera: Vespidae)), along with three genera of coleopterans. Of these species, the velvet ant *Dasymutilla occidentalis* was able to withstand the highest absolute crushing force (27.8 N) before "skeletal collapse", and, when compared to other hymenopterans, was able to withstand 78% more force relative to its mass (247 N/g).

In Wang *et al.* (2018a,b), the aposematic weevil *Pachyrhynchus sarcitis* also had its physical properties measured, and what's more, was compared to a diverse selection of sympatric insects in order to place it within the context of its local prey community (Wang *et al.* 2018b). Using the dissected jaws of a lizard predator (*Japalura swinhonis*) embedded in resin as the crushing tool, the maximum force (N) at exoskeleton failure was used to quantify the "hardness" of the different insects, which included *P. sarcitis* as well as other coleopterans and insects in the orders Odonata, Orthoptera, Hemiptera, Blattodea, Neuroptera, and Hymenoptera. Although aposematic and non-aposematic taxa were not explicitly compared, it was noted that the aposematic *P. sarcitis* took

more force to crush on average than any of the other weevils (Curculionidae) in the same habitat, often by a wide margin. The majority of these sympatric weevils appear to have been cryptic, although this was not stated explicitly in the paper and is instead a judgement based on a cursory examination of type specimen photographs. Including the weevils, 28 different insect families were sampled and measured, and although several families only had a single representative specimen, this is still one of the most diverse datasets to date in regard to resistance to handling as an anti-predator defence.

Finally, DeVries (2002, 2003) explored the relationship between tensile wing "toughness" and palatability. In his 2002 paper, he examined five species of sympatric nymphalid butterflies, two palatable and three unpalatable, and measured the "toughness" of their wings by attaching a small metal electrical clip to the hindwing margin and adding weight until the clip ripped free. He found that the wings of unpalatable species took more weight to rip compared to those of palatable species, and also that this "toughness" was variable between different species. He suggested that this difference may be due in part to interspecific variation in palatability, with more unpalatable species having "tougher" wings, and completed a follow-up study to examine this idea further.

In DeVries (2003), he examined the wings of three nymphalid species in the same manner, finding the aposematic species to have the highest tear weight, the cryptic species the lowest, and the "putative" Batesian mimic a tear weight which was intermediate between the two. From this, DeVries concluded that the Batesian mimic was likely on the palatability spectrum, being less palatable than the cryptic species but more palatable than the aposematic species. If this is true, then it would make

Pseudacraea lucretia a mislabelled Mullerian mimic, although we could not find any study that has since evaluated its palatability to confirm this. It is also worth noting that the species tested varied significantly in wing length, with the aposematic species being the largest and the cryptic species being the smallest, making this a potentially confounding variable.

Although studying the physical properties of organisms comes with a number of difficulties, these studies have been integral in expanding our understanding of the relationship between resistance to handling and aposematism. While the structural and material properties of organisms have been studied in some detail, all the way down to the microscale (Vincent & Wegst 2004; Wang *et al.* 2018a, 2019; van de Kamp *et al.* 2015; Parle *et al.* 2017; Xing & Yang 2020), placing that research in the ecological context in which these organisms evolved is a relatively new endeavour, and there is still much we don't understand about the selective pressures and trade-offs that lead to the variation we see in resistance to handling.

1.3 Variables and trade-offs influencing resistance to handling

Organisms exist and evolve in complex environments, which provide a wide variety of selective pressures on a specific organism's physical properties, including those which determine how much force an organism can withstand before suffering a functionally mortal injury (Parle *et al.* 2017; Clark & Triblehorn 2014). It is insufficient to solely consider predation when examining the relative resistance to handling of different specimens; factors such as environment, life-history strategies, phylogeny, and intraspecific variation must also be weighed and, whenever possible, controlled and accounted for. In addition, there are likely to be trade-offs between different

functionalities in regard to an organism's physical properties, with mobility in particular being likely to be negatively impacted by increased protective structures (Vincent & Wegst 2004). This section will cover a few of the factors and trade-offs which should be considered when evaluating an organism's resistance to handling, with a particular focus on insects.

Environment and life-history strategies are both certain to impact the degree of resistance to handling an organism possesses, as both will come with their own associated stressors and pressures. For example, large beetles living in arid environments have been found to be generally more resistant to crushing than those living in temperate areas, which may be related to protection against predators and/or desiccation (Fisher & Dickman 1993a,b). Additionally, certain life-history strategies are likely to necessitate more resistant bodies: many ectoparasites are known to be resistant to damage from grooming hosts (Gullan & Cranston 2014), and insects that frequently come into contact with abrasive substrates, such as those which burrow underground, must be able to resist excessive friction damage (Sun *et al.* 2008). It has even been suggested that, for certain chemically-defended species, a "harder" integument may be necessary to protect the organism from its own defensive compounds (Matthews & Matthews 2010).

Phylogenetic relationships also important to consider, as certain groups of organisms, such as beetles, tend to be generally more resistant to plastic deformation relative to others, such as grasshoppers, with members of each group bearing a degree of morphological resemblance on account of their relatedness (Wang *et al*. 2018a,b; Herrel *et al.* 2001). Even when considering variation *within* a species, differences such

as sex (Davis & Holden 2015), age (Hill & Vaca 2004), and time since an arthropod's last moult can make a significant difference in a specimen's ability to survive sampling (Wang *et al.* 2018a,b).

Variation in diet may also affect the eventual physical properties of an insect's cuticle, as an organism can only synthesize materials and build body structures using the resources available to them (Hopkins 1992). It is known that intraspecific differences can occur due to diet (Tsao & Richards 1952), and this has implications for variation between species that are different types of consumers (e.g., carnivores, herbivores, detritivores, etc.), as one might expect diets which result in the acquisition of different proportions of resources to lead to different cuticle properties (Kramer & Hopkins 1987).

While the above variables are important to consider, one of the strongest predictors of resistance to handling in insects is body size; both within and between species, larger insects tend to be more resistant to compressive forces than smaller insects (Schmidt & Blum 1977; Fisher & Dickman 1993a,b; Herrel *et al.* 2001; Aguirre *et al.* 2003; Evans & Sanson 2005; Freeman & Lemen 2007; Wang *et al.* 2018b). One possibility is that this correlation is due to increased predation pressure selecting for more resistant bodies, as larger insects are likely to be both easier to detect and potentially represent a meal of higher caloric value (Hossie *et al.* 2015). However, one would also expect to find this trend when solely considering the structural needs of a larger body, as determined by basic geometric scaling and biomechanical constraints (Williams *et al.* 2012).

To demonstrate this, let us use a simplified example in which we assume an idealised insect which is a perfect cube composed of a homogenous, isotropic material.

It is known that the amount of force a three-dimensional structure can withstand without permanent deformation is approximately proportional to its cross-sectional area (Williams *et al.* 2012). When we increase the length (a) of the insect, the cross-sectional area will increase by a^2, while volume increases by a^3. This means that doubling the length of an insect will increase its cross-sectional area by a factor of 4, while mass (assumed to be proportional to volume) would increase by a factor of 8. If we exclusively consider the object's ability to hold up its own mass against gravity and ignore other biomechanical or environmental stresses, this alone is enough to necessitate a disproportionate increase in the strength of any supportive structures as the organism increases in size (Lease 2008; Williams *et al.* 2012). This example is, of course, an oversimplification of the complex geometry and structures that make up an insect's body, and we cannot assume that cuticle alone is responsible for structural support (muscles, tendons, and even hydrostatic pressure are likely to also play a role; Clark & Triblehorn 2014), but the basic principles of scaling are still expected to apply.

Finally, it is important to consider potential trade-offs between certain cuticle properties and different beneficial traits, most notably impacting flight and locomotion (Marden & Chai 1991; Vincent & Wegst 2004; van de Kamp *et al.* 2016; Jafarpour *et al.* 2020; Wang *et al.* 2019). Some of the most resistant beetle species, such as weevils, are completely flightless, their apterous body plan allowing their elytra to thicken and fuse into a more robust structure (van de Kamp *et al.* 2015, 2016; Wang *et al.* 2018a,b, 2019). Meanwhile, for flighted insects, the properties which make a cuticle more protective against predation may also negatively impact their flight ability in terms of speed, manoeuvrability, efficiency, and energetic cost. For example, one of the simplest

ways to make a cuticle more resistant to damage is to make it thicker (Evans & Sanson 2005), but this also adds extra weight which must be overcome and carried around in flight, which is energetically expensive (Vincent & Wegst 2004). In addition, it is known that the thoraxes of insects flex and distort during flight, contributing to the efficiency and mechanics of flight through such properties as the capacity to store and transmit elastic strain (Ennos 1987; Vincent & Wegst 2004). The traits which allow flighted insects to perform at the necessary proficiency for survival may be irreconcilable with the traits that increase their resistance to handling. What's more, if a species is a skilful enough flier to escape the majority of attacks before the predator even makes contact, then the post-capture defence of a protective cuticle may not be strongly selected for (Brodie *et al.* 1991).

1.4 Predator cognition & tactile cues

The nature of aposematism, in which a predator learns to associate a signal with an unprofitable prey item, requires that the predator have the ability to learn and make informed decisions about which prey are worthwhile and which are not. In order to do this, predators collect information on their prey by perceiving various signals and cues, through different sensory inputs, and then interpret the combined stimuli to make a decision informed by both innate biases and knowledge from past experiences (Ashby & Maddox 1990; Ruxton *et al.* 2018). Different stimuli have different levels of "salience" for predators; that is, the amount they stand out individually from the whole. The salience of a given stimulus is highly subjective and can vary depending on the context of the encounter –lighting conditions, amount of sensory "noise", weather, etc.— and the cognitive abilities of the predator (Taylor et al. 2016b). Highly salient signals, such

as the black and yellow stripes on many stinging hymenopterans, may be generalised by a predator, which could, in turn, give rise to a greater diversity of imperfect mimics (Svádová *et al.* 2009; Chittka & Osorio 2007; Easley & Hassall 2014; Pekár 2021).

While the bulk of research has focused on how visual and gustatory stimuli are interpreted, particularly by avian predators, there is a growing awareness of the potential importance of non-visual and multimodal cues and signals, including those using auditory (Bura *et al.* 2016), olfactory (Winters *et al.* 2021), and even thermal sensory pathways (Rundus *et al.* 2007). But perhaps the least-explored sensory component of prey evaluation is tactile cues, in which the predator detects and receives information through the feeling, texture, and other material properties of the prey item. While it has long been recognised that predators engage in sampling behaviour, making exploratory contact with a prey item without consuming it, this has been largely evaluated in the context of gustatory taste-rejection (Sillen-Tullberg 1985; Halpin & Rowe 2017), and is rarely considered to be part of tactile exploration, although this possibility has been raised in several papers (Swynnerton 1926; Hogan-Warburg & Hogan 1981; Kassarov 1999; DeVries 2003).

In the case of avian predators, tactile cues are certainly being perceived, as birds are known to have various types of sensorimotor neurons in their beaks, jaws, and oral cavities which allow them to feel and manipulate the objects and food items they grasp (Kuenzel 1989; Schneider *et al.* 2014; Soliman & Madkour 2017). These mechanoreceptive structures are particularly well-developed in “tactile foragers”, such as waterfowl, shorebirds, and spoonbills, which locate food through touch and thus require highly sensitive beaks (Schneider *et al.* 2014; Martill *et al* 2021). While the

majority of birds must have enough tactile sensitivity to perform the manipulations we observe in regard to nest building, prey handling, and preening behaviours, it is not yet known whether or not the sensory input is precise enough to detect small differences between objects, and whether or not such differences would have very much salience when evaluating the relative profitability of prey. I would speculate that the mechanoreceptive abilities of a bird's beak are extremely likely to have such a sensitivity, due to the beak being, for most species, the primary tool with which birds are able to interact with and manipulate items in their environment, but this has yet to be tested.

The salience of tactile stimuli in terms of prey evaluation is also an avenue well worth pursuing. Even if protective cuticle properties did not specifically evolve as a signal to communicate unpalatability to predators, cues can still be interpreted to have meaning by an organism if there is benefit in doing so (Ruxton *et al*. 2018). It seems plausible that a predator might use tactile stimuli as one sensory input of many that contribute to their recognition of unprofitable prey, especially in cases where mimicry makes organisms otherwise virtually indistinguishable. In fact, we have an example of one type of animal employing tactile cues in order to differentiate butterfly models from mimics: humans. In an 1883 letter, referenced by both Trimen & Bowker (1887) and Haase (1896), Bowker commented on two species of South African butterflies which he could only distinguish once he pinched their thorax. Reportedly, the Batesian mimics of the genus *Pseudacraea* were "brittle" and would die at once, while the aposematic *Acraea* were "leathery" and could be squeezed "as long as hard as you like without effect." From this, we know that it is at least possible to use tactile cues to differentiate

visually similar butterfly species, and although there is a human tendency to believe that our sensory experiences are somehow exceptional to those of other animals, we have not yet even looked for evidence that birds and other predators would be unable to employ similar strategies.

1.5 Future directions

The topic of resistance to handling is incredibly broad and covers many different disciplines of science, and so it is not surprising that there are still many avenues left to explore. In order to further expand our understanding of how resistance to handling relates to aposematism and other evolutionary and ecological processes, the first step is to broaden our pool of data, ideally using a standardised measure of resistance to handling, and continue work in the same thread as Wang *et al*. (2018b) and Herrel *et al.* (2001), in which a wide variety of species from the same ecosystem are tested in a way which is relevant in the context of predator-prey interactions. This is not a small undertaking; there are thousands of examples of aposematic species and mimicry rings in the literature, of which only a scant few have been examined in the context of their resistance to handling.

Additionally, this review primarily focused on resistance to handling in insects, owing to the overwhelming tendency in the literature to consider resistance to handling in arthropods rather than other invertebrates or vertebrate species. This is likely due in part to ethical considerations, as well as the fact that arthropod exoskeletons are retained after death in a more-or-less similar condition to how they were when alive. However, it would be highly beneficial to consider humane ways in which resistance to handling may be studied in vertebrates, such as observational and morphological

studies, as well as re-examining some of the existing research through the lens of resistance to handling. In particular, reptiles, amphibians, and fish have an incredible diversity of aposematic species which could be explored (Caro & Ruxton 2019), especially in species possessing easily quantifiable morphological traits which are likely to increase their resistance to handling. For example, *Brachycephalus* (Anura: Brachycephalidae) is a genus of toads that includes both aposematic and cryptic members; several species have been found to exhibit hyperossification, in which the skin, skull, and postcranial skeleton undergo a high degree of mineralisation (Condez *et al.* 2020). It is noteworthy that this trait only occurs in aposematic species of this genus and also that the degree of hyperossification exists on a continuum, being absent, intermediate, and extreme in different species. Taxa such as these, in which a group of closely related species display a variety of states relevant to aposematism and resistance to handling, should continue to be identified and examined as we consider the diversity of forms and functions that may lend themselves to this proposed association.

Finally, the role of resistance to handling needs to be further explored from the perspective of tactile cues experienced and interpreted by a sampling predator. Experiments are required to determine whether or not predators are able to differentiate prey solely on the basis of tactile stimuli, as well as the salience of such stimuli compared to other cues and signals of unpalatability that influence a predator's ultimate decision regarding a prey item. For example, one might test whether or not birds treat unpalatable prey items differently when they are visually identical, but one is stiffer than the other. Such research would be of particular interest in regard to tactile foragers and

species which lack visual organs altogether, relying instead on tactile cues to determine whether or not a potential prey item will make a suitable meal. A series of experiments of this type may add another sensory dimension to research on predator learning, multimodal signalling, and the trade-offs of different defences.

These are only a few of the directions that we may choose to explore in the years to come, as there are countless questions left to answer, and even more that we have not yet thought to ask. Are aposematic species always more resistant to handling than closely related non-aposemes? What are the interactions of resistance to handling with Mullerian mimicry and the palatability spectrum? Are Batesian mimics more resistant to handling than non-mimics, despite being undefended? How does resistance to handling vary between environments, predator communities, levels of predation pressure, diet, and so forth? There are many more avenues of research that we may wish to explore as we move forward in this field, and untangling the intricacies of aposematism, mimicry, and resistance to handling can only serve to further improve our understanding of the ecological and evolutionary factors which allow such systems to arise and persist.

Chapter 2: Experiments in Resistance to Handling: Measuring Survivorship & Physical Characteristics

In this chapter, I will be presenting the experimental results from my research on resistance to handling in Batesian mimics. First, basic background information is provided on the gaps in research pertaining to Batesian mimics and resistance to handling, as well as information about flies in the family Syrphidae (Diptera), the model Batesian mimics for these experiments. Then, the two experiments are presented: the titration experiment, in which sampling survivorship is quantified by determining a "kill weight" for a large number of specimens, and the micrometre experiment, in which the physical property of "resistance magnitude" is determined and compared to the kill weights from the titration experiment. The results of the two experiments will then be discussed together, particularly in the context of Batesian mimicry.

2.1 Introduction

Mimicry and resistance to handling

While the relationship between aposematism and resistance to handling has been discussed in the literature (Chapter 1), the role of mimicry in this relationship has rarely been considered. Several papers have suggested that mimics, and in particular good mimics, are more resistant to handling than non-mimetic species (e.g., DeVries 2002, 2003; Gilbert 2004). Gilbert (2004) in particular, suggested that high-fidelity mimics in the family Syrphidae (Diptera)—commonly known as "flower flies" or "hoverflies"—are "harder" and "more durable" than non-mimetic dipterans and low-fidelity mimics, citing rounded, emarginate abdomens, a punctate cuticle, and strong

joints between overlapping tergites as traits common in especially good mimics which may result in a greater resistance to handling. Theoretically, this association would enable the mimics to withstand a greater level of scrutiny from predators, as it provides tactile stimuli that resemble the model in combination with visual signals, potentially increasing the likelihood of being incorrectly identified by the predator as undesirable even after direct contact is made. However, there are no known experiments comparing the mimetic fidelity and resistance to handling of syrphids. In addition, there has been little research into the specific physical properties associated with resistance to handling, with both “elasticity” (Trimen 1868; Trimen & Bowker 1887; Poulton 1909; Fisher 1930), and “hardness” (Rettenmeyer 1970; Gilbert 2004) suggested as metrics for resistance to handling but not tested empirically (see Appendix A, Table A.2 for technical definitions of biomechanical and material science terms).

Elucidating the relationship between resistance to handling and mimetic fidelity may help to improve our understanding of how aposematism and Batesian mimicry evolve in relation to other defences. Meanwhile, exploring how the specific physical properties of an organism influence their resistance to handling will help us better understand the physiological and evolutionary basis upon which such defences function, evolve, and are maintained, and potentially represent an alternative way to quantify resistance other than sampling survival experiments.

Batesian mimics: Syrphidae

The vast majority of species in the family Syrphidae are Batesian mimics of stinging Hymenoptera; that is, they are an undefended species which mimics the defended Hymenoptera in order to gain the protection of their aposematic signals

(Gilbert 2005; Bain *et al.* 2007; Penney *et al.* 2012; Rashed & Sherratt 2006; Rashed *et al*. 2009; Golding *et al.* 2001). The diversity of mimics in this family is high; numerous types of hymenopterans are mimicked in varying degrees of accuracy, including so-called “perfect mimics,” which resemble the model species so closely that, at a glance, they could fool even the most seasoned of entomologists (Howarth & Edmunds 2000; Edmunds 2000; Marchini *et al.* 2017; Skevington *et al.* 2019). However, many other mimetic syrphids seem to be imperfect, or even quite poor mimics (at least, poor to human eyes; Cuthill & Bennett 1993). The reason for the existence of poor mimics is still a topic of debate, and hypotheses on how poor mimics evolve are numerous and varied (Pfennig & Kikuchi 2012; Ruxton *et al.* 2018).

Syrphids are an excellent candidate for research into Batesian mimicry, largely due to the ample research which already exists about their biology and ecology. In addition to a thorough phylogeny established in the literature (Mengual 2019), the models for the different mimetic species have been widely determined (Howarth & Edmunds 2000; Holloway *et al.* 2002; Rashed *et al.* 2009; Taylor *et al.* 2016), and the spectrum of mimetic fidelity among syrphid flies has been quantified experimentally, using both human and avian predator models (Dittrich *et al.* 1993; Bain *et al.* 2007; Penney *et al.* 2012, 2014; Hassall *et al.* 2019).

Experiments in resistance

In the past, there have been two basic ways in which resistance to handling has been measured. First, in terms of the prey species’ ability to survive being sampled and rejected by a predator (e.g., Wiklund & Järvi 1982; see section 1.2.4: Surviving sampling), and secondly, more rarely, in terms of their physical characteristics (e.g.,

Wang *et al.* 2018a; see section 1.2.5: Testing the physical properties of insects). Unfortunately, these two methods have seldom been used in tandem. While the ability to survive being sampled can be considered the “brass tacks” of resistance to handling, the physical properties associated with increased survival rates are likely to be at the root of this resistance. Some organisms possess physical properties that make them more resistant to handling, and if we can identify and measure those characteristics in relation to their ability to survive sampling, then this will not only give us a more well-rounded understanding of the mechanics of resistance to handling, but may also allow us to measure resistance in a variety of taxa without necessarily resorting to sampling survival experiments.

The next section of this thesis will be an overview of my experiments testing syrphids, along with a variety of hymenopterans and non-syrphid dipterans, in two experiments meant to quantify sampling survival and the physical properties of these organisms.

In my first experiment, the ability of individuals to survive sampling was titrated by testing live insects with a series of standard weights as model “predators”. This essentially measures insect’s ability to withstand a given amount of compressive force and still be able to survive, and is meant to simulate the types of forces insects may experience upon being sampled by a predator.

The second experiment sought to measure the physical properties of these same species of insects by compressing dead specimens in discrete steps and measuring their stress-strain responses. This gives a more detailed picture of how the structure of the insect body responds to the sorts of mechanical stresses they may encounter, and

allows us to determine what physical traits, if any, are most closely linked with the resistance to handling measured by the titration experiments.

2.2 Methodology

2.2.1 Field sites

Working within the limitations posed by the COVID-19 pandemic, field research was undertaken from May to October 2020 to collect a diverse selection of insects, with the assistance of Emily Moynes. The primary site of sampling was the Fletcher Wildlife Garden in Ottawa, as well as two other sites, namely Carleton University campus and Mont Rigaud, QC.

2.2.2 Data collection

Titration data collection

Specimens for the titration experiment were captured from July 7, 2020, to October 16, 2020. After capture via hand net, the live insects were placed into glassine envelopes (Crystal Clear Bags Canada), sized 59 mm x 92 mm (listed as 2 $^{5}/_{16}$" x 3 ⅝"). The date, location, and the specimen's ID number and tentative field identification were written on the outside of the envelope and the open end was folded twice to prevent escape. Insects were usually tested within minutes of capture. Over the course of the field season, 876 specimens were collected and tested in this manner, including Syrphidae (n=367), non-syrphid Diptera (n=219), and Hymenoptera (n=280).

The range of weights used in the titration experiment were chosen based on the bite forces of insectivorous birds. Generally, birds that primarily eat insects have lower bite forces (Lederer 1975); the weakest bite force measured by Corbin et al. (2015) was that of yellow-rumped warbler (*Setophaga coronata*) at 69 gf (0.68 N), while the red-bellied woodpecker (*Melanerpes carolinus*) had a bite force of 488 gf (4.8 N). The titration weights used (20 g, 50 g, 100 g, 200 g, 500 g) cover this range of bite forces exerted by the birds categorised as insectivorous by Gionfriddo & Best (1996), but it is worth noting that birds which specialize in other food sources are known to eat insects when available and can have a bite force far exceeding this range (e.g., the northern cardinal, *Cardinalis cardinalis*, has a maximum bite force exceeding 2000 gf, 22.91 N). Simulating these forces, rather than using live predators, allowed us to simplify the quantification of sampling survival by eliminating the variables introduced by the predator, such as variable beak or tooth structures, variable bite force used, predator cognition, sampling psychology, hunger level, individual personality, and other variables which can complicate the outcomes of sampling survival studies (Halpin *et al.* 2008; Winters *et al.* 2021).

The titration method involved the discrete increase of compressive force, using standard weights, until the insect was killed. Standard steel weights of 20 g, 50 g, 100 g, 200 g, and 500 g (Neewer® brand) were glued to wooden blocks (75x21x16 mm l-w-h), making each titration level functionally higher than the listed numbers, due to the weight of the wooden block, but by a relatively fixed amount of 10-17 g, depending on the specific block.

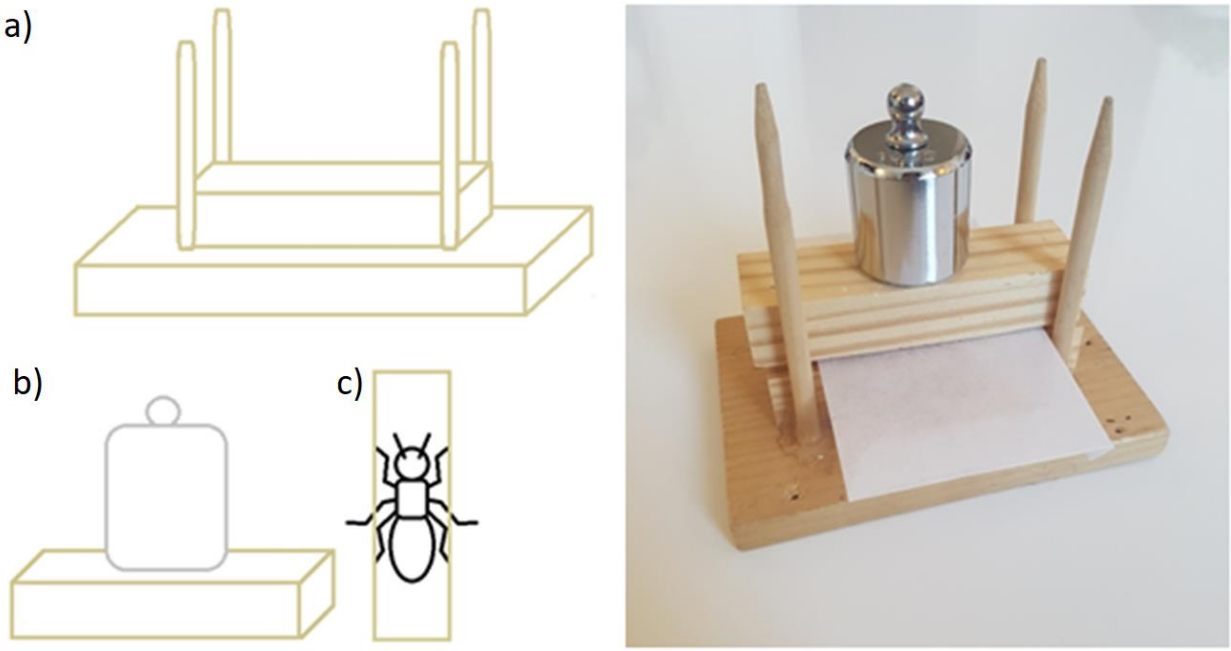

Figure 2.1: Diagram of the Titration compression stand (a), compression block with weight (b), and orientation of insect relative to compression block during titration (c). For each titration level, the weighted block was placed on the insect for 5 seconds, taking approximately 3 seconds to transfer the weight.

The live captured specimens were allowed a minimum of 5 minutes to habituate to the envelope before titration began in-field. The envelope containing the insect was placed on a wooden stand with its body oriented so that the block would be set on it lengthwise (Figure 2.1c). The blocks were carefully placed atop the specimen by hand, taking approximately three seconds to transfer the weight completely, and left for five seconds before being removed. The specimens were then placed in a recovery location for a minimum of five minutes before their state was evaluated and recorded.

The insect's state was categorized in one of four levels: Unharmed (no visible injury), Injured (limping or otherwise visibly damaged but still mobile), Twitching (very slight movements, such as the twitching of leg or antennae, but no other obvious movements), and Killed (no detectable movement). The five-minute wait served two

functions: to allow some recovery from a suspected “I” (Injured) status – which sometimes appeared to be the case immediately after “squishing” but didn’t always continue for the full five minutes – and to reduce the possibility of thanatosis confounding the results, where the insect “plays dead” for a short time after being handled (Ruxton *et al.* 2018). Titration only continued to the next level for insects categorized as “U” (Unharmed), since “I” (Injured) was defined as the insect being unlikely to be able to thrive and survive long enough to reproduce.

The titration started at 20 g and moved up through the five weights if the specimen continued to be unharmed. Some insects were unharmed even after the 500 g weight, and these specimens were classified as having a kill weight of N, where N > 500 g. All insects tested were transported to the laboratory and placed in a freezer for several hours before being photographed and pinned. Thorax diameter and body length were measured from these photographs using ImageJ software (Abràmoff *et al.* 2004), as the titration very rarely distorted the insects’ bodies significantly.

Micrometre data collection

Specimens for the micrometre experiment were captured from August 24th to October 14th 2020, using the same method and locations as the titration experiments, with the only difference being that the specimens were immediately killed in the field after capture via exposure to potassium cyanide for approximately 30 minutes prior to being placed in labelled glassine envelopes and transported to the lab. 167 specimens were tested for this analysis from the groups Syrphidae (n=78), non-syrphid Diptera (n=43), and Hymenoptera (n=46). Specimens were photographed and tested on the

same day as they were captured. Thorax diameter and body length of each insect was measured from their pre-compression photographs using ImageJ software (Abràmoff *et al.* 2004).

The experimental crushing apparatus consisted of a load cell (780 gm) attached to a metal stand upon which a barrel micrometre was held (Figure 2.2). To test each specimen, the glassine envelope containing the insect was clipped in place atop the force sensor using a fold-back binder clip, which was weighed down with a 200 g standard weight to prevent any shifting during measurement. The placement of the specimen within the envelope was adjusted so that the head of the specimen faced away from the clip and the thorax was directly under the micrometre, the barrel of which was then depressed until just slightly above the specimen to the nearest 0.01 inch (0.254mm) reading on the micrometre. Because of this, the first displacement step for each specimen could be anywhere between 0.254mm and 0.0mm.

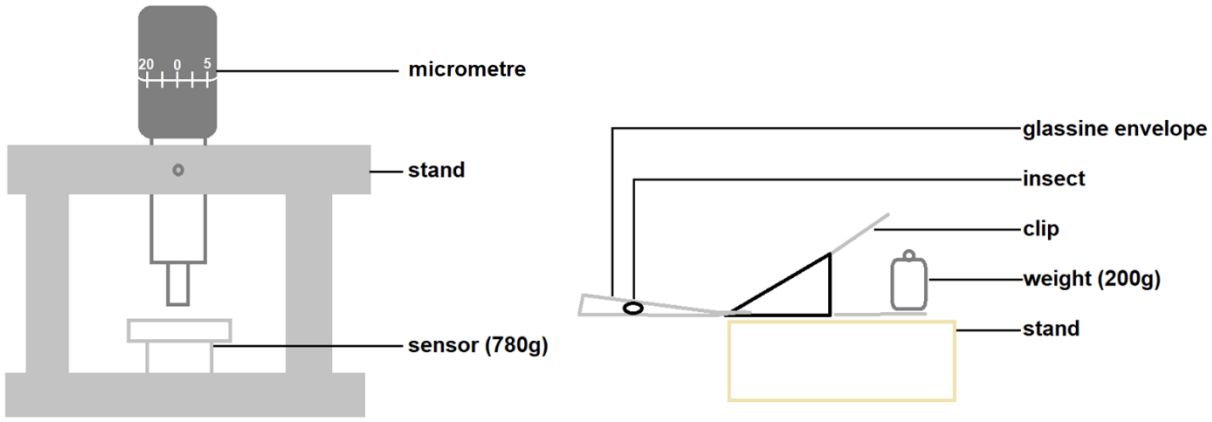

Figure 2.2: Micrometre setup; to take a measurement, the insect is placed between sensor and micrometre, held in place by the stand apparatus. The barrel of the micrometre was then lowered to within 0.254mm of the insect before the program “Squasher_v1” was run and data collection commenced; built by Prof. Jeff Dawson, Carleton University.

Data were collected using the MATLAB program “Squasher_v1” created by Prof. Jeff Dawson, Carleton University. The software was set to the “780 gm” load cell and the number of samples specified at 9000, which automatically set the sampling rate at ~60 samples per second. 10 seconds after data acquisition was initiated, the micrometre was depressed 0.254 mm (0.01 inches). 10 seconds were allowed to pass before the micrometre was depressed another 0.254 mm. This was continued until the sensor read 1000 g of force or until the specimen was completely crushed, whichever came first.

2.2.3 Data analysis

Within my sample there were three large groups of interest: flies in the family Syrphidae, non-syrphid dipterans, and hymenopterans; since these taxonomic ranks are not equivalent, they will be referred to simply as “groups”. Individual specimens were identified to the relevant taxonomic level for their group; flies in the family Syrphidae, being the primary taxon of interest, were identified to the species level (Skevington *et al.* 2019; McAlpine *et al.* 1981-1987), while non-syrphid dipterans were identified to family (McAlpine *et al.* 1981-1987) and hymenopterans to subfamily (Goulet & Huber 1993). These relevant taxonomic levels within the larger groups will be referred to as “subgroups” from this point on.

All specimens were pinned and labelled using standard methods (Gullan & Cranston 2014), and will be deposited in the Canadian National Collection of Insects as voucher specimens, with corresponding collection data available through the Dryad Digital Repository.

All statistical analyses were completed with R (version 4.0.5; R Core Team 2021) through RStudio (version. 2021.09.1-372; RStudio Team 2021). All code, as generated by Rmarkdown (Allaire *et al.* 2021), can be found in Appendices C and D.

Since the response variable for the titration experiment, kill weight, is an ordered factor in which the highest level, “N”, represents all values greater than 500 and is thus numerically undefined, kill weight was treated as an ordered categorical factor with levels 20, 50, 100, 200, 500, and N. These levels were assigned the ranks of 1, 2, 3, 4, 5, and 6, respectively, in order to calculate a median kill rank for each subgroup, as well as to use in certain statistical tests that required a numeric dependent variable (Appendix C.2).

The overall approach to the following statistical analysis, whenever kill weight was used as the response variable, was to complete the primary analysis using an ordinal logistic regression, and then to use a linear model to help interpret the results. Converting the ordinal variable into a numeric one leads to some of the information contained in the data being lost and can lead to misleading results, especially when the data being analysed include many individuals with a kill weight of “N”. However, the results of an ordinal logistic regression can be difficult to visualise and interpret on their own, and the linear models were able to further support the conclusions of the primary analyses.

The ordinal analyses had two main steps: first, several different ordinal logistic regressions were fitted to the data using the *polr* function from the MASS package (Venables & Ripley 2002), with each candidate model treating the predictor variables

slightly differently. For each predictor variable, a separate model was generated with that variable as the sole predictor of kill weight. Then the predictors were combined into an additive model, with each predictor affecting kill weight independently, and finally, into an interaction model, in which the effect of each variable is dependent on the value of the other(s). Second, these models were compared using the Akaike Information Criterion (AIC) to determine which best explained the data, and the best predictive model (lowest out of sample deviance) was used to interpret the data. Generating multiple models allows us to better determine how (or if) each variable affected the response variable, as well as whether or not one variable alone better explained the response than both together, and whether or not the interactions truly improved the fit of the model.

Titration data analysis

The topic of interest for the titration experiments is how aposematic species, mimetic species, and non-mimetic species differ in terms of resistance to handling. First, I predict that, given their aposematism, insects in the order Hymenoptera will have the highest resistance to handling, non-syrphid dipterans the lowest, and flies in the family Syrphidae will be intermediate between the two. I also predict that body length will be a significant predictor of resistance to handling, both overall and within groups, as has been found in previous studies (Schmidt & Blum 1977; Fisher & Dickman 1993a,b; Herrel *et al.* 2001; Aguirre *et al.* 2003; Evans & Sanson 2005; Freeman & Lemen 2007; Wang *et al.* 2018b). Finally, I predict that mimicry will be associated with a greater resistance to handling, with mimetic dipteran families being more resistant than non-

mimetic families, and higher-fidelity mimics being more resistant than lower-fidelity mimics.

Group and body size vs kill weight

An ordinal logistic regression was used to compare kill weight to body length and group, comparing hymenopterans, non-syrphid dipterans, and syrphids. The four candidate models fitted to the data were body length as the sole predictor for kill weight, group as the sole predictor, body length and group as additive predictors, and body length and group as interacting predictors. These four models were compared using AIC and the best-predictive model was then used to calculate the confidence interval and odds ratios, and predictions were generated and visualised (Appendix C.2 & C.3).

To evaluate the robustness of the conclusions of the ordinal logistic regression, a linear model, with numeric kill rank as the response variable and body length and group as interacting predictors, was also fitted to the data based on a simple ANCOVA (Appendix C.4).

In order to visualise the relationship between kill rank and body length, and how they differ between the three groups, mean kill rank and mean body length were calculated for each subgroup and plotted on a scatter plot, with linear models fitted to show the general trends within each of the three main groups. The linear models used mean body length as the predictor variable and mean kill rank as the response variable (section 2.3.1: Titration results, Figure 2.4; Appendix C.1).

Size and kill weight within species

In order to determine whether or not body length was associated with kill weight *within* species, an additional analysis was completed. Since many subgroups contained only one or two representative specimens, a new category was created for this analysis. Seventeen taxa were selected, including seven syrphid species, six hymenopteran genera, and four non-syrphid dipteran taxa. The hymenopterans and non-syrphid dipterans were identified to a higher level of classification for the purpose of this analysis. Although the taxonomic ranks of identification were quite varied, these "intra" groups were selected for their relative morphological homogeneity, as well as for their frequency, with all groups containing a minimum of 7 specimens. For a complete table of included intra groups, the number of specimens found within each, and their range of body lengths and kill ranks, see Appendix B, Table B.1.

After creating a subset of the data that included only these intra groups, a linear model was fitted using the lm function, with "intra" and body length as interacting predictors of kill rank, and run through an ANCOVA. Three additional subsets of these data were then created, each containing only one of the three groups (hymenopterans, syrphids, and non-syrphid dipterans). Linear interaction models were fitted and each run through an independent ANCOVA to determine which groups, if any, had a significant intraspecific effect of body length on kill rank (Appendix C.5). An ordinal regression was not completed due to small sample sizes, which led to probabilities of 1 or 0 being generated by the interaction model (i.e., certain "intra" groups *always* having the same predicted outcome for a given body length).

Mimicry status and resistance to handling.

In order to compare the resistance to handling of mimetic and non-mimetic dipterans, dipteran families were categorised by their mimicry status as either mimetic (Syrphidae, Bombyliidae, Conopidae, Asilidae, and Stratiomyidae), or non-mimetic (Muscidae, Sarcophagidae, Calliphoridae, Dolichopodidae, Anthomyiidae, Fannidae, Polleniidae). This categorisation was based on a subjective visual evaluation of the specimens collected for this study and may not reflect the mimicry status of the family as a whole. The family Tachinidae was excluded from this analysis due to being a highly diverse family containing both mimetic and non-mimetic species, while the families Tephritidae, Platystomatidae, and Heleomyzidae were excluded due to the ambiguity of their mimicry status. Non-mimetic dipterans were assumed to be palatable and non-aposematic.

The mean kill weight and body lengths of mimetic and non-mimetic dipteran families were plotted on a scatter plot to visualise the relationship (section 2.3.1: Titration results, Figure 2.6; Appendix C.6). A one-way ANOVA was fitted to determine whether or not the mean kill rank of mimetic families was different at the population level than the mean kill rank of non-mimetic families.

An ordinal logistic regression, with kill weight as the response variable, was fitted to the data using four candidate models: body length as the sole predictor for kill weight, mimetic status as the sole predictor, body length and mimetic status as independent additive predictors, and body length and mimetic status as interacting predictors. The

candidate models were compared, using the AIC test, and the confidence intervals and p-values generated (Appendix C.6).

Mimetic fidelity and resistance to handling

The estimates of mimetic fidelity for each syrphid species (or the subgroup to which they belong if species were not distinguishable) were drawn from Penney *et al*. (2014). Due to this, all specimens of a given species have identical mimetic fidelities. To avoid pseudo-replication, the data were analysed in terms of subgroup, with median kill ranks and average body lengths for each. In addition, several morphologically indistinguishable species of syrphid flies were combined together and their mimetic fidelities (MF) averaged. The resulting subgroups were *Eupeodes* AP (*E. americanus* and *E. pomus,* average MF=7.02), *Sphaerophoria* APA (*S. asymmetrica, S. philanthus,* and *S. abbreviata,* average MF=4.99), and *Syrphus* VR (*S. vitripennis* and *S. rectus,* average MF=6.72). A complete list of the syrphid subgroups used in this study, their mimetic fidelities, their median kill ranks, and their mean body lengths can be found in Appendix B, Table B.2.

In order to visualise the relationship between mimetic fidelity, body length, and kill rank, three different scatter plots were generated comparing median kill rank and mimetic fidelity, median kill rank and average body length, and mimetic fidelity and average body length (section 2.3.1: Titration results, Figure 2.7).

Median kill rank was converted into an ordered factor, and four different ordinal logistic regressions were fitted to the data: body length as the sole predictor for kill rank, mimetic fidelity as the sole predictor, body length and mimetic fidelity as independent

additive predictors, and body length and mimetic fidelity as interacting predictors. The optimal model was determined using AIC, and this model was used to calculate confidence intervals and p-values (Appendix C.7).

Phylogenetic control with MCMCglmm

Due to the phylogenetic relatedness of different taxa, data points from different species cannot be treated as independent, a key assumption of most statistical analyses (Symonds et al. 2014). There are various phylogenetic comparative methods that can be used to model and correct for this lack of independence via shared ancestry, commonly using quantitative genetics methods and a Brownian motion model of evolution, though only a few exist for ordinal data (eg. Baliga 2019).

Unfortunately, non-syrphid dipterans and hymenopterans could not be included in this method, as they were not identified to species and thus could not be placed on a phylogeny using genetic information. For this reason, and to improve focus, this method was only used for syrphids.

The mitochondrial DNA data used to generate the tree were obtained from Genbank™ and imported into R using the ape package version 5.5 (Paradis *et al*. 2019; GenBank™ Accession Numbers can be found in Appendix B, Table B.3). The phylogenetic data were extracted and matched with the experimental data using the picante package (Kembel *et al.* 2010) and aligned with the msa and seqinr packages (Bodenhofer *et al.* 2015; Charif & Lobry 2007; Appendix C.8).

A Markov chain Monte Carlo generalised linear mixed model (MCMCglmm) was fitted to the data, with the phylogenetic tree built into the model, using the MCMCglmm function from the MCMCglmm package (Hadfield 2010). The priors for the R-structure (residual variance) were V=1, nu=0.002, and fix=1, and for the G-structure (random effects) were V=1 and nu=0.002; these priors correspond with an inverse-gamma distribution and are recommended when dealing with an ordinal response variable (Hadfield 2021). The model was run for 600,000 iterations with a burn-in period of 100,000 and a thinning interval of 50 (Appendix C.9).

Micrometre data analysis

The prediction of the micrometre experiment is that insect subgroups which are more resistant to deformation will also be more resistant to handling, as determined by the kill weights measured in the titration experiment.

This portion of the analysis has three parts. First, the raw data, as collected by the Squasher_v1 software, are processed in order to extract the force values associated with each discrete step of deformation from the micrometre, matching and transforming the variables where needed. Second, the relationship between these force values and the proportion of the insect's thorax being displaced is analysed using a linear mixed-effect model with specimen ID number included as a random effect. This allows us to extract a different intercept for each individual specimen, which represents the magnitude of force needed to displace a given proportion of that insect's thorax. Finally, the mean "resistance magnitudes" are calculated for each subgroup and compared to

their median kill ranks, as determined in the titration experiment, in order to analyse the relationship between these two values.

Data processing

When a specimen is placed upon the force transducer and the Squasher_v1 software run, the raw data which are produced consist of a rapid sequence of force (gf) samples over time, with each depression of the micrometre appearing as a peak followed by a settling period (Figure 2.3a). The settling period is due to the viscoelastic body of the specimen deforming and relieving some of the force being applied to it

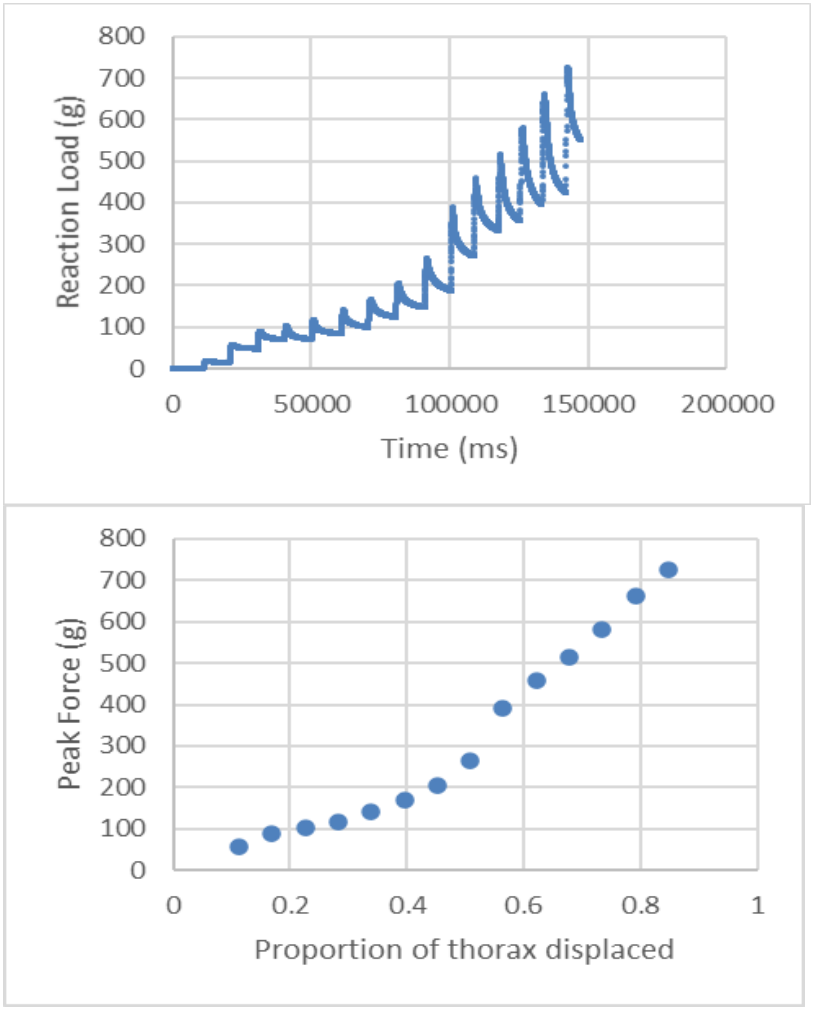

Figure 2.3: Relationship between stress and strain for AF95 (*Eristalis tenax* female) a) raw data — Force-time graph for AF95 (*Eristalis tenax* female); b) processed data — peak forces (g) per proportion of thorax displaced; thorax diameter=4.5mm

(Vincent 2012). The peak values were extracted from the raw data and paired with the displacement associated with each step (peak 1 with 0.254 mm, peak 2 with 0.508 mm, etc.); the displacement was then converted to the proportion of the total thorax diameter of the insect (mm displacement/ mm thorax diameter) and the first peak dropped due to the uncertainty introduced by the methodology (see Appendix D.1 for code). The resulting processed data for each specimen consist of a series of peak force (gf) values and the associated proportion of the thorax displaced (Figure 2.3b).

The displacement was expressed as a proportion in order to make comparisons between differently sized specimens possible, as well as more relevant to their biological context, as a displacement of 0.254mm is more likely to be injurious to a 1mm wide insect than a 5mm wide insect. Thorax compression proportions greater than 1.0 are due to the height added by the specimens' legs, the envelope, the flexing of the force transducer, and imprecise measurements.

Extracting resistance magnitude using a linear mixed-effects model

Next, a single value for resistance to deformation needed to be extracted from each individual specimen, rather than a series of peak forces over the course of the thorax being displaced. A visual evaluation of the data found the relationship between peak force (gf) and displacement to be logarithmic rather than linear, and so the response variable of peak force (gf) was log-transformed in order to meet the assumption of linearity (section 2.3.2: Micrometre results, Figure 2.8; Appendix D.2). A linear mixed-effects model was fitted to the data, with log(peak force (gf)) as the response variable, proportion of thorax displaced as the predictor variable, and

specimen ID as a random effect to remove the non-independence caused by each specimen contributing multiple data points (Appendix D.3). This allowed a separate intercept to be generated for each individual specimen using this model. The intercept represents the magnitude of force (gf) required to deform a given proportion of a given insect, with a higher intercept indicating a higher resistance to deformation (i.e., a stiffer and/or harder insect, though the point at which elastic deformation becomes plastic deformation is not known). From this point forward, this value will be referred to as "resistance magnitude".

The slopes generated by the linear mixed-effects model were not examined due to the viscoelastic nature of biological structures leading to a relationship between slope and body length. This relationship was confirmed using a linear regression of body length and slope for each specimen ($p=0.0192$; Appendix D.4); the slopes were extracted from a linear mixed-effects model in which the random effect of ID included the correlated intercepts and slopes for the fixed effect of displacement (disp|ID). The slopes of these lines essentially represent the amount of force needed to compress an insect over its entire diameter, and since insects, as with most biological materials, are viscoelastic, they display nonlinear elastic behaviour in response to compression (Vincent 2012). The stress-strain curves measured in this dataset generally showed increasing slopes as displacement approached 100% of the thorax diameter (section 2.3.2: Micrometre results, Figure 2.8), which occurred far more often with smaller specimens due to larger insects generally reaching 1000 g of force before approaching 100% compression. In addition, I did not record when (or if) structural collapse occurred, which also would have had an influence on the slope. For these reasons, this study is

not well-suited to measure the elastic moduli and non-Hookean behaviours of different insects in a meaningful way, and slope was excluded from the analyses.

Resistance magnitude and kill rank

The resistance magnitudes for each individual were extracted from the model and aggregated into an average resistance magnitude for each subgroup, which were then paired with their associated median kill ranks, as determined by the titration experiment (Appendix D.5). The relationship between resistance magnitude and median kill rank was then analysed using a generalised linear model, with resistance magnitude as the predictor variable and median kill rank as the response variable, and the data visualised with a scatter plot (Appendix D.5 & D.6).

An ordinal logistic regression, with kill rank treated as an ordered factor, was also completed in order to support the findings of the generalised linear model (Appendix D.7).

2.3 Results

2.3.1 Titration results

Group and kill weight

The scatter plot in Figure 2.4 shows that hymenopteran subgroups tended to have higher mean kill ranks than syrphid or non-syrphid dipteran subgroups, while syrphids and non-syrphid dipterans appear to be similar in this regard. While this graph may appear to show that the impact of body length on kill rank is less for

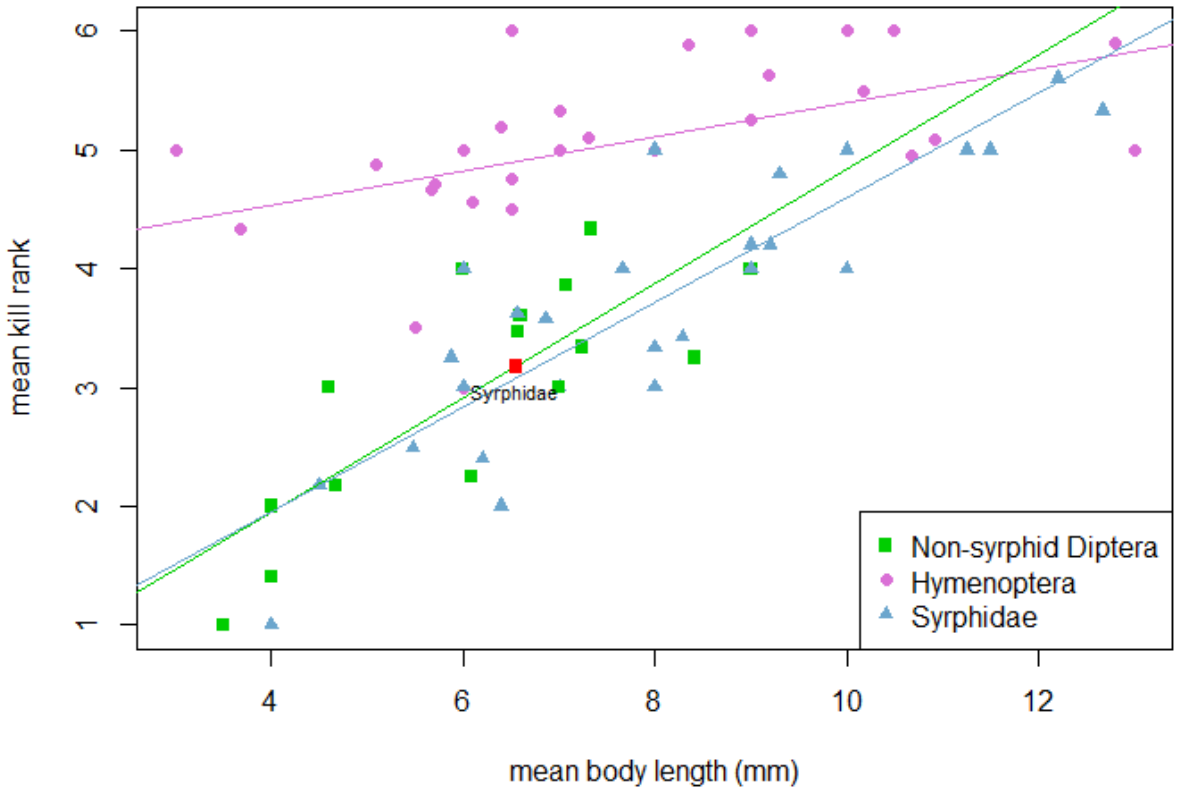

Figure 2.4: Relationships between the average kill rank and average body lengths of subgroups for each group; linear models for the relationship among hymenopterans (purple), non-syrphid dipterans (green), and syrphid flies (blue) are shown; red square represents Syrphidae as a subgroup within the non-syrphid dipteran group. Kill ranks represent kill weights of 20 g, 50 g, 100 g, 200 g, 500 g, and >500 g, sequentially.

hymenopterans, it is worth noting that the y-axis is non-linear. The summary outputs for these linear models can be found in Appendix C.1.

The results of the AIC comparing the different ordinal logistic regression models indicated that the interaction model was the best fit (Table 2.1). The results of this model found that body length and group were significant predictors of kill weight, with both predictors mediating one another (Table 2.2).

The odds ratio shows that, for every increase of 1mm to body length, we would expect the odds of having a higher kill weight to increase by 2.10 times, assuming group is constant. Meanwhile, compared to an insect in the group Diptera of the same size, an insect in the group Hymenoptera is ~492 times more likely to have a higher kill weight, while a syrphid is 4.16 times *less* likely to have a higher kill weight.

Figure 2.5 visualises this model's predicted values as probabilities, showing that, for all three groups, the probability of having a higher kill weight increases as body length increases. It also shows that hymenopterans are more likely to have a higher kill weight across all body lengths compared to the other two groups, while syrphids and

Table 2.1: AIC for different proportional odds logistic regression models predicting kill weight; bodyL = body length (mm); degrees of freedom are equal to the number of parameters estimated in the model.

Model	**Df**	**AIC**
bodyL * group	10	2013.41
bodyL + group	8	2058.15
group	7	2405.09
bodyL	6	2524.37

Table 2.2: Proportional odds logistic regression summary output with p-values, confidence intervals (CI), and odds ratio (OR). Non-syrphid Diptera is the reference group; bodyL = body length (mm).

	Value	**Std. Error**	**t value**	**CI 2.5%**	**CI 97.5%**	**OR**
bodyL	0.74	0.08	9.56	0.59	0.9	2.10
Hymenoptera	6.20	0.68	9.07	4.87	7.55	491.95
Syrphidae	-1.43	0.62	-2.30	-2.64	-0.21	0.24
bodyL: Hymenoptera	-0.33	0.09	-3.56	-0.51	-0.15	0.72
bodyL: Syrphidae	0.21	0.09	2.28	0.03	0.39	1.23

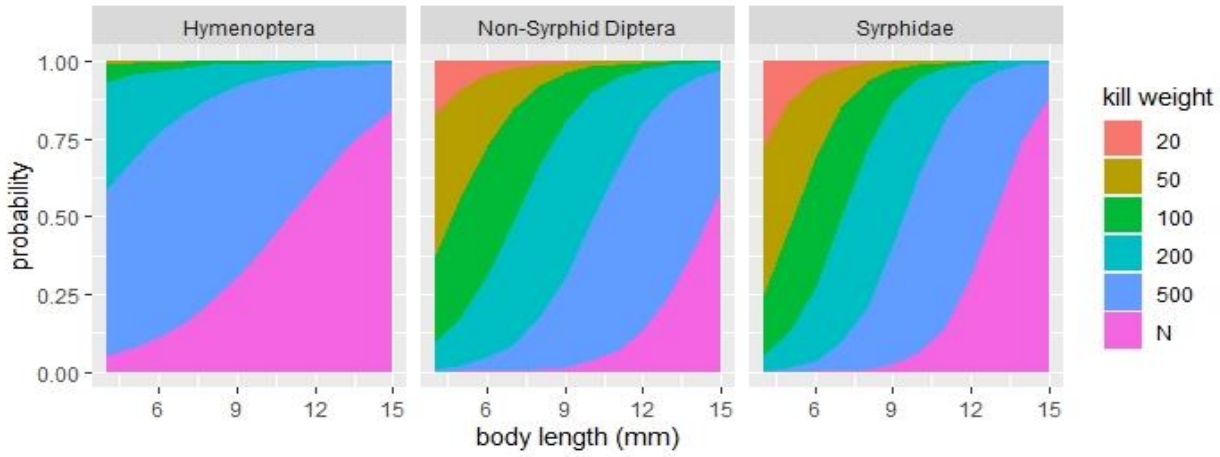

Figure 2.5: Predicted probabilities of kill weight per mm body length (+/- 0.5 mm) for each group, as generated by the ordinal logistic regression; see Appendix C.3 for code.

non-syrphid Diptera appear to be more similar to one another, although the ordinal logistic regression found the two groups to have a difference which was statistically significant once body length was taken into account (CI 2.5% = 0.03, CI 95% = 0.39; see Appendix C.2 for code).

These findings are further reinforced by the results of the linear model. The ANCOVA performed on the linear interaction model found that both group and body length were significant predictors of kill rank, including when the two predictors were interacting (Table 2.3). The residuals of this model were normally distributed and homogenous in variance (Appendix C.4).

Table 2.3: ANCOVA output of the linear model for group and body length (aov(killrank~bodyL*group)); bodyL = body length (mm)

	Df	**Sum Sq**	**Mean Sq**	**F value**	**p-value**
group	2	623.47	311.736	458.906	<2e-16
bodyL	1	263.25	263.253	387.534	<2e-16
group:bodyL	2	78.67	39.334	57.904	<2e-16
Residuals	851	578.09	0.679		

Size and kill weight within species

Examining whether or not body length was a significant predictor of kill weight *within* intra groups, the ANCOVA of the linear interaction model showed that, when all groups were considered, the effect of body length on kill rank was significant within intra groups (p=0.03). However, while this held true for species in the family Syrphidae (p=0.01), this interaction was non-significant for both hymenopterans and non-syrphid dipterans (p=0.27 and p=0.29, respectively; see Appendix C.5 for results summary).

Table 2.4: ANCOVA output for four different linear interaction models, one including all groups (non-syrphid Diptera, Syrphidae, and Hymenoptera), and separate models for each group, respectively.

	Df	Sum Sq	Mean Sq	F value	p-value
All Groups					
bodyL	1	241.66	241.66	478.67	<2e-16
intra	16	287.97	18	35.65	<2e-16
bodyL:intra	16	14.48	0.91	1.79	0.03
Residuals	467	235.77	0.5		
Non-syrphid Diptera-only					
bodyL	1	8.51	8.51	14.26	0.0003
intra	3	12.92	4.31	7.21	0.0002
bodyL:intra	3	2.26	0.76	1.27	0.29
Residuals	80	47.76	0.6		
Syrphidae-only					
bodyL	1	150.52	150.52	316.38	<2e-16
intra	6	48.07	8.01	16.84	<2e-16
bodyL:intra	6	8.12	1.35	2.85	0.011
Residuals	268	127.5	0.48		
Hymenoptera-only					
bodyL	1	16.41	16.41	32.09	9.4e-08
intra	5	13.09	2.62	5.12	0.0003
bodyL:intra	5	3.3	0.66	1.29	0.27
Residuals	127	64.95	0.51		

Mimicry status and resistance to handling.

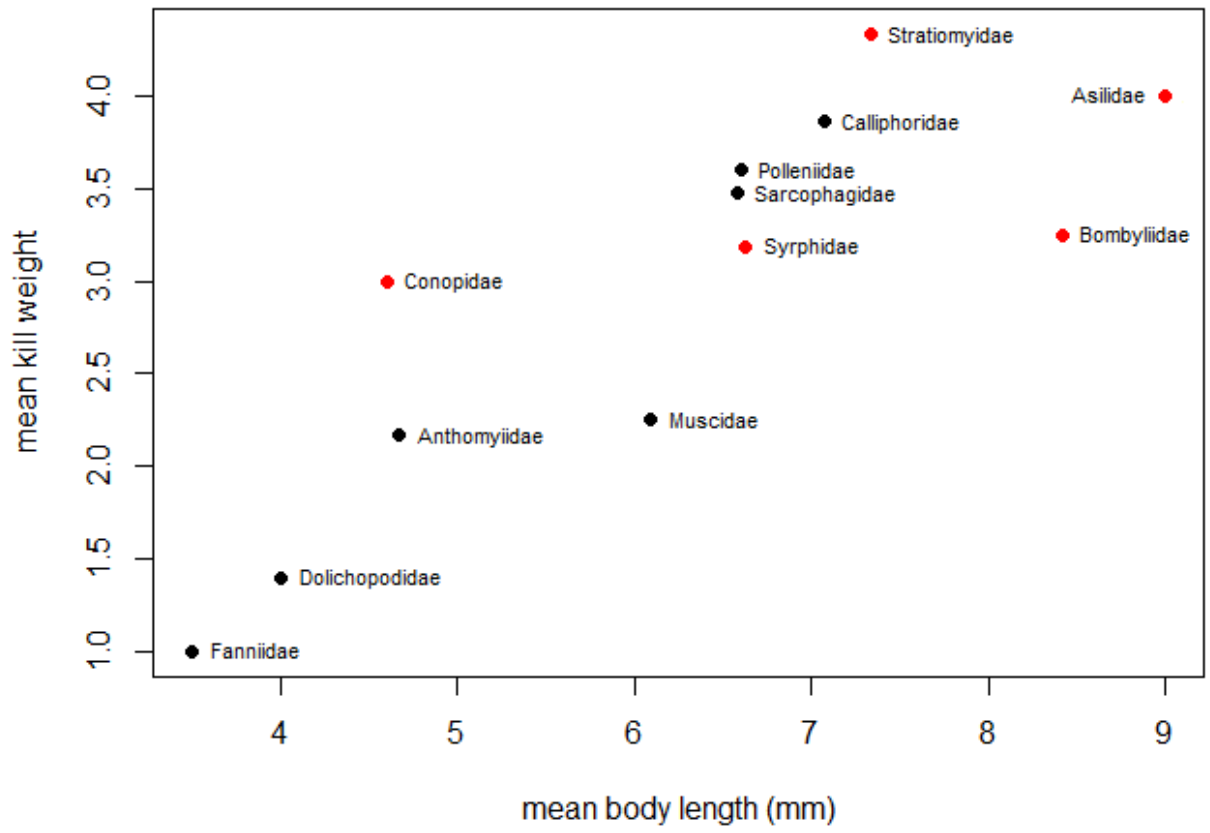

Figure 2.6: Scatter plot of mean kill rank vs mean body length (mm) of Diptera families; mimetic families (red circles) and non-mimetic families (black circles).

The one-way ANOVA did not find a significant difference between the mean kill weights of mimetic and non-mimetic dipteran families (df=1; p=0.84).

Of the candidate ordinal logistic regression models, the AIC found that the model with body length as the sole predictor was the best fit to the data (AIC=1333.57), although it was very similar to the AIC for the additive model (AIC=1333.86; Table 2.5).

The ordinal logistic regression, performed using the additive model, confirmed the finding that we cannot reject the null hypothesis that mimetic status has no effect on kill weight, once body length is taken into account (Table 2.6, Figure 2.6, Appendix C.6)

Table 2.5: Mimetic status and kill weight; AIC for different proportional odds logistic regression models predicting kill weight; bodyL = body length (mm), MimDip = mimetic status.

Model	**Df**	**AIC**
bodyL	6	1333.57
bodyL + MimDip	7	1333.86
bodyL * MimDip	8	1335.77
MimDip	6	1656.00

Table 2.6: Mimetic status and kill weight; proportional odds logistic regression summary output with p-values, confidence intervals (CI), and odds ratio (OR); non-mimetic is the reference group.

	Value	**Std. Error**	**t value**	**CI 2.5%**	**CI 97.5%**	**P-value**
bodyL	0.97	0.06	15.49	0.85	1.09	<0.0001
MimdipY	-0.24	0.18	-1.31	-0.6	0.12	0.190

Mimetic fidelity and resistance to handling

The scatter plots (Figure 2.7) appear to show a general trend of mimetic fidelity being related to mean kill rank; however, mean body length is a potentially confounding variable, as it also appears to be related to both mean kill rank *and* mimetic fidelity.

The optimal ordinal logistic regression model, as determined by the AIC test, indicated the additive model, in which body length and mimetic fidelity both influence the reaction variable independently, was the best fit to the data (Table 2.7). This model

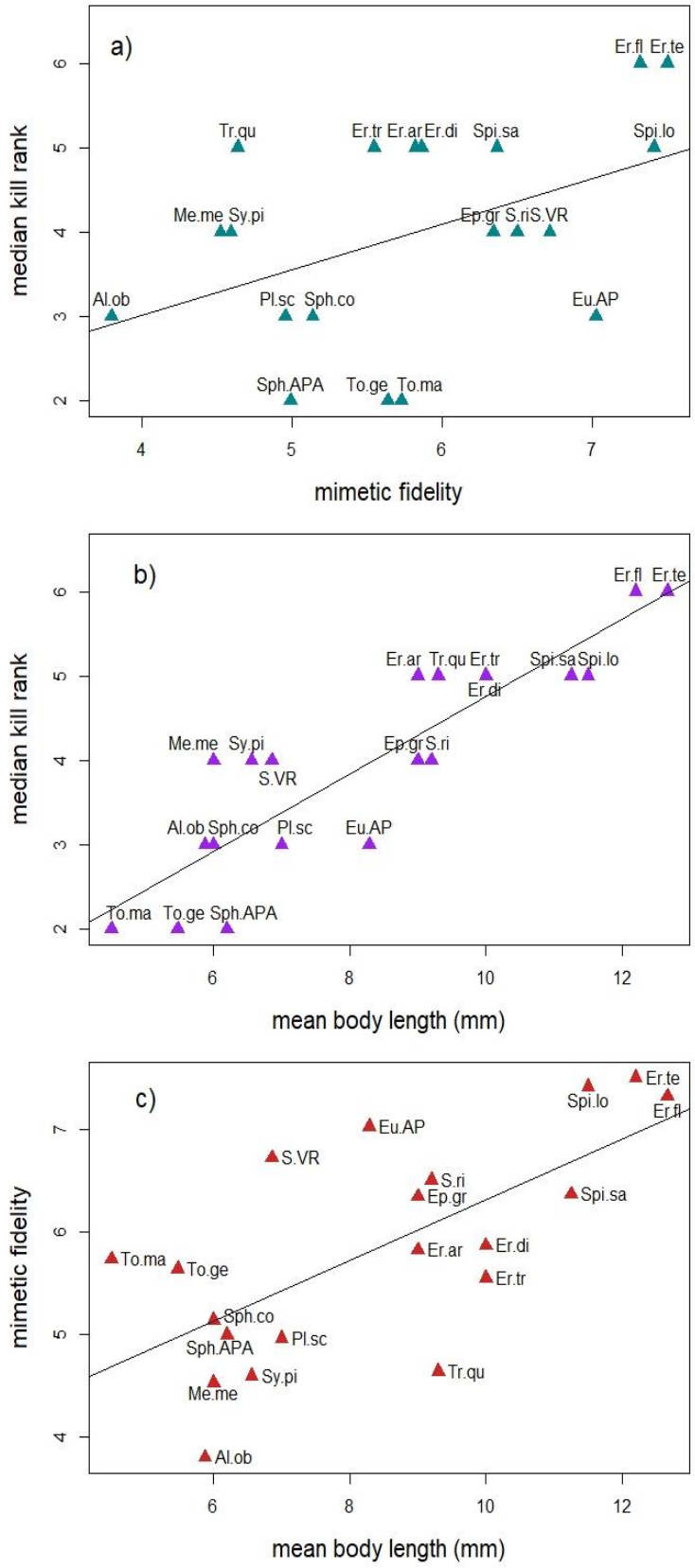

Figure 2.7: The relationships between median kill rank, average body length, and mimetic fidelities among syrphid species; lines are a general linear model of each relationship. a) scatter plot of median kill rank vs mimetic fidelity; b) scatter plot of median kill rank vs mean body length (mm); c) scatter plot of mimetic fidelity vs mean body length (mm). Abbreviations: *Al.ob = Allograpta obliqua, Ep.gr = Epistrophe grossulariae, Er.ar = Eristalis arbustorum, Er.di = E. dimidiata, Er.fl = E. flavipes, Er.te = E. tenax, Er.tr = E. transversa, Eu.AP = Eupeodes AP, Me.me = Melanostoma mellinum, Pl.sc = Platycheirus scutatus, Sph.APA = Sphaerophoria APA, Sph.co = S. contigua, Spi.lo = Spilomyia longicornis, Spi.sa = S. sayii, Sy.pi = Syritta pipiens, S.ri = Syrphus ribesii, S.VR = S. VR, To.ge = Toxomerus geminatus, To.ma = T. marginatus, Tr.qu = Tropidia quadrata*

found that, although species with higher mimetic fidelities tended to have higher kill ranks (Figure 2.7a), once body length was taken into account, the relationship between mimetic fidelity and kill rank was non-significant (p-value = 0.09, CI 2.5% = -3.34, CI 95% = 0.03; Table 2.8).

Table 2.7: AIC for different proportional odds logistic regression models predicting kill weight; bodyL = body length (mm), mimfid = mimetic fidelity.

Model	Df	AIC
bodyL + mimfid	6	39.18
bodyL	5	40.85
bodyL * mimfid	7	40.87
mimfid	5	67.28

Table 2.8: Mimetic fidelity and body length; proportional odds logistic regression summary output with confidence intervals (CI) and p-value; bodyL = body length (mm), mimfid = mimetic fidelity.

	Value	Std. Error	t value	CI 2.5%	CI 97.5%	p-value
bodyL	2.59	0.83	3.14	1.33	4.71	0.002
mimfid	-1.41	0.83	-1.71	-3.34	0.03	0.09

Phylogenetic control with MCMCglmm

The phylogenetic MCMCglmm analysis returned similar results as the ordinal logistic regression, with mimetic fidelity being non-significant as a predictor of kill rank once body length was considered (p-value = 0.76, CI 2.5% = -6.62, CI 95% = 4.59; Table 2.9). See Appendix C.9 for the complete summary table and trace plots.

Table 2.9: Markov Chain Monte Carlo generalised linear mixed model summary output

	Posterior mean	CI 2.5%	CI 95%	Effective sample	pMCMC
Intercept	-38.25	-146.67	50.27	74.72	0.38
Body length	15.9	5.67	31.33	9.2	0.0002
Mimetic Fidelity	-0.77	-6.62	4.59	141.81	0.76

2.3.2 Micrometre results

Extracting resistance magnitude from a linear mixed-effects model

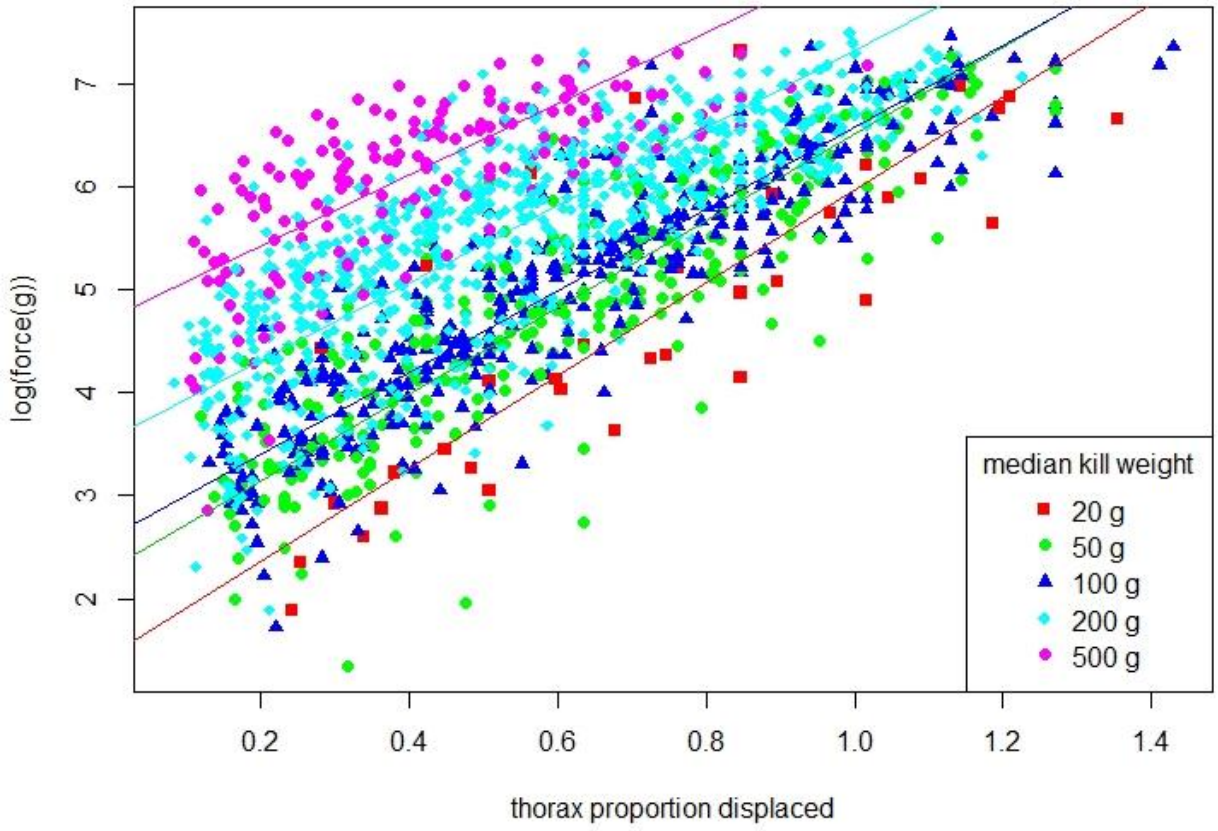

Figure 2.8: Relationship between log(peak force(g)) and thorax proportion displacement for all specimens over the course of compression. Colours indicate median kill rank for the subgroup to which each specimen belongs. Lines represent a linear mixed-effects model, interaction with median kill rank and (1|subgroup/ID) as a random effect. Intercepts for each kill weight are: 20 g = 1.46, 50 g = 2.30, 100 g = 2.57, 200 g = 3.56, 500 g = 4.74 (see Appendix D.8 for code).

The relationship between the force (log(gf)) and thorax proportion displaced for all specimens over the course of the compression test is visualised in Figure 2.8, with different linear models shown for each median kill weight. Increasing kill weight appears to be associated with an increased magnitude of force required to displace the thorax by a given amount.

The results of the linear mixed-effects model, using ID as a random effect, can be seen in Table 2.10; the overall intercept for the model is 3.15 (CI 2.5% = 3.01, CI 95% = 3.29). The standard deviation of the random effect (individual ID) was 0.89 (Variance = 0.80). Summary of data can be found in Appendix D.3.

Table 2.10: log(force) per proportion of thorax displacement. Linear mixed-effects model data summary with specimen ID as a random effect.

	Estimate	**Std. Error**	**CI 2.5%**	**CI 97.5%**
Intercept (log(force(gf)))	3.15	0.072	3.01	3.29
Displacement	3.92	0.030	3.86	3.98

Resistance magnitude and kill rank

The generalised linear model found median kill rank and average resistance magnitude to have a statistically significant relationship (Table 2.11; Appendix D.6 for complete summary output). This means that subgroups that required more force on average to displace a given proportion of their thorax also tended to have higher kill ranks in the titration experiment. These results were confirmed with an ordinal logistic regression, which found mean resistance magnitude to be a significant predictor of median kill rank (p=0.0005; CI 2.5% = 3.16, CI 95% = 9.68; Appendix D.7).

Table 2.11: Micrometre generalised linear model data summary. Predictor variable is mean resistance magnitude, response variable median kill rank.

	Estimate	Std. Error	CI 2.5%	CI 97.5%	p-value
Intercept	1.01	0.43	0.18	1.85	0.025
Mean resistance magnitude	1.07	0.13	0.82	1.32	5.0e-09

2.4 Discussion

Limitations of the research

There were several constraints to this research imposed by the COVID-19 pandemic and the nature of fieldwork. Particularly for the titration experiments, the methodology was unavoidably inexact, and we cannot be certain that certain variables, such as the loading speed of the weights, were kept constant between specimens and not influenced by the unconscious biases that can be introduced by measurements taken by unblinded experimenters. In addition, a certain amount of shear stress was likely introduced in titration by the occasional “tipping” of the blocks forward and backwards; while this was reduced as much as possible, examining the squashed specimens often displayed the tipped-house shape characteristic of shear stress damage. Ideally, future research would find a way to standardise and automate the application of force to avoid these issues.

Additionally, it should be noted that the mimetic status classifications assigned to different dipteran families was based on a subjective visual evaluation of the specimens collected and may be considered debateable for the families Asilidae and Bombyliidae in particular.

Group and kill weight

As predicted, these results show that hymenopterans were the most resistant to handling of the three groups sampled. They had significantly higher kill weights at all body lengths compared to the other groups, while syrphid flies and non-syrphid dipterans were more similar to one another. However, syrphid flies were *less* resistant than non-syrphid dipterans in a statistically significant way, once body size was accounted for.

The proposed reason for why hymenopterans are more resistant to handling than dipterans is that they are aposematic, and that aposematic species are more resilient against physical forces than their non-mimetic, palatable counterparts. However, while the findings of this study appear to be in line with this hypothesis, it cannot be said to be supported or unsupported for several reasons. First, having a sample of “unpalatable” insects coming only from Hymenoptera while the sample of “palatable” insects comes only from Diptera ignores several important variables that cannot be dis-entangled. We cannot know if the differences in resistance to handling are truly due to aposematism rather than differences in phylogeny, life-history, and other factors; a comparison between more closely related taxa would be more appropriate for that purpose. In addition, not all hymenopterans tested were chemically defended with stingers, and many would not be considered to be truly aposematic. A comparison between stinging and non-stinging hymenopterans, with their defences quantified in terms of costliness for a predator, may be beneficial to further elucidate this relationship *within* the order of Hymenoptera.

Body length and kill weight

Of all the variables analysed in this study, the strongest predictor of kill weight was body length; it was found that, in all groups tested, larger insects had a significantly greater resistance to handling, a relationship which is supported by the results of numerous studies (Schmidt & Blum 1977; Fisher & Dickman 1993a,b; Herrel *et al.* 2001; Aguirre *et al.* 2003; Evans & Sanson 2005; Freeman & Lemen 2007; Wang *et al.* 2018b). This relationship may exist due to the biomechanical needs of having a larger body (see section 1.2.6: Variables and trade-offs in resistance to handling), or perhaps as an anti-predator defence, because larger insects may be attractive to predators as a meal of greater caloric value, as well as potentially being easier to detect and capture compared to smaller insects (Hossie *et al*. 2015). These results reinforce the importance of taking body size into consideration when completing studies on resistance to handling, which may be especially true for studies involving mimicry and mimetic fidelity, as mimetic fidelity has been shown to be correlated with body size within Syrphidae (Penney *et al.* 2012).

Surprisingly, the relationship between body length and kill weight *within* species was not always significant. Although this relationship was significant overall, as well as for species in the family Syrphidae, it was found to be non-significant for non-syrphid dipterans and hymenopterans. This may be due to different selective pressures experienced by these groups (e.g., some groups being influenced more by biomechanical constraints and others by predation pressure) but may also have been influenced by the small sample sizes and loose taxonomic classifications in the study.

Mimicry and kill weight

If a greater resistance to handling co-evolves with Batesian mimicry, rather than with other traits, then we may expect mimics to be of intermediate resistance between aposematic models and non-mimetic species. However, this was not found to be the case for the species evaluated in this study; flies in the family Syrphidae were found to have lower resistance compared to non-syrphid dipterans, and mimetic fidelity and mimicry status were found to be non-significant as predictors of kill weight, once body length was taken into consideration. This suggests that, for dipterans mimicking hymenopterans, resistance to handling may not be a trait which is strongly selected for.

There are several possible explanations for why mimicry was not a significant predictor of kill weight, including the hypothesis, proposed by DeVries (2003), that resistance to handling is correlated with unpalatability. Since Batesian mimics are not unpalatable, they are less likely to be rejected on the basis of taste, potentially making the benefit of a more resistant integument limited. As was said by Rothschild (1971), “unlike the model, with its tough cuticle and other disagreeable qualities, the mimic cannot afford to be examined at close quarters.”

However, the selective pressures which determine resistance to handling are quite complex, and can include many selective forces unrelated to anti-predator defences. For example, diet may be a predictor for resistance to handling in syrphids. *Intra*specific variation in diet has been shown to affect the cuticle properties of certain insects (Tsao & Richards 1952; Hopkins 1992), and while all adult syrphids are nectar and pollen specialists, as larvae they are known to have many diverse life-history strategies, including aphid predators, detritivores, herbivores, fungivores, tree borers,

and even parasitoids of hymenopteran hosts (Rupp 1989; Thompson & Rotheray 1998; Dziock 2006; Skevington *et al.* 2019). Additionally, several species are known to pupate underground, which would provide very strong selection for an abrasion-resistant cuticle to facilitate emergence from the substrate (Sun *et al.* 2008). A comprehensive comparison between these differences within Syrphidae, and their relation to resistance to handling, may be a worthwhile avenue of future research.

Trade-offs: flight ability and cuticle resistance

One noteworthy reason to forgo a greater resistance to handling is also the reason why syrphid flies may rarely require it: they are excellent fliers (Gilbert 2004; Skevington *et al*. 2019). Although syrphids are vulnerable when feeding on flowers, when compared to hymenopterans, they are quicker to flee upon approach and nearly impossible to track visually through the air as they escape (Gilbert 2004; personal observations). With species so adept at escape, one must wonder: how often would a syrphid even *need* to make use of a greater resistance to handling?

We must also consider the fact that there are certain trade-offs between mobility and cuticle properties in flying insects (see section 1.3: Variables and trade-offs in resistance to handling). Thicker cuticle, which is more resistant to deformation (Evans & Sanson 2005), is also heavier and may represent a major cost when considering the energetics of flight. In addition, the changes to the other properties of the cuticle may result in a loss of efficiency, particularly among dipterans, as the cuticles of their thoraxes are known to “flex” and contribute mechanically to flight (Ennos 1987; Vincent & Wegst 2004). This suggests that an investment in quick, agile flight may come at the expense of some resistance to handling, while simultaneously reducing the need for

post-capture defences at all, if it facilitates escape from the majority of attacks (Brodie *et al*. 1991).

In addition to a speedy getaway, it is also possible that fleeing behaviour may enhance, or act in concert with, mimicry in syrphid flies. It has been shown that perceived mimetic fidelity increases when the mimic is in motion (Chittka & Osorio 2007; Pekár 2021), and since the black-and-yellow stripes of hymenopterans have been shown to be a highly salient signal to predators (Schuler & Hesse 1985), even an imperfect mimic may cause predators to delay their attack long enough for the syrphid to escape. There are a few possible mechanisms which may cause predators to hesitate, including in order to collect more information to better make a decision about ambiguous or uncertain stimuli (Mappes *et al.* 2005; Abbott & Sherratt 2013; Leavell & Bernal 2019), general neophobia (Marples & Kelly 1999), or “go-slow” predation, in which potentially costly prey are examined longer and sampled slower than familiar prey out of caution (Guilford 1994; Holen & Svennungsen 2012). Even a small delay in attack may give speedy syrphids ample time to escape, leaving behind the flower and the less-wary hymenopterans, potentially to be sampled and further reinforce the predators’ learned association between black and yellow stripes and unpalatability (Gilbert 2004).

Regardless of the mechanism, if mimicry and fleeing behaviours interact synergistically as defences, then we would not necessarily expect selection for greater resistance to handling in high-fidelity mimics, and may even expect a certain amount of selection *against* such a trait. Further research on the flight and fleeing behaviours of Batesian mimics may help to round out our knowledge on this topic, including predator reactions to different syrphid species in live or dynamic contexts compared to

hymenopterans and other dipterans, as well as approach behaviours and success rates of predators attacking nectar-feeding insects in the field.

Resistance magnitude and kill rank

In the micrometre experiments, a statistically significant relationship was found between the subgroups' mean resistance magnitude and the median kill ranks, as determined by the titration experiments. In other words, subgroups that were generally able to survive higher titration levels also required a greater amount of force to deform their thorax by a given proportion. While the survival of an organism after a sampling event is likely a complex interplay of many physical characteristics, these results show that the dipterans and hymenopterans which are more resistant to deformation, or "stiffer", are also more resistant to fatal damage from the compressive forces of a simulated predator, and likely to be better able to survive sampling events from predators in the field. There may be several reasons why stiffness, rather than elasticity, is associated with this trait, but one possibility is that certain structures which are integral to the survival of the insect, such as the trachea used in gas exchange, may be biomechanically constrained by their function and thus unable to withstand deformation without permanent damage.

While stating that "harder" insects are more difficult to crush may seem to be an intuitive statement, it has been suggested in the past that the *elasticity* of an animal's body was the most important factor determining survival, with some specimens able to withstand a significant amount of deformation before dying, especially when compared to "harder", more "brittle" specimens (Trimen 1868; Trimen & Bowker 1887; Fisher 1930, p. 196; Poulton 1908, p. 316). This study provides some direct evidence that an

insect's resistance to handling is related to its ability to withstand compressive forces without deforming.

There is a fair amount of information still contained within the raw data collected from the micrometre experiments which has the potential to be further explored. For example, the amount of "settling" between each compressive step, in which the viscoelastic specimens deformed and distributed the compressive stress to relieve the force without damage, was not quantified in this analysis, and could be used to examine the relative viscoelastic properties of different taxa. However, further analyses such as these are outside the scope of this thesis and are better suited for future projects.

2.4.1 Conclusion

This study is the first to attempt to systematically examine resistance to handling in Canadian Hymenoptera and Diptera species, including measuring the physical properties associated with different survival rates. The results indicate that body length was a significant predictor of kill weight, with large insect species being more resistant than smaller species. In addition, within the family Syrphidae, mimetic fidelity was *not* a significant predictor of kill weight, once size was taken into consideration. Finally, insect taxa which took more force on average to deform by a given proportion also had higher median kill weights in the titration experiments.

While we have yet to evaluate the vast majority of model-mimic complexes in the context of resistance to handling, this thesis is an important addition to the existing body of research, expanding the current pool of data to include a valuable handful of additional species. In addition to reinforcing the importance of accounting for size when

researching the resistance to handling of different insects, this study also opens further avenues of inquiry in regard to how different factors and ecosystem dynamics interact to produce the diversity of resistance we observe. Finally, the relationship between resistance magnitude and sampling survival presents a potential metric with which to measure an organism's resistance to handling indirectly. Sampling survival experiments are often complicated and can have several ethical concerns, but if resistance magnitude is a reliable predictor of resistance to handling, then this may be an alternative method which avoids such difficulties.

As we move forward in this field, studying and collecting data on more diverse taxa, patterns and relationships are sure to become apparent, helping to enhance our knowledge of how resistance to handling evolves and persists in the context of other ecological factors and selective pressures.

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Appendices

Appendix A

Table A.1: Mentions of the connection between aposematism and resistance to handling in the literature.

Citation	Quotations and summary of experiments.
Trimen 1868	"…*species of Danais and Acraea…[posses a] remarkable elasticity of their entire structure*" (pg. 498) "…*however bent and distorted the wings may become… I have never known a fracture of nervures or membrane to result, the organs resuming their natural position even after having been bent double for some hours.*" (pg. 499) "*That birds…may occasionally capture a Butterfly of these malodorous tribes before discovering its distasteful character is not an unreasonable supposition…in such a case… the chances are very greatly in favour of a Danais or an Acraea escaping, if not wholly unhurt, yet without serious injury, after rough treatment that would have proved fatal to a harder but less elastic animal*" (pg. 499)
Trimen & Bowker 1887	"*The Araeinae are extremely tenacious of life, and their structure is so elastic that no pressure of the thorax, short of absolute crushing of the tissue, suffices to kill, or even paralyse them*." (pg. 295) "*Colonel Bowker…, wrote: 'It is quite impossible to distinguish the difference between this butterfly and Aganice… the first notice you get is the brittle crunch between finger and thumb of Imitator, or the soft*

	leathery feel of Aganice. Death is, moreover, instantaneous with the former, while you may squeeze Aganice as long and as hard as you like without effect; nothing but the poison-bottle will settle him!'" (pg. 295)
Haase 1896	"*'They are so tenacious of life as to be able to bear considerable pressure between the finger and thumb without being killed. Birds and other insectivorous animals do not appear to be partial to these butterflies as food; they are probably unpalatable to them owing to their possessing a peculiar odor*.'" (pg. 21) "…*remark on the great tenacity of life and conclude 'that any individual which might be accidentally seized and afterwards dropped by a bird, has a good chance of escaping with impunity, when more delicately formed insects would be killed or hopelessly maimed*.'" (pg. 21; quote from Marshall & Nicéville 1882-86) "*The tenacity of life of the Danaidae is extraordinary. They are still able to fly off even when the thorax has been badly crushed*"(pg. 23) "*Chalcosia papilionaris… emitted an extremely unpleasant odor when the thorax was pressed. The pinned insect was more tenacious of life than any other butterfly with which I am acquainted*." (pg. 37; n.b., likely a synonym of *Cyclosia papilionaris*, which is both aposematic AND a Mullerian mimic of hymenopterans) [Regarding *Battus philenor,* an aposematic species, called *Papilio philenor*]: "*As regards its tenacity of life…a specimen which had lain for half an hour in the potassium glass, and was then pinned out, still*

	lived three days. Moreover, an imago whose thorax I had completely crushed by continued pressure I saw fly about again after a short time." (pg. 47) "*In general the tenacity of life is much less among the edible forms than among the immune forms*" (pg. 101) Mentioned in passing: pg. 3, 12, 22.
Poulton 1908	"*Great tenacity of life is usually possessed by animals with Warning Colours. The tissues of insects with an Aposematic appearance often possess great elasticity, toughness, and power of resistance, so that large numbers of individuals can recover after very severe treatment*." (pg. 316)
Carpenter 1929	"*A typically aposematic insect… is of an extremely tough physique. It will be uninjured by treatment which would break the wings of another butterfly such as the Nymphaline or Papilionine mimic… This resistance to injury is part and parcel of the process whereby an aposematic insect teaches an enemy that it is harmful or unpalatable. It almost invites attack, and if it is seized and handled, suffers little injury, and when released after a pinch or a lick is often undamaged*." (pg. 662)
Carpenter 1938	"*The fact that these aposematic butterflies were caught and released many times, apparently without suffering any vital damage, is in accordance with the theory which demands that such insects should invite, but be enabled to resist, experimental tasting*." (pg. 101)

Carpenter & Eltringham 1938	"*The toughness of this insect is an example of the general occurrence of unusual toughness and resistance to injury among conspicuous and sluggish insects which by their bright colours attract attention and invite examination. When the enemy has discovered their unpalatability and releases them they may still be little damaged*." (pg. 247) *"…Acridid grasshoppers in Africa; observations and experiments with young monkeys show their distastefulness…There is evidence for some of them that they are unusually tough*." (pg. 249-250)
Cott 1940	"*Another characteristic shared by [aposematic] animals…* *is their savage disposition when attacked, their toughness, and their remarkable tenacity of life when injured*." (pg. 245) "*intimately associated with the possession of a warning apparatus, is the possession of a tough physique….conspicuous appearance does, in a sense, actually invite attack by those enemies who have not learned, or are unable to learn, by experience to avoid them at sight. … 'toughness', when associated with a special means of defence, will greatly increase the chances of escape or survival of experimental attack*." (pg. 259)

Carpenter 1941	Beak mark study. Compares the beak mark frequency on museum specimen aposematic butterflies (*Euplaea* and African Danainae) to palatable butterflies (African *Colotis*); beak mark frequency higher in unpalatable species. Models had more beak marks than mimics. "*These facts are interpreted as evidence of the greater destruction of species not furnished with aposematic characters, which, when attacked, do not escape like the tougher, more distasteful species*." (pg. 226)
Blest 1963	"*It is a familiar fact… that aposematic insects are commonly tough and heavily sclerotized in comparison with non-aposematic forms, and it is usually argued that aposematic species have to survive sampling by inexperienced predators*" (pg. 1047; n.b.: Blest mentions that this hasn't been "searched for critically", and that "their confirmation would be of some interest.")
Rettenmeyer 1970	"…*aposematic insects, models, and mimics often have harder, more durable bodies than other insects, which was interpreted partly as an adaptation that allowed them to be tasted and still survive.*" (pg. 58)
Rothschild 1971	*"…unlike the model, with its tough cuticle and other disagreeable qualities, the mimic cannot afford to be examined at close quarters*." (pg. 211) *"...characteristic of the majority of aposematic insects...[is] the ability to withstand injury*..." (pg. 216)

Smith 1979	Beak-mark study; the aposematic species *Danaus chrysippus* were beak-marked more often (7.4%) than their Batesian mimics (*Hypolimnas misippus;* 3.2%). "*If the initial taste is repellent, the butterfly may be allowed to escape more or less uninjured*" (pg. 215) "*Thus, the similar proportion of beak marks… probably results from the larger individuals of each sex containing more cardenolide, being more distasteful and, consequently, more often rejected unharmed*." (pg. 216)
Järvi et al. 1981	Sampling survival experiment. Wild-caught great tits (*Parus major*) were allowed to chose between mealworms and *Papilio machaon*, an aposematic caterpillar. Of the caterpillars attacked, 100% survived the encounter (n=32).
Wiklund & Järvi 1982	Sampling survival experiment. Four species of naïve, hand-reared birds were presented with five species of aposematic insects; the survival rate of aposematic species after an encounter was 92%, and the survival rate after being seized was 84%.
Vermeij 1982	"*The fundamental assumption underlying the last prediction [that aposematic, tough-bodied species have higher incidences of beak marks than palatable, 'more delicate' species] is that predators must catch and injure butterflies before the edibility of the butterflies can be ascertained by the predator*." (pg. 708-709)

Chai 1986	Sampling survival experiment. Two jacamars (*Galbula ruficauda*) were presented with over 1000 individuals from 114 different morphs of butterflies. 97% of butterflies survived being rejected by sight or taste; the majority of taste-rejected butterflies survived. "…*butterflies were seldom killed after taste rejection. Indeed, the large, unacceptable Parides species and danaids appeared to be especially tenacious, and could still fly well after several days of multiple attacks.*" (pg. 175)
Malcolm 1986	"*The classical view is that aposematism evolves through in- dividual selection in which aposematic prey individuals survive predator encounters because they are tough-bodied*" (pg. 387)
Evans & Schmidt 1990	"*Like many toxic and aposematic butterflies… many stinging Hymenoptera possess tough integuments*." (pg. 405)
Brower 1995	"*It has long been recognized that unpalatable butterfly taxa tend to be tough and hard to kill*" (pg.415)
Kassarov 1999	"…*the great majority of aposematic insects, including butterflies, are released unharmed after being seized by birds.*" (pg. 973) "*Most unacceptable butterflies have a tough and flexible body…*" (pg. 973)
Lindström 1999b	"…*a tough cuticle that some insects have, which allows them to survive even when attacked…. Given that a toughened cuticle can enhance survivorship of prey, we need to know: (1) How did the tough cuticle*

	evolve?… (2) Can predators taste the unpalatability or noxiousness without eating the prey?... (3) Are there any interactions between coloration and toughness, i.e. would aposematically coloured individuals with a tough cuticle be more easily avoided than if they were brown?" (pg. 609-610)
DeVries 2002	Physical property experiment. Comparing wing tear weights of five nymphalid butterfly species of varying palatability; wing tear weight was positively correlated with palatability "*A simple extension of unpalatable theory suggests that natural selection should favor aposematic phenotypes possessing a physical toughness that makes them resistant to handling by predators*." (pg. 176)
DeVries 2003	Physical property experiment. Mean hindwing tear weight was measured and compared between an aposematic model, a Batesian mimic, and a palatable, non-mimetic relative. The model had the highest wing tear weight, and the palatable species had the lowest, the mimic being intermediate.
Hill & Vaca 2004	"…*wing strength is correlated with the palatability spectrum, suggesting that wing strength may be a general part of adult butterfly defenses against predators.*" (pg. 368)
Gilbert 2004	"*One characteristic of aposematic models and their mimics is that they often have harder, more durable bodies than other insects, toughened to withstand attack by predators so that the predators taste them but the prey still survive.*" (pg. 246)

Halpin *et al.* 2008	"*The degree to which prey can survive attacks will be crucial for the evolution of conspicuous coloration because conspicuous signals will attract visually hunting predators*." (pg. 1016)
Pinheiro & Campos 2013	"*In contrast to palatable species (including Batesian mimics), which usually exhibit soft wings, aposematic butterflies have tough wings that help them to resist sampling by birds*" (pg. 366)

Table A.2: Technical definitions of terms from biomechanics and material science, with associated equations and sources.

Term	**Definition**	**Equation**
Stress	Force (N) per unit area (m^2) upon which the force is acting (Vincent 2012)	$\sigma_C = \frac{f}{A_0}$
True stress	Actual stress experienced by complex, non-isotropic materials; stops being true after material yields. Equation is for relating true stress to conventional stress (Vincent 2012)	$\sigma_H = \frac{f}{A} = (1 + \varepsilon_C)\sigma_C$
Strain (Cauchy strain)	Amount of extension under force per unit length; can be expressed as Cauchy strain (Vincent 2012)	$\varepsilon_C = \frac{\Delta l}{L_0}$

True strain (Hencky strain)	Actual strain experienced by complex, non-isotropic materials; stops being true after material yields. Equation is for relating true strain to conventional strain (Vincent 2012)	$\varepsilon_H = \ln\left(\frac{L_0 + \Delta l}{L_0}\right)$ $= \ln(1 + \varepsilon_C)$
Stiffness	Resistance to deformation in its elastic range (i.e., where there's non-permanent deformation); extremes of stiff and pliant; represented by Young's modulus (E), the ratio of stress to strain (Vincent 2012)	$E = \frac{\sigma}{\varepsilon}$
Hardness	Definitions are varied depending on field and context (Boyer 1987); degree of resistance to indentation or scratching, abrasion and wear (Wright & Vincent 1996)	
Strength	Amount of force required to initiate a crack (Wright & Vincent 1996)	Force per area (N/m2)
Toughness	Amount of energy required to propagate a crack (Wright & Vincent 1996; Miller *et al.* 2009)	Energy per unit area (J/m2)

Plasticity	A material's ability to undergo plastic deformation without breaking, i.e. ability to deform permanently; extremes of brittle and ductile (Vincent 2012)	
Elasticity	A material's ability to return to its original shape after a deforming force is removed. (Vincent 2012)	
Viscoelastic	Materials which exhibit both elastic and viscous properties; causes biomechanical properties to be dependant on such factors as speed of loading (Vincent 2012)	
Resilience	The ability of a material to store strain energy via elastic deformation without breaking (Vincent 2012)	
Newton	Unit of force; function of mass and acceleration due to gravity; 1N = ~100 g of force in earth's gravity (Vincent 2012)	$1N/1m^2 = 1Pa$
Shear stress	The ratio of stress to strain (Vincent 2012)	$\tau = \frac{f}{A_S}$

Shear modulus	Strain (y) per unit shear stress (τ); works for isotropic materials (structure is the same in all directions) (Vincent 2012)	$G = \frac{\tau}{y}$

Appendix B Extra Figures & Tables

Table B.1: Taxa included in the analysis of the intraspecific effect of body length on kill rank, the group to which they belong, and the size and kill rank ranges within each intra group.

Intra group	Group	Count	Size range (mm)	Kill rank range
Cylindromyia bicolor	Diptera	8	8-10	3-5
Calliphoridae	Diptera	7	4-10	1-5
Polleniidae	Diptera	57	8-14	2-6
Bombyliidae	Diptera	8	8-11	4-5
Halictus	Hymenoptera	11	6-9	4-6
Apis mellifera	Hymenoptera	27	10-12	3-6
Hylaeus	Hymenoptera	32	4-6	2-6
Lasioglossum	Hymenoptera	38	4-8	3-6
Polistes	Hymenoptera	21	6-8	3-4
Vespula	Hymenoptera	10	11-15	5-6
Toxomerus geminatus	Syrphidae	34	4-5	1-4
Eupeodes AP	Syrphidae	14	7-9	3-4
Sphaerophoria APA	Syrphidae	93	6-9	2-3
Syritta pipiens	Syrphidae	112	5-9	1-5
Eristalis tenax	Syrphidae	10	4-7	1-4
Toxomerus marginatus	Syrphidae	9	11-14	5-6
Tropidia quadrata	Syrphidae	10	5-7	1-3

Table B.2: Syrphidae subgroups, mimetic fidelities from Penney et al. 2014, median kill rank, and average body length.

Species	Average mimetic fidelity	Median kill rank	Average body length (mm)
Allograpta obliqua	3.80	3	5.88
Epistrophe grossulariae	6.34	4	9
Eristalis arbustorum	5.82	5	9
Eristalis dimidiata	5.86	5	10
Eristalis flavipes	7.32	6	12.67
Eristalis tenax	7.5	6	12.2
Eristalis transversa	5.55	5	10
Eupeodes AP	7.02	3	8.23
Melanostoma mellinum	4.52	4	6
Platycheirus scutatus	4.95	3	7
Sphaerophoria APA	4.99	2	6
Sphaerophoria contigua	5.14	3	6
Spilomyia longicornis	7.41	5	11.5
Spilomyia sayii	6.36	5	11.25
Syritta pipiens	4.59	4	6.56
Syrphus ribesii	6.50	4	9.2
Syrphus VR	6.72	4	6.5
Toxomerus geminatus	5.64	2	5.47
Toxomerus marginatus	5.73	2	4.5
Tropidia quadrata	4.64	5	9.3

Table B.3: GenBank accession numbers for the species in the phylogenetically controlled MCMCglmm analysis.

Species	Genbank™ Accession Number
Allograpta_obliqua	JF871064.1
Epistrophe_grossulariae	JF869380.1
Eristalis_arbustorum	JN991982.1

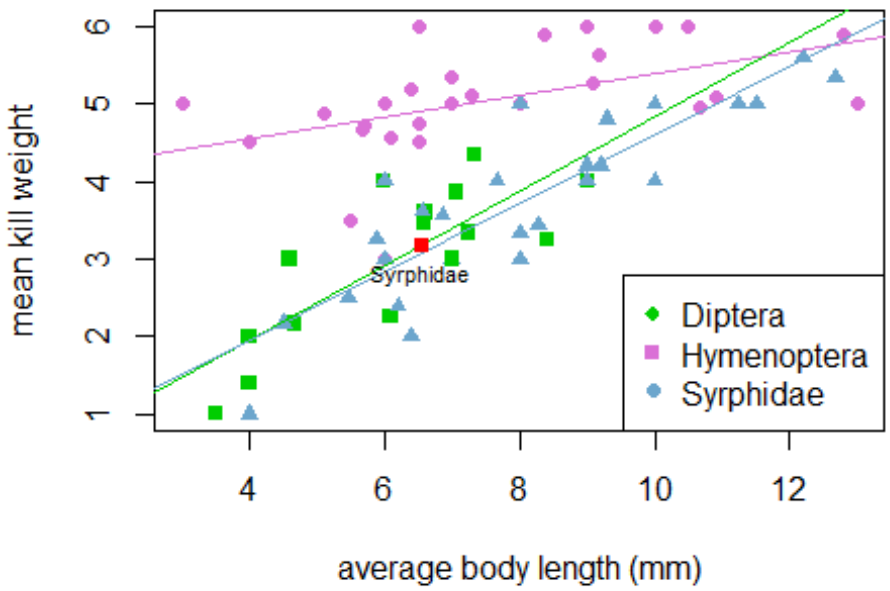
Eristalis_dimidiata	JF876135.1
Eristalis_flavipes	HQ944876.1
Eristalis_tenax	MN565029.1
Eristalis_transversa	JF873742.1
Eupeodes_AP_pomus	MK037251.1
Melanostoma_mellinum	JN285895.1
Platycheirus_scutatus	KX281065.1
Sphaerophoria_APA_asymmetrica	KC900483.1
Sphaerophoria_contigua	KC900440.1
Spilomyia_longicornis	HQ982380.1
Spilomyia_sayi	JF869220.1
Syritta_pipiens	JF869267.1
Syrphus_ribesii	HQ944943.1
Syrphus_VR_rectus	GU803811.1
Toxomerus_geminatus	JF871029.1
Toxomerus_marginatus	JF872382.1
Tropidia_quadrata	JN302515.1

Appendix C R markdown: titration experiment code & output

C.1 Size vs kill weight: visualised for each group

```
library(readxl)

syrph4R <- read_excel("C:/Users/amand/OneDrive - Carleton University/Master's Research/statistics & data/syrphRnov2.xlsx", sheet = "fixed data")
syrph4R$killweight<-factor(syrph4R$killweight,levels=c("20","50","100","200","500","N"))
syrph4R$groupvis<-factor(syrph4R$group,levels=c("Hymenoptera","Syrphidae","Diptera"))
```

```
smimd1<-subset(syrph4R,syrph4R$mimfid !='NA')
syrph4R<-subset(syrph4R,syrph4R$group !='Coleoptera')
tdata1<-aggregate(bodyL~subgroup+groupvis, data=syrph4R,FUN=mean)
tdata2<-aggregate(killrank~subgroup+groupvis,data=syrph4R,FUN=mean)
syrphdot1<-aggregate(bodyL~group,data=smimd1,FUN=mean)
syrphdot2<-aggregate(killrank~group,data=smimd1,FUN=mean)
syrphdot<-merge(syrphdot1,syrphdot2)

avttr<-(merge(tdata1,tdata2))


hymdat<-subset(avttr,avttr$group=="Hymenoptera")
dipdat<-subset(avttr,avttr$group=="Diptera")
syrdat<-subset(avttr,avttr$group=="Syrphidae")
lmhym<-lm(killrank~bodyL, data=hymdat)
lmdip<-lm(killrank~bodyL, data=dipdat)
lmsyr<-lm(killrank~bodyL, data=syrdat)

#average size vs average kill rank
plot(avttr$bodyL,avttr$killrank,col=(c("orchid","skyblue3","green3")[av
ttr$group]),
     xlab="average body length (mm)",pch=(c(16,17,15)[avttr$group]),
     ylab="mean kill weight")
legend("bottomright",legend=c("Diptera", "Hymenoptera","Syrphidae"),
       pch=c(18,15,16,17),col=c("green3","orchid","skyblue3"))
abline(lmhym,col="orchid")
abline(lmdip,col="green3")
abline(lmsyr,col="skyblue3")
points(syrphdot$bodyL,syrphdot$killrank, col="red",pch=15)
text(syrphdot$bodyL,syrphdot$killrank,labels=syrphdot$group,cex=0.7,pos
=1)
```




```
summary(lmhym)

##
## Call:
## lm(formula = killrank ~ bodyL, data = hymdat)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.82488 -0.15591  0.07577  0.35380  1.10320
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  3.96182    0.37241   10.64  3.7e-11 ***
## bodyL        0.14384    0.04641    3.10   0.0045 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.6139 on 27 degrees of freedom
## Multiple R-squared:  0.2624, Adjusted R-squared:  0.2351
## F-statistic: 9.607 on 1 and 27 DF,  p-value: 0.004496

summary(lmdip)

##
## Call:
## lm(formula = killrank ~ bodyL, data = dipdat)
##
```

```
## Residuals:
##     Min      1Q  Median      3Q     Max
## -0.8253 -0.4701 -0.1019  0.4180  1.0890
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)   0.0201     0.6396   0.031 0.975402
## bodyL         0.4818     0.1008   4.781 0.000359 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.6269 on 13 degrees of freedom
## Multiple R-squared:  0.6375, Adjusted R-squared:  0.6096
## F-statistic: 22.86 on 1 and 13 DF,  p-value: 0.0003587

summary(lmsyr)

##
## Call:
## lm(formula = killrank ~ bodyL, data = syrdat)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.01111 -0.38358 -0.04626  0.40083  1.28309
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)   0.1879     0.4001   0.470    0.642
## bodyL         0.4411     0.0471   9.365 5.69e-10 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.5587 on 27 degrees of freedom
## Multiple R-squared:  0.7646, Adjusted R-squared:  0.7559
## F-statistic: 87.71 on 1 and 27 DF,  p-value: 5.687e-10
```

C.2 Group vs kill weight: ordinal logistic model

```
library(readxl)

syrph4R <- read_excel("C:/Users/OneDrive - Carleton University/syrphRnov2.xlsx", sheet = "fixed data")

#define ordinal variable
syrph4R$killweight<-factor(syrph4R$killweight,levels=c("20","50","100","200","500","N"))


#OLR model
library(MASS)
bg1<-polr(killweight~bodyL+group, data=syrph4R, Hess = TRUE)
bg2<-polr(killweight~bodyL*group, data=syrph4R, Hess = TRUE)
bg3<-polr(killweight~bodyL, data=syrph4R, Hess = TRUE)
```

```r
bg4<-polr(killweight~group, data=syrph4R, Hess = TRUE)


#Determine best OLR model

anova(bg1,bg2,bg3,bg4)
```

```
## Likelihood ratio tests of ordinal regression models
##
## Response: killweight
##           Model Resid. df Resid. Dev   Test    Df  LR stat.      Pr(Chi)
## 1         bodyL       851   2512.373
## 2         group       850   2391.091 1 vs 2     1 121.28112 0.000000e+00
## 3 bodyL + group       849   2042.148 2 vs 3     1 348.94325 0.000000e+00
## 4 bodyL * group       847   1993.414 3 vs 4     2  48.73365 2.615896e-11
```

```r
AIC(bg1,bg2,bg3,bg4)
```

```
##     df      AIC
## bg1  8 2058.148
## bg2 10 2013.414
## bg3  6 2524.373
## bg4  7 2405.091
```

```r
#OLR results

summary(bg2)
```

```
## Call:
## polr(formula = killweight ~ bodyL * group, data = syrph4R, Hess = TRUE)
##
## Coefficients:
##                           Value Std. Error t value
## bodyL                    0.7421    0.07764   9.558
## groupHymenoptera         6.1984    0.68364   9.067
## groupSyrphidae          -1.4257    0.61959  -2.301
## bodyL:groupHymenoptera -0.3255    0.09135  -3.564
## bodyL:groupSyrphidae    0.2069    0.09083   2.277
##
## Intercepts:
##         Value   Std. Error t value
## 20|50    1.4078  0.5193     2.7110
## 50|100   3.4778  0.5142     6.7633
## 100|200  5.2563  0.5369     9.7893
## 200|500  7.5079  0.5842    12.8526
## 500|N   10.7776  0.6256    17.2285
##
## Residual Deviance: 1993.414
## AIC: 2013.414
```

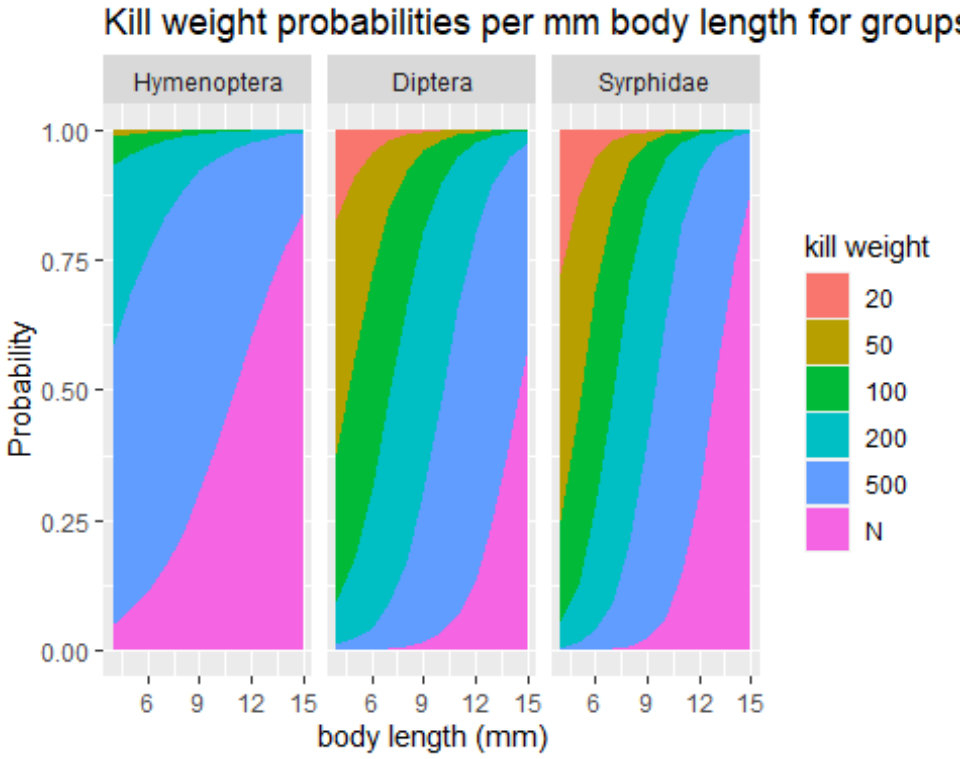
```r
#confidence interval
ci<-confint(bg2)
ci
```

```
##                              2.5 %     97.5 %
## bodyL                     0.5914620  0.8960165
## groupHymenoptera          4.8685187  7.5497661
## groupSyrphidae           -2.6423343 -0.2120102
## bodyL:groupHymenoptera -0.5050149 -0.1467092
## bodyL:groupSyrphidae      0.0289499  0.3852531

#odds ratio
exp(coef(bg2))

##                   bodyL       groupHymenoptera          groupSyrphidae
##               2.1004188             491.9501077                 0.2403420
## bodyL:groupHymenoptera   bodyL:groupSyrphidae
##               0.7221327               1.2298235

exp(cbind((OR = coef(bg2)), ci))

##                                          2.5 %       97.5 %
## bodyL                     2.1004188   1.80662771    2.4498248
## groupHymenoptera        491.9501077 130.12801061 1900.2981505
## groupSyrphidae            0.2403420   0.07119489    0.8089564
## bodyL:groupHymenoptera    0.7221327   0.60349661    0.8635451
## bodyL:groupSyrphidae      1.2298235   1.02937302    1.4699863

#pvalue
st <- coef(summary(bg2))
pval <- pnorm(abs(st[, "t value"]),lower.tail = FALSE)* 2
st <- cbind(st, "p value" = round(pval,3))
st

##                              Value Std. Error   t value p value
## bodyL                     0.7421368 0.07764280  9.558346   0.000
## groupHymenoptera          6.1983773 0.68364295  9.066688   0.000
## groupSyrphidae           -1.4256926 0.61959047 -2.301024   0.021
## bodyL:groupHymenoptera -0.3255464 0.09134824 -3.563794   0.000
## bodyL:groupSyrphidae      0.2068706 0.09083413  2.277455   0.023
## 20|50                     1.4078278 0.51929986  2.711011   0.007
## 50|100                    3.4777615 0.51420880  6.763326   0.000
## 100|200                   5.2562892 0.53694162  9.789312   0.000
## 200|500                   7.5078588 0.58415230 12.852571   0.000
## 500|N                    10.7775898 0.62556739 17.228503   0.000
```

C.3 Visualise OLR predicted probabilities

```
#visualise probabilities & predictions (adapted from UCLA 2021)
library(ggplot2)
library(reshape2)
library(Hmisc)
library(foreign)

j<-c("Hymenoptera","Diptera","Syrphidae")
s<-c(4,5,6,7,8,9,10,11,12,13,14,15)
```

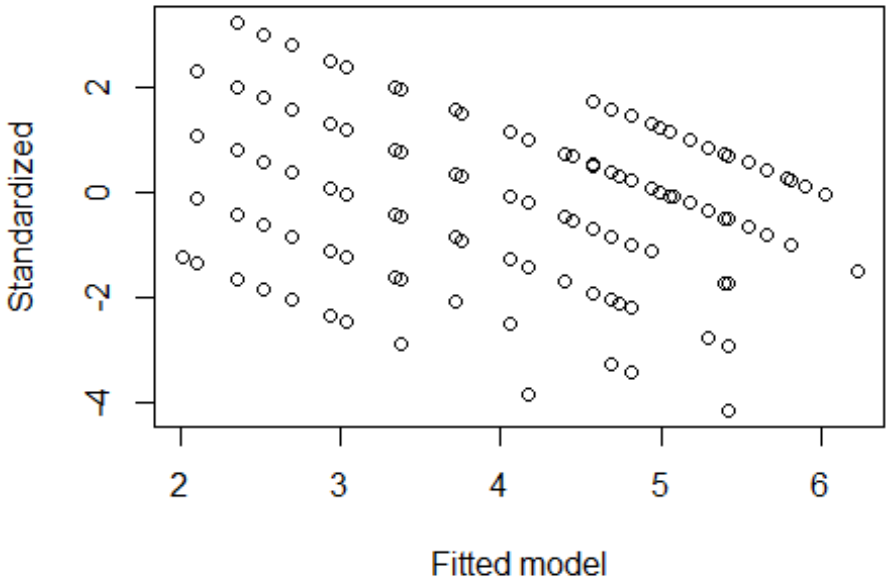
```
newdat<-data.frame()
for (oVar in j) {
  for (iVar in s){
    newdat<-rbind(newdat,data.frame(group=oVar,bodyL=iVar))
  }
}

newdat1 <- cbind(newdat, predict(bg2, newdat, type = "probs"))
lnewdat <- melt(newdat1, id.vars = c("group", "bodyL"),
                variable.name = "KillWeight", value.name="Probability")
Body_length<-round(lnewdat$bodyL)

#Killrank probabilities per body length for groups
ggplot(lnewdat, aes(x = Body_length, y = Probability, fill = KillWeight
)) +
  geom_area() +facet_wrap(lnewdat$group)+
  labs(x="body length (mm)",fill= "kill weight",title="Kill weight prob
abilities per mm body length for groups")
```



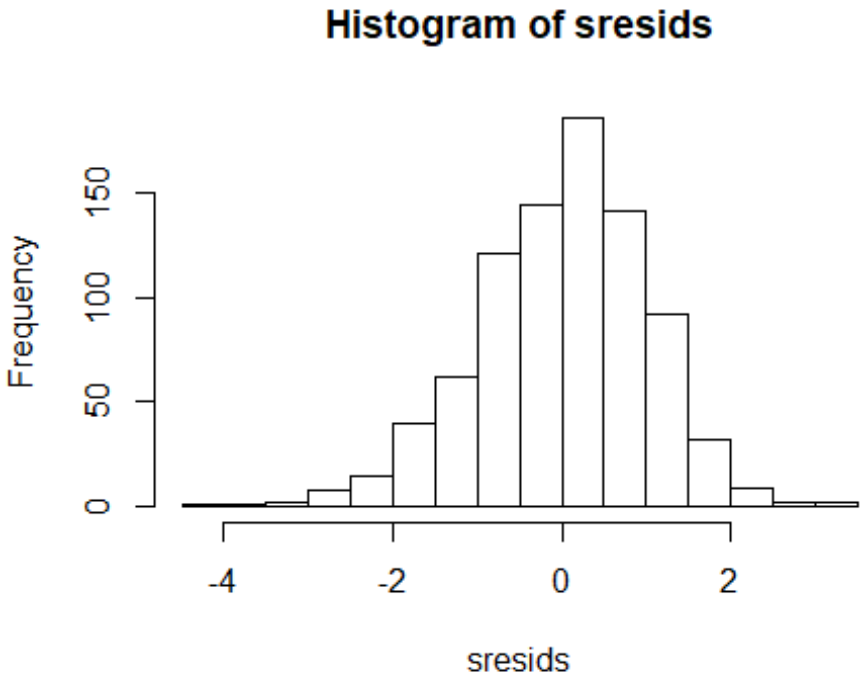
C.4 Group & size vs kill weight: linear model

```
#linear model: kill weight predicted by size and group
bglm<-lm(killrank~group*bodyL, data=syrph4R)
anova(bglm)

## Analysis of Variance Table
##
```

```
## Response: killrank
##              Df Sum Sq Mean Sq F value    Pr(>F)
## group         2 623.47 311.736 458.906 < 2.2e-16 ***
## bodyL         1 263.25 263.253 387.534 < 2.2e-16 ***
## group:bodyL   2  78.67  39.334  57.904 < 2.2e-16 ***
## Residuals   851 578.09   0.679
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(bglm)

##
## Call:
## lm(formula = killrank ~ group * bodyL, data = syrph4R)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -3.4193 -0.5164  0.0716  0.6076  2.6516
##
## Coefficients:
##                         Estimate Std. Error t value Pr(>|t|)
## (Intercept)              0.98575    0.21086   4.675 3.42e-06 ***
## groupHymenoptera         3.09925    0.25504  12.152  < 2e-16 ***
## groupSyrphidae          -0.52933    0.26265  -2.015   0.0442 *
## bodyL                    0.34067    0.03058  11.139  < 2e-16 ***
## groupHymenoptera:bodyL -0.21937    0.03507  -6.255 6.30e-10 ***
## groupSyrphidae:bodyL     0.07133    0.03809   1.873   0.0614 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.8242 on 851 degrees of freedom
## Multiple R-squared:  0.6255, Adjusted R-squared:  0.6233
## F-statistic: 284.2 on 5 and 851 DF,  p-value: < 2.2e-16

#check residuals
sresids<- rstandard(bglm)
fittedv<- fitted(bglm)
plot(fittedv,sresids, xlab = "Fitted model", ylab = "Standardized")
```




```
hist(sresids)
```



C.5 Intraspecific trends: size vs kill weight

```
#create subset

subdat<-subset(syrph4R,syrph4R$intra !='NA')
subdat<-subset(subdat,subdat$intra1 !='NA')

subdat$killweight<-factor(subdat$killweight,levels=c("20","50","100","2
00","500","N"))

hymint<-subset(subdat,subdat$group=="Hymenoptera")
dipint<-subset(subdat,subdat$group=="Diptera")
syrint<-subset(subdat,subdat$group=="Syrphidae")


##linear model
inlmi<-(lm(killrank~bodyL*intra, data=subdat))
inlma<-(lm(killrank~bodyL+intra, data=subdat))
AIC(inlmi,inlma)

##       df      AIC
## inlmi 35 1114.143
## inlma 19 1112.009

##linear model ANCOVA

#all groups ANCOVA

insb1<-aov(killrank~bodyL*subgroup1, data=subdat)
summary(insb1)

##                  Df Sum Sq Mean Sq F value Pr(>F)
## bodyL             1  346.8   346.8 670.355 <2e-16 ***
## subgroup1        20  437.7    21.9  42.312 <2e-16 ***
## bodyL:subgroup1  20   19.2     1.0   1.859  0.013 *
## Residuals       602  311.4     0.5
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

#non-syrphid dipterans ANCOVA

insbd<-aov(killrank~bodyL*subgroup1, data=dipint)
summary(insbd)

##                  Df Sum Sq Mean Sq F value   Pr(>F)
## bodyL             1  48.05   48.05  68.991 3.53e-14 ***
## subgroup1         6  42.51    7.09  10.173 1.48e-09 ***
## bodyL:subgroup1   6   1.91    0.32   0.458    0.839
## Residuals       164 114.22    0.70
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

#Syrphidae ANCOVA

insbs<-aov(killrank~bodyL*subgroup1, data=syrint)
summary(insbs)
```

```
##                   Df Sum Sq Mean Sq F value   Pr(>F)
## bodyL              1 145.00  145.00 303.248  < 2e-16 ***
## subgroup1          5  43.63    8.73  18.247 1.68e-15 ***
## bodyL:subgroup1    5   6.52    1.30   2.726   0.0202 *
## Residuals        258 123.37    0.48
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
#Hymenoptera ANCOVA

insbh<-aov(killrank~bodyL*subgroup1, data=hymint)
summary(insbh)
```

```
##                   Df Sum Sq Mean Sq F value   Pr(>F)
## bodyL              1  23.92  23.920  58.337 1.26e-12 ***
## subgroup1          7  31.21   4.458  10.873 2.05e-11 ***
## bodyL:subgroup1    7   6.06   0.866   2.112   0.0445 *
## Residuals        180  73.81   0.410
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
##linear model summaries

#all groups linear additive model summary

summary(inlma)
```

```
##
## Call:
## lm(formula = killrank ~ bodyL + intra, data = subdat)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -2.99396 -0.39258  0.09898  0.39929  1.95371
##
## Coefficients:
##                             Estimate Std. Error t value Pr(>|t|)
## (Intercept)                 2.698182   0.422985   6.379 4.18e-10 ***
## bodyL                       0.208128   0.037338   5.574 4.14e-08 ***
## intraBombyliidae           -1.387135   0.328878  -4.218 2.95e-05 ***
## intraCalliphoridae         -0.313688   0.215658  -1.455 0.146439
## intraCylindromyia_bicolor  -0.998369   0.294788  -3.387 0.000765 ***
## intraEristalis_tenax        0.362652   0.272254   1.332 0.183477
## intraEupeodes_AP           -0.994102   0.253671  -3.919 0.000102 ***
## intraHalictus               0.735090   0.290668   2.529 0.011757 *
## intraHylaeus                1.116664   0.281526   3.966 8.40e-05 ***
## intraLasioglossum           0.515597   0.274846   1.876 0.061265 .
## intraPolistes               0.537775   0.277719   1.936 0.053401 .
## intraPolleniidae           -0.254064   0.323096  -0.786 0.432052
## intraSphaerophoria_APA     -1.835841   0.328029  -5.597 3.67e-08 ***
## intraSyritta_pipiens       -0.439669   0.220700  -1.992 0.046917 *
## intraToxomerus_geminatus   -1.346242   0.248911  -5.409 1.00e-07 ***
## intraToxomerus_marginatus  -1.458289   0.296789  -4.914 1.23e-06 ***
## intraTropidia_quadrata      0.166225   0.271566   0.612 0.540762
## intraVespula                0.006366   0.209490   0.030 0.975769
```

```
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.7198 on 483 degrees of freedom
## Multiple R-squared:  0.6791, Adjusted R-squared:  0.6678
## F-statistic: 60.13 on 17 and 483 DF,  p-value: < 2.2e-16
```

```
#all groups linear interaction model summary
```

```
summary(inlmi)
```

```
##
## Call:
## lm(formula = killrank ~ bodyL * intra, data = subdat)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -2.95833 -0.44406  0.08475  0.46670  2.23803
##
## Coefficients:
##                                  Estimate Std. Error t value Pr(>|t|)
## (Intercept)                      3.630573   2.234343   1.625   0.1049
## bodyL                            0.121019   0.208354   0.581   0.5616
## intraBombyliidae                -1.330573   3.152758  -0.422   0.6732
## intraCalliphoridae              -2.122563   2.293135  -0.926   0.3551
## intraCylindromyia_bicolor        0.655141   4.171010   0.157   0.8753
## intraEristalis_tenax             5.737074   3.250843   1.765   0.0783 .
## intraEupeodes_AP                -0.547240   3.175362  -0.172   0.8632
## intraHalictus                    0.284061   2.596308   0.109   0.9129
## intraHylaeus                     0.441111   2.551564   0.173   0.8628
## intraLasioglossum               -1.674490   2.309512  -0.725   0.4688
## intraPolistes                    1.293156   2.923641   0.442   0.6585
## intraPolleniidae                -1.847965   3.654496  -0.506   0.6133
## intraSphaerophoria_APA          -7.519462   3.759760  -2.000   0.0461 *
## intraSyritta_pipiens            -2.183277   2.336790  -0.934   0.3506
## intraToxomerus_geminatus        -0.651105   2.332951  -0.279   0.7803
## intraToxomerus_marginatus       -1.454103   2.491964  -0.584   0.5598
## intraTropidia_quadrata          -1.815758   3.230096  -0.562   0.5743
## intraVespula                    -0.047240   2.527381  -0.019   0.9851
## bodyL:intraBombyliidae          -0.046019   0.362989  -0.127   0.8992
## bodyL:intraCalliphoridae         0.210772   0.220303   0.957   0.3392
## bodyL:intraCylindromyia_bicolor -0.192448   0.433193  -0.444   0.6571
## bodyL:intraEristalis_tenax      -0.429843   0.283783  -1.515   0.1305
## bodyL:intraEupeodes_AP          -0.079352   0.342105  -0.232   0.8167
## bodyL:intraHalictus              0.019225   0.277981   0.069   0.9449
## bodyL:intraHylaeus               0.036687   0.318302   0.115   0.9083
## bodyL:intraLasioglossum          0.330452   0.236009   1.400   0.1621
## bodyL:intraPolistes             -0.044748   0.254565  -0.176   0.8605
## bodyL:intraPolleniidae           0.183329   0.467989   0.392   0.6954
## bodyL:intraSphaerophoria_APA     0.878981   0.543911   1.616   0.1068
## bodyL:intraSyritta_pipiens       0.210787   0.232745   0.906   0.3656
## bodyL:intraToxomerus_geminatus  -0.210253   0.241444  -0.871   0.3843
## bodyL:intraToxomerus_marginatus -0.121019   0.320634  -0.377   0.7060
## bodyL:intraTropidia_quadrata     0.199969   0.325176   0.615   0.5389
## bodyL:intraVespula               0.003981   0.235970   0.017   0.9865
```

```
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.7105 on 467 degrees of freedom
## Multiple R-squared:  0.6977, Adjusted R-squared:  0.6763
## F-statistic: 32.66 on 33 and 467 DF,  p-value: < 2.2e-16
```

```
#diptera linear interaction model
```

```
summary(lm(killrank~bodyL*intra, data=dipint))
```

```
##
## Call:
## lm(formula = killrank ~ bodyL * intra, data = dipint)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -2.8396 -0.4976  0.1604  0.4869  1.4869
##
## Coefficients:
##                                  Estimate Std. Error t value Pr(>|t|)
## (Intercept)                       0.05121    1.19062   0.043  0.96580
## bodyL                             0.38005    0.13896   2.735  0.00768 **
## intraCalliphoridae                1.50342    1.31415   1.144  0.25602
## intraCylindromyia_bicolor         4.23450    4.01068   1.056  0.29424
## intraPolleniidae                 -2.01996    2.35378  -0.858  0.39336
## bodyL:intraCalliphoridae         -0.05364    0.15904  -0.337  0.73682
## bodyL:intraCylindromyia_bicolor -0.45148    0.43574  -1.036  0.30326
## bodyL:intraPolleniidae            0.46370    0.33553   1.382  0.17083
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.7726 on 80 degrees of freedom
## Multiple R-squared:  0.3317, Adjusted R-squared:  0.2732
## F-statistic: 5.671 on 7 and 80 DF,  p-value: 2.429e-05
```

```
#syrphid linear interaction model
```

```
summary(lm(killrank~bodyL*intra, data=syrint))
```

```
##
## Call:
## lm(formula = killrank ~ bodyL * intra, data = syrint)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -2.4381 -0.4441 -0.1017  0.5524  1.8235
##
## Coefficients:
##                                  Estimate Std. Error t value Pr(>|t|)
## (Intercept)                       9.3676     2.2922   4.087 5.78e-05 ***
## bodyL                            -0.3088     0.1870  -1.651 0.099872 .
## intraEupeodes_AP                 -6.2843     3.1704  -1.982 0.048477 *
## intraSphaerophoria_APA          -13.2565     3.7243  -3.559 0.000439 ***
## intraSyritta_pipiens             -7.9204     2.3865  -3.319 0.001029 **
```

```
## intraToxomerus_geminatus          -6.3882     2.3830  -2.681 0.007801 **
## intraToxomerus_marginatus         -7.1912     2.5301  -2.842 0.004824 **
## intraTropidia_quadrata            -7.5528     3.2220  -2.344 0.019803 *
## bodyL:intraEupeodes_AP             0.3505     0.3230   1.085 0.278918
## bodyL:intraSphaerophoria_APA       1.3088     0.5224   2.506 0.012816 *
## bodyL:intraSyritta_pipiens         0.6406     0.2124   3.016 0.002808 **
## bodyL:intraToxomerus_geminatus     0.2196     0.2214   0.992 0.322121
## bodyL:intraToxomerus_marginatus    0.3088     0.3016   1.024 0.306749
## bodyL:intraTropidia_quadrata       0.6298     0.3061   2.057 0.040620 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.6897 on 268 degrees of freedom
## Multiple R-squared:  0.6185, Adjusted R-squared:    0.6
## F-statistic: 33.42 on 13 and 268 DF,  p-value: < 2.2e-16
```

```
#hymenoptera linear interaction model

summary(lm(killrank~bodyL*intra, data=hymint))
```

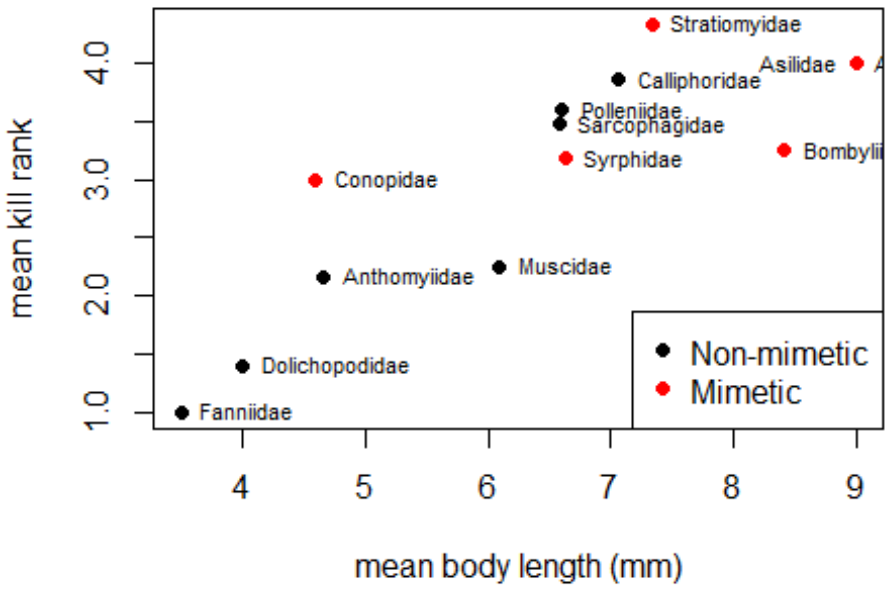
```
##
## Call:
## lm(formula = killrank ~ bodyL * intra, data = hymint)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -2.9583 -0.1466  0.1398  0.2380  2.2380
##
## Coefficients:
##                            Estimate Std. Error t value Pr(>|t|)
## (Intercept)                3.630573   2.248851   1.614    0.109
## bodyL                      0.121019   0.209706   0.577    0.565
## intraHalictus              0.284061   2.613167   0.109    0.914
## intraHylaeus               0.441111   2.568132   0.172    0.864
## intraLasioglossum         -1.674490   2.324508  -0.720    0.473
## intraPolistes              1.293156   2.942625   0.439    0.661
## intraVespula              -0.047240   2.543792  -0.019    0.985
## bodyL:intraHalictus        0.019225   0.279786   0.069    0.945
## bodyL:intraHylaeus         0.036687   0.320368   0.115    0.909
## bodyL:intraLasioglossum    0.330452   0.237541   1.391    0.167
## bodyL:intraPolistes       -0.044748   0.256218  -0.175    0.862
## bodyL:intraVespula         0.003981   0.237503   0.017    0.987
##
## Residual standard error: 0.7151 on 127 degrees of freedom
## Multiple R-squared:  0.3356, Adjusted R-squared:  0.278
## F-statistic: 5.831 on 11 and 127 DF,  p-value: 1.349e-07
```

```
#OLR optimal model
subolrA<-polr(killweight~bodyL+subgroup1, data=subdat, Hess = TRUE)
subolrB<-polr(killweight~bodyL*subgroup1, data=subdat, Hess = TRUE)

anova(subolrA,subolrB)
```

```
## Likelihood ratio tests of ordinal regression models
##
## Response: killweight
##                Model Resid. df Resid. Dev   Test    Df LR stat.    Pr(Chi)
## 1 bodyL + subgroup1       618   1278.106
## 2 bodyL * subgroup1       598   1240.918 1 vs 2    20 37.18747 0.01111072

AIC(subolrA,subolrB)

##         df      AIC
## subolrA 26 1330.106
## subolrB 46 1332.918
```

C.6 Diptera mimetic status and kill weight

```
mimdat<-subset(syrph4R,syrph4R$MimDip!='NA')

#Visualise averages for each family
Ddata1<-aggregate(bodyL~Family, data=mimdat,FUN=mean)
Ddata2<-aggregate(killrank~Family+MimDip,data=mimdat,FUN=mean)
mimav<-merge(Ddata1,Ddata2)

plot(mimav$bodyL,mimav$killrank,xlab="mean body length (mm)",ylab="mean
kill rank",
     pch=c(16), col=c(as.factor(mimav$MimDip)))+
  text(mimav$bodyL,mimav$killrank,labels=mimav$Family,cex=0.7,pos=4)+
  text(9,4,labels="Asilidae",cex=0.7,pos=2)

## integer(0)

legend("bottomright",legend = c("Non-mimetic", "Mimetic"),pch=c(16),col
=c("black","red"),cex=1)
```




```
#compare mimetic and non-mimetic families
bmaov<-aov(killrank~MimDip, data=mimdat)
summary(bmaov)

##              Df Sum Sq Mean Sq F value Pr(>F)
## MimDip        1    0.1  0.0509   0.039  0.843
## Residuals   535  692.8  1.2950

#OLR
syrph4R$killweight<-factor(syrph4R$killweight,levels=c("20","50","100",
"200","500","N"))
mdip1<-polr(killweight~bodyL+MimDip, data=mimdat)
mdip2<-polr(killweight~bodyL*MimDip, data=mimdat)
mdip3<-polr(killweight~bodyL, data=mimdat)
mdip4<-polr(killweight~MimDip, data=mimdat)

AIC(mdip1,mdip2,mdip3,mdip4)

##       df      AIC
## mdip1  7 1333.856
## mdip2  8 1335.767
## mdip3  6 1333.572
## mdip4  6 1656.001

summary(mdip1)

## Call:
## polr(formula = killweight ~ bodyL + MimDip, data = mimdat)
```

```
##
## Coefficients:
##           Value Std. Error t value
## bodyL    0.9688    0.06256  15.487
## MimDipY -0.2395    0.18292  -1.309
##
## Intercepts:
##         Value   Std. Error t value
## 20|50    2.6421  0.3997     6.6107
## 50|100   4.7442  0.3967    11.9590
## 100|200  6.5051  0.4326    15.0370
## 200|500  9.1554  0.5352    17.1060
## 500|N   12.5947  0.7849    16.0453
##
## Residual Deviance: 1319.856
## AIC: 1333.856
```

```
st <- coef(summary(mdip1))
pval <- pnorm(abs(st[, "t value"]),lower.tail = FALSE)* 2
st <- cbind(st, "p value" = round(pval,9))
st
```

```
##              Value Std. Error   t value   p value
## bodyL    0.9688448 0.06255752 15.487262 0.0000000
## MimDipY -0.2394962 0.18292370 -1.309269 0.1904434
## 20|50    2.6421316 0.39967261  6.610740 0.0000000
## 50|100   4.7441838 0.39670440 11.958990 0.0000000
## 100|200  6.5050827 0.43260465 15.037015 0.0000000
## 200|500  9.1554168 0.53521819 17.105952 0.0000000
## 500|N   12.5947465 0.78494790 16.045328 0.0000000
```

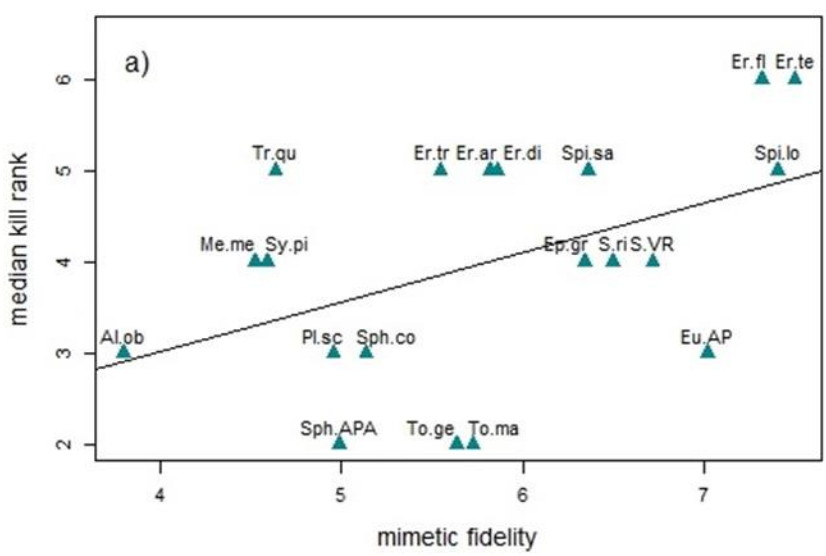
```
ci<-confint(mdip1)
ci
```

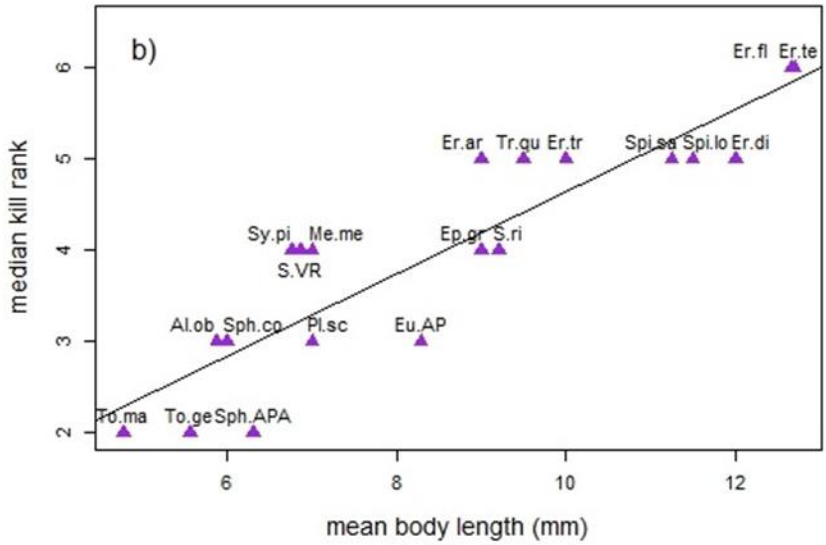
```
##              2.5 %    97.5 %
## bodyL    0.8489468 1.0943523
## MimDipY -0.5988346 0.1186921
```

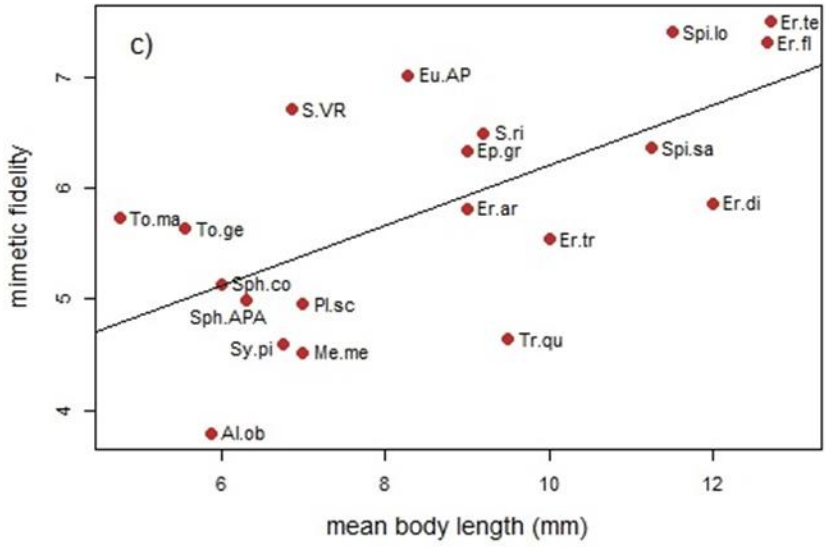
C.7 Syrphidae OLR model: median kill rank, mimetic fidelity, & body size

```
##visualise & plot mimetic fidelity, body size, kill rank
#aggregate data

smimd1<-subset(syrph4R,syrph4R$mimfid !='NA')
tdata3<-aggregate(mimfid~subgroup,data=smimd1,FUN=mean)
tdata4<-aggregate(killrank~subgroup,data=smimd1,FUN=median)
tdata6<-aggregate(bodyL~subgroup,data=smimd1,FUN=mean)
tdata7<-aggregate(killnum~subgroup,data=smimd1,FUN=median)
avmim<-merge(tdata3,tdata4)
avmim$bodyL<-tdata6$bodyL
avmim$killnum<-tdata7$killnum
```

```
#plotting relationship between these 3 variables

lmKM<-lm(as.numeric(killrank)~mimfid, data=avmim)
lmKS<-lm(as.numeric(killrank)~bodyL, data=avmim)
lmSM<-lm(mimfid~bodyL, data=avmim)


par(mfrow=c(3,1))

plot(avmim$mimfid,avmim$killrank,xlab="mimetic fidelity",ylab="median k
ill rank", pch=17, cex=1.5,col="turquoise4",ylim=c(2,6.5),cex.lab=1.35)
avmim$labpos1<-c(3.795455, 6.25, 5.75, 6.0, 7.25, 7.5, 5.5, 7.022727, 4
.38, 4.9, 4.988636, 5.25, 7.409091, 6.363636, 4.7, 6.5, 6.715909, 5.5,
5.85, 4.636364)
text(avmim$labpos1,avmim$killrank,labels=avmim$label,cex=1, pos=3)
text(4,6.2,labels="a)", cex=1.5,pos=2)
abline(lmKM)

plot(avmim$bodyL,avmim$killrank,xlab="mean body length (mm)",ylab="medi
an kill rank", pch=17,col="purple",ylim=c(2,6.5), cex=1.5,cex.lab=1.35)
avmim$labpos2<-c(5.6, 8.8, 8.8, 12.2, 12.2, 12.75, 10, 8.285714, 7.3, 7
.2, 6.3, 6.3, 11.65, 11, 6.5, 9.3, 0, 5.5625, 4.764706, 9.45)
text(avmim$labpos2,avmim$killrank,labels=avmim$label,cex=1, pos=3)
text(6.857143,4,labels="S.VR",cex=1, pos=1)
text(5.3,6.2,labels="b)", cex=1.5,pos=2)
abline(lmKS)

plot(avmim$bodyL,avmim$mimfid,xlab="mean body length (mm)",ylab="mimeti
c fidelity", pch=16, col="firebrick3",cex=1.5, xlim=c(4.8,13),cex.lab=1
.35)
avmim$labpos3<-c(3.795455, 6.340909, 5.818182, 5.863636, 7.318182, 7.5,
5.545455, 7.022727, 4.522727, 4.954545, 0, 5.136364, 7.409091, 6.363636
, 0, 6.5, 6.715909, 5.636364, 5.727273, 4.636364)
text(avmim$bodyL,avmim$labpos3,avmim$label,cex=1, pos=4)
text(6.763441,4.56,labels="Sy.pi",cex=1, pos=2)
text(6.1,5.01,labels="Sph.APA",cex=1, pos=1)
text(5.3,7.3,labels="c)", cex=1.5,pos=2)
abline(lmSM)
```


a)
median kill rank
mimetic fidelity
Er.fl
Er.te
Tr.qu
Er.tr
Er.ar
Er.di
Spi.sa
Spi.lo
Me.me
Sy.pi
Ep.gr
S.ri
S.VR
Al.ob
Pl.sc
Sph.co
Eu.AP
Sph.APA
To.ge
To.ma


b)
median kill rank
mean body length (mm)
Er.fl
Er.te
Er.ar
Tr.qu
Er.tr
Spi.sa
Spi.lo
Er.di
Sy.pi
Me.me
Ep.gr
S.ri
S.VR
Al.ob
Sph.co
Pl.sc
Eu.AP
To.ma
To.ge
Sph.APA


c)
mimetic fidelity
mean body length (mm)
Spi.lo
Er.te
Er.fl
Eu.AP
S.VR
S.ri
Ep.gr
Spi.sa
Er.di
Er.ar
To.ma
To.ge
Er.tr
Sph.co
Pl.sc
Sph.APA
Sy.pi
Me.me
Tr.qu
Al.ob

```
###OLR model
library(MASS)
smimd1<-subset(syrph4R,syrph4R$mimfid !='NA')
tdata3<-aggregate(mimfid~subgroup,data=smimd1,FUN=mean)
tdata4<-aggregate(killrank~subgroup,data=smimd1,FUN=median)
tdata6<-aggregate(bodyL~subgroup,data=smimd1,FUN=mean)
tdata7<-aggregate(killrank~subgroup,data=smimd1,FUN=median)
avmim<-merge(tdata3,tdata4)
avmim$bodyL<-tdata6$bodyL
avmim$killrank<-tdata7$killrank

library(MASS)
avmim$killrank<-factor(avmim$killrank,levels=c("2","3","4","5","6"),ord
ered = TRUE)

m1<-polr(killrank~bodyL+mimfid, data=avmim, Hess = TRUE)
m2<-polr(killrank~mimfid*bodyL, data=avmim, Hess = TRUE)
m3<-polr(killrank~mimfid, data=avmim, Hess = TRUE)
m4<-polr(killrank~bodyL, data=avmim, Hess = TRUE)

AIC(m1,m4,m2,m3)

##    df      AIC
## m1  6 39.17859
## m4  5 40.84625
## m2  7 40.87119
## m3  5 67.27544

summary(m1)

## Call:
## polr(formula = killrank ~ bodyL + mimfid, data = avmim, Hess = TRUE)
##
## Coefficients:
##         Value Std. Error t value
## bodyL   2.590     0.8262   3.135
## mimfid -1.414     0.8277  -1.708
##
## Intercepts:
##     Value   Std. Error t value
## 2|3  7.8766  3.9909     1.9736
## 3|4 10.4161  4.1755     2.4946
## 4|5 15.1201  5.6528     2.6748
## 5|6 20.8851  7.1318     2.9284
##
## Residual Deviance: 27.17859
## AIC: 39.17859

st <- coef(summary(m1))
pval <- pnorm(abs(st[, "t value"]),lower.tail = FALSE)* 2
st <- cbind(st, "p value" = round(pval,3))
st
```

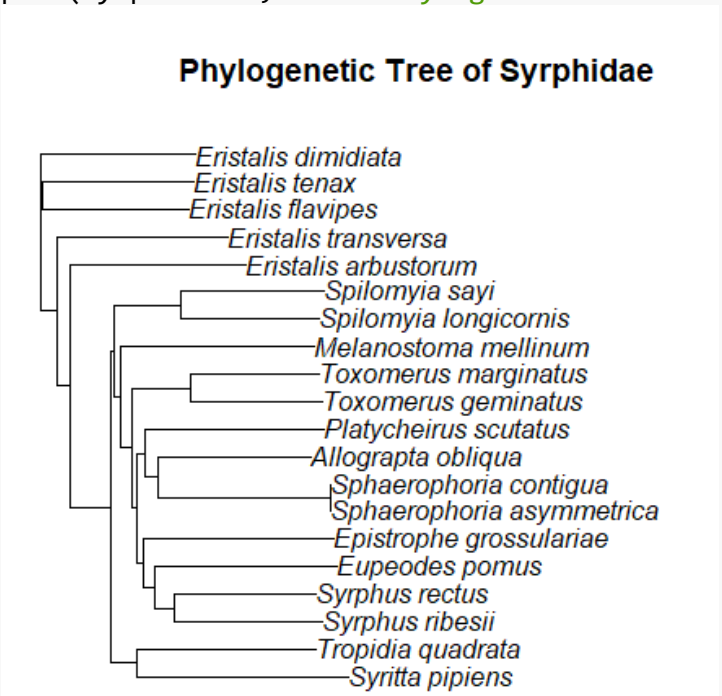
```
##            Value Std. Error   t value p value
## bodyL   2.590316  0.8261593  3.135371   0.002
## mimfid -1.413913  0.8276871 -1.708271   0.088
## 2|3     7.876562  3.9908933  1.973634   0.048
## 3|4    10.416063  4.1755247  2.494552   0.013
## 4|5    15.120088  5.6527927  2.674800   0.007
## 5|6    20.885059  7.1318445  2.928423   0.003

#confidence interval
ci<-confint(m1)
ci

##            2.5 %     97.5 %
## bodyL   1.329412 4.71408045
## mimfid -3.343876 0.03028389

exp(coef(m1))

##      bodyL     mimfid
## 13.3339824  0.2431897

exp(cbind((OR = coef(m1)), ci))

##                       2.5 %     97.5 %
## bodyL  13.3339824 3.77882104 111.506229
## mimfid  0.2431897 0.03529986   1.030747
```

C.8 Phylogenetic control: Sequence data & phylogeny

```
#load the packages

library(BiocManager)
BiocManager::install("msa")
library("ape")
library("rentrez")
library("seqinr")
library("picante")
library("MCMCglmm")
library("readxl")
library("geiger")
library("nlme")
library("phytools")


##Get sequence data from GenBank
#name the species needed + accession number

SyrphidAccessions<-matrix(ncol=2,byrow=TRUE,
                          c("Allograpta_obliqua","JF871064.1",
                          "Epistrophe_grossulariae","JF869380.1",
                          "Eristalis_arbustorum","JN991982.1",
                          "Eristalis_dimidiata","JF876135.1",
                          "Eristalis_flavipes", "HQ944876.1",
```

```
                            "Eristalis_tenax","MN565029.1",
                            "Eristalis_transversa","JF873742.1",
                            "Eupeodes_AP_pomus","MK037251.1",
                            "Melanostoma_mellinum","JN285895.1",
                            "Platycheirus_scutatus","KX281065.1",
                            "Sphaerophoria_APA_asymmetrica","KC900483.1",
                            "Sphaerophoria_contigua","KC900440.1",
                            "Spilomyia_longicornis","HQ982380.1",
                            "Spilomyia_sayi","JF869220.1",
                            "Syritta_pipiens","JF869267.1",
                            "Syrphus_ribesii","HQ944943.1",
                            "Syrphus_VR_rectus","GU803811.1",
                            "Toxomerus_geminatus","JF871029.1",
                            "Toxomerus_marginatus","JF872382.1",
                            "Tropidia_quadrata","JN302515.1"))

colnames(SyrphidAccessions)<-c("Species","Accession")

#aquire sequences & attach species names to Accession number
coi_gen <- read.GenBank(SyrphidAccessions[,2], species.names = TRUE)

names_coi <- data.frame(species = attr(coi_gen, "species"), accs = name
s(coi_gen))

names(coi_gen) <- attr(coi_gen, "species")

##make data readable to the packages
#write data as FASTA

write.FASTA(coi_gen,"SyrphidFASTA.fasta")

# from the msa handbook (Bonatesta et al. 2015)
# Read in FASTA file
syprhidSeq<-readDNAStringSet("SyrphidFASTA.fasta")

syprhidAln<-msa(syprhidSeq)
syprhidAln2<-msaConvert(syprhidAln,type="seqinr::alignment")

d<-dist.alignment(syprhidAln2,"identity")

syrphidTree <-nj(d)

# Plot the tree
```

```
plot(syrphidTree, main="Phylogenetic Tree of Syrphidae")
```

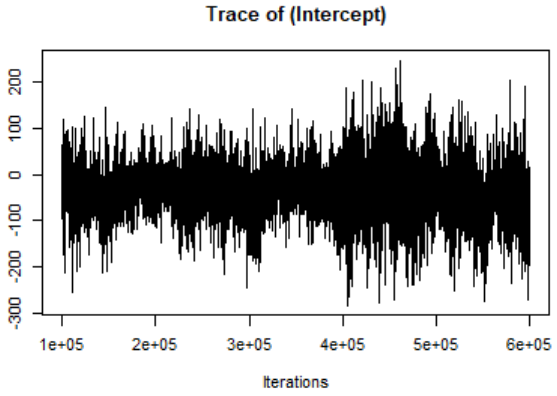


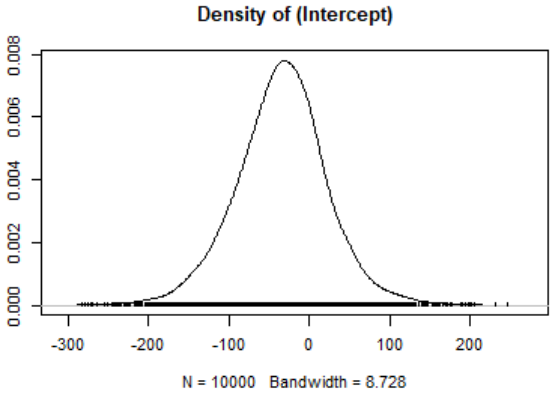
C.9 Phylogenetic control: MCMCglmm

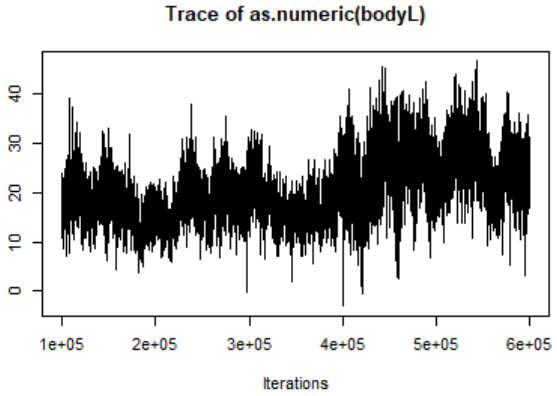
```
load the data

syrphidData <- read_excel("C:/Users/amand/OneDrive - Carleton University/Master's Research/statistics & data/Copy of syrphid resilience data.xlsx", range = "A1:f21")
syrphidData<-as.data.frame(syrphidData)

rownames(syrphidData)<-syrphidData$TreeName

syrphidData<-transform(syrphidData, mimfid = as.numeric(mimfid),
                       bodyL = as.numeric(bodyL),
                       intercepts = as.numeric(intercepts))

# remove missing values & root tree with Eristalis as the outgroup
syrphidDataComplete<-syrphidData[complete.cases(syrphidData),]
# resolve root fix from spiral01 (2016); https://www.biostars.org/p/188923/#189130
syrphidTree<-root(syrphidTree,outgroup="Eristalis_dimidiata", resolve.root = TRUE)

# make tree ultrametric (Chang 2021)
```

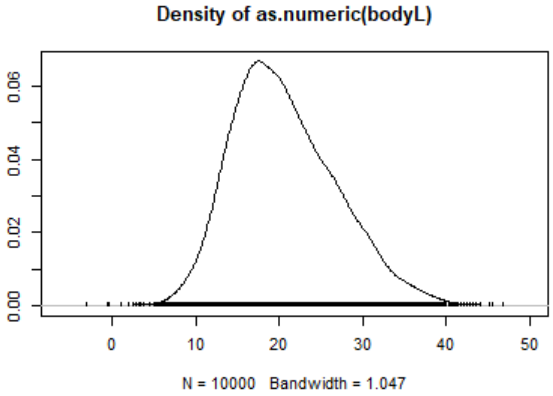
```
syrphidTree$edge.length[which(syrphidTree$edge.length==0)]<-0.0001

syrphidTree_UM <- force.ultrametric(syrphidTree,method="extend")

syrphidTree_UM<-match.phylo.data(syrphidTree_UM,syrphidData)$phy
syrphidData_sorted2<-match.phylo.data(syrphidTree_UM,syrphidData)$data

inv.syrphidTree<-inverseA(syrphidTree_UM,nodes="TIPS",scale=TRUE)

#base code for model from Garamszegi (2018)

syrphidData_sorted2$killrank<-factor(syrphidData_sorted2$killrank,level
s=c("2","3","4","5","6"),ordered = TRUE)

prior<-list(G=list(G1=list(V=1,nu=0.002)),R=list(V=1,nu=0.002,fix=1))

MCMCmod<-MCMCglmm(killrank~as.numeric(bodyL)+as.numeric(mimfid),
                  random=~TreeName,
                  data=syrphidData_sorted2,
                  family = "ordinal",
                  prior=prior,nitt=600000,burnin=100000,thin=50,verbose
=FALSE,
                  ginverse=list(TreeName=inv.syrphidTree$Ainv))
summary(MCMCmod)

##
##  Iterations = 100001:599951
##  Thinning interval  = 50
##  Sample size  = 10000
##
##  DIC: NaN
##
##  G-structure:  ~TreeName
##
##          post.mean l-95% CI u-95% CI eff.samp
## TreeName      2640    136.2     7323    22.51
##
##  R-structure:  ~units
##
##       post.mean l-95% CI u-95% CI eff.samp
## units         1        1        1        0
##
##  Location effects: killrank ~ as.numeric(bodyL) + as.numeric(mimfid)
##
##                    post.mean  l-95% CI  u-95% CI eff.samp  pMCMC
## (Intercept)         -38.2519 -146.6748   50.2692    74.72 0.3820
## as.numeric(bodyL)    15.8976    5.6746   31.3268     9.20 0.0002 ***
## as.numeric(mimfid)   -0.7693   -6.6164    4.5917   141.81 0.7596
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##  Cutpoints:
##                          post.mean l-95% CI u-95% CI eff.samp
## cutpoint.traitkillrank.1     45.76    9.521     82.8   52.131
```

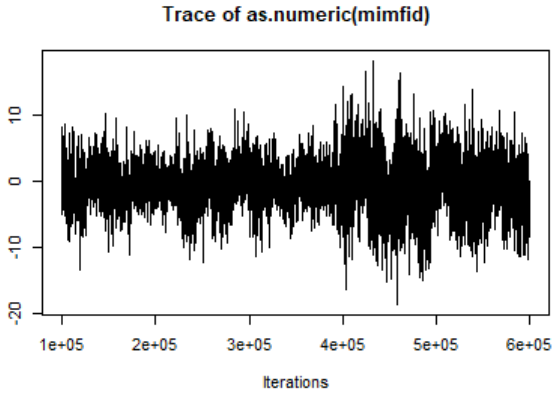
```
## cutpoint.traitkillrank.2    110.13   53.573    183.6   14.983
## cutpoint.traitkillrank.3    218.87  111.631    354.6    7.153

plot(MCMCmod)
```

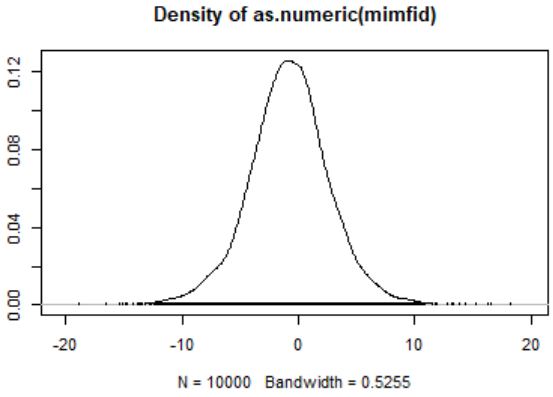

Trace of (Intercept)
200
100
0
-100
-200
-300
1e+05
2e+05
3e+05
4e+05
5e+05
6e+05
Iterations

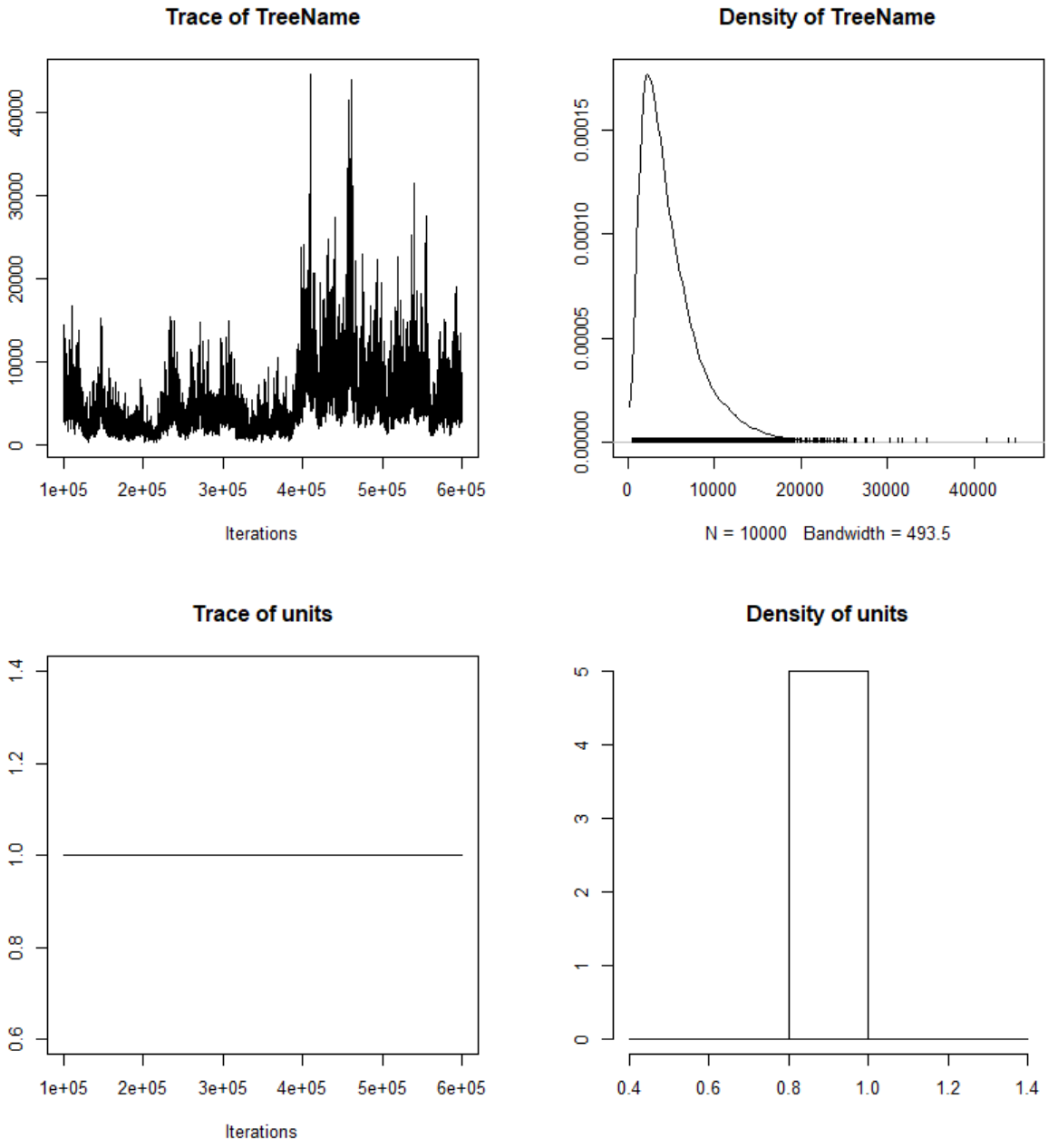

Density of (Intercept)
0.008
0.006
0.004
0.002
0.000
-300
-200
-100
0
100
200
N = 10000 Bandwidth = 8.728


Trace of as.numeric(bodyL)
40
30
20
10
0
1e+05
2e+05
3e+05
4e+05
5e+05
6e+05
Iterations


Density of as.numeric(bodyL)
0.06
0.04
0.02
0.00
0
10
20
30
40
50
N = 10000 Bandwidth = 1.047


Trace of as.numeric(mimfid)
10
0
-10
-20
1e+05
2e+05
3e+05
4e+05
5e+05
6e+05
Iterations


Density of as.numeric(mimfid)
0.12
0.08
0.04
0.00
-20
-10
0
10
20
N = 10000 Bandwidth = 0.5255



Appendix D R markdown: micrometre experiment code & output

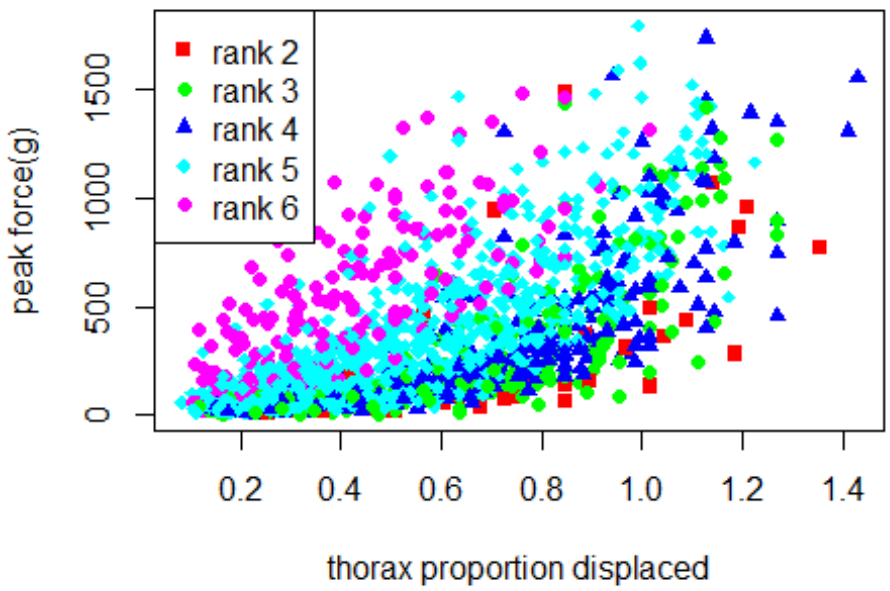
D.1 Importing and pairing data

```
#DATA AND LIBRARIES
library(ggplot2)
library(tidyverse)
```

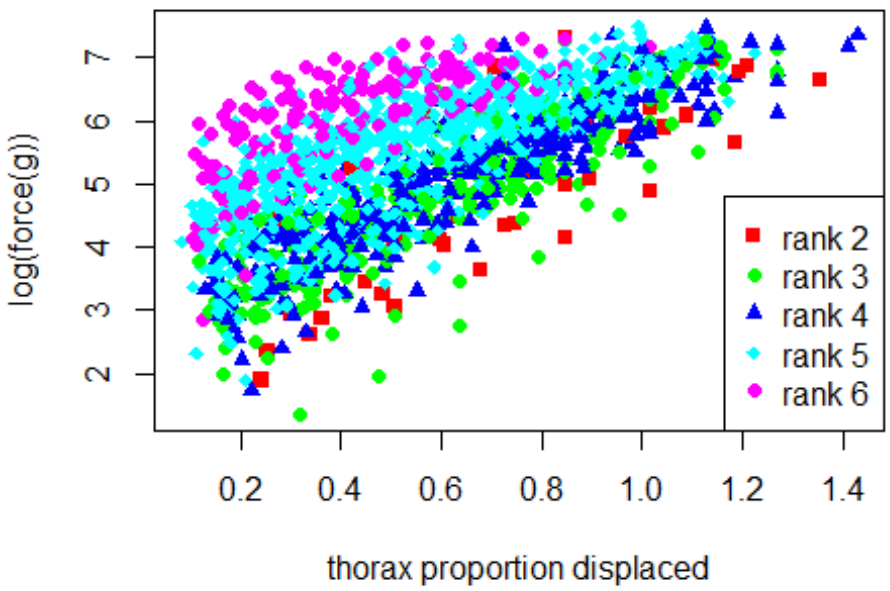
```
library(magrittr)
dy <- read_excel("C:/Users/statistics & data/micrm4R5.xlsx",
                 sheet = "y values")
dx <- read_excel("C:/Users/statistics & data/micrm4R4.xlsx",
                 sheet = "x values")
#place y-values in one column
y_long<-dy %>%
  pivot_longer(-c(Group:ID),names_prefix = "peak",
               names_transform = list(name= as.integer, values_to
="peak"))
#place x-values in one column
x_long<-  dx %>%
  pivot_longer(-ID, names_prefix = "disp",
               names_transform = list(name = as.integer),
               values_to = "disp")

#join the two datasets & define variables
xybind<-cbind(y_long,x_long)
togxy<-subset(xybind,xybind$value !='NA')
togxy<- subset(togxy, togxy$mkillr != "NA")
disp<-togxy$disp
force<-togxy$value
logforce<-log(togxy$value)
size<-togxy$`Thorax diameter`
togxy['logforce']=logforce
```

D.2 Visualise data

```
#graph force per displacement w ranks
plot(disp,force, pch=c(14:19)[togxy$mkillr], col=c("black","red","green
","blue","cyan","magenta")[togxy$mkillr],
       xlab="thorax proportion displaced",ylab="peak force(g)")
legend("topleft",legend=c("rank 2", "rank 3","rank 4","rank 5","rank 6"
),
       pch=c(15:19),col=c("red","green","blue","cyan","magenta"))
```




```
#graph log(force) per displacement w ranks
plot(togxy$disp,togxy$logforce, pch=c(14:19)[togxy$mkillr], col=c("blac
k","red","green","blue","cyan","magenta")[togxy$mkillr],
       xlab="thorax proportion displaced",ylab="log(force(g))")
legend("bottomright",legend=c("rank 2", "rank 3","rank 4","rank 5","ran
k 6"),
       pch=c(15:19),col=c("red","green","blue","cyan","magenta"))
```



D.3 Micrometre linear mixed-effects model

```
#linear mixed-effects model
library(statmod)
library(Matrix)
library(lme4)

model3<-lmer(logforce~disp + (1/ID), data = togxy)
model4<-lmer(logforce~disp + (disp/ID), data = togxy)
anova(model4,model3, test="LRT")

## refitting model(s) with ML (instead of REML)

## Data: togxy
## Models:
## model3: logforce ~ disp + (1 | ID)
## model4: logforce ~ disp + (disp | ID)
##        npar     AIC     BIC  logLik deviance  Chisq Df Pr(>Chisq)
## model3    4 1399.84 1421.25 -695.92  1391.84
## model4    6  578.95  611.06 -283.47   566.95 824.89  2  < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

AIC(model4,model3)

##        df       AIC
## model4  6  585.6463
## model3  4 1408.4627
```

```
res<-coef(model3)$ID[,1]
summary(model3)
```

```
## Linear mixed model fit by REML ['lmerMod']
## Formula: logforce ~ disp + (1 | ID)
##    Data: togxy
##
## REML criterion at convergence: 1400.5
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -6.6940 -0.5245  0.0397  0.5649  3.3689
##
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  ID       (Intercept) 0.79794  0.8933
##  Residual             0.09019  0.3003
## Number of obs: 1559, groups:  ID, 163
##
## Fixed effects:
##             Estimate Std. Error t value
## (Intercept)  3.15251    0.07242   43.53
## disp         3.91990    0.03037  129.06
##
## Correlation of Fixed Effects:
##      (Intr)
## disp -0.234
```

```
confint(model3)
```

```
##                 2.5 %    97.5 %
## .sig01      0.8003960 0.9982557
## .sigma      0.2894067 0.3117057
## (Intercept) 3.0100833 3.2946459
## disp        3.8600902 3.9794405
```

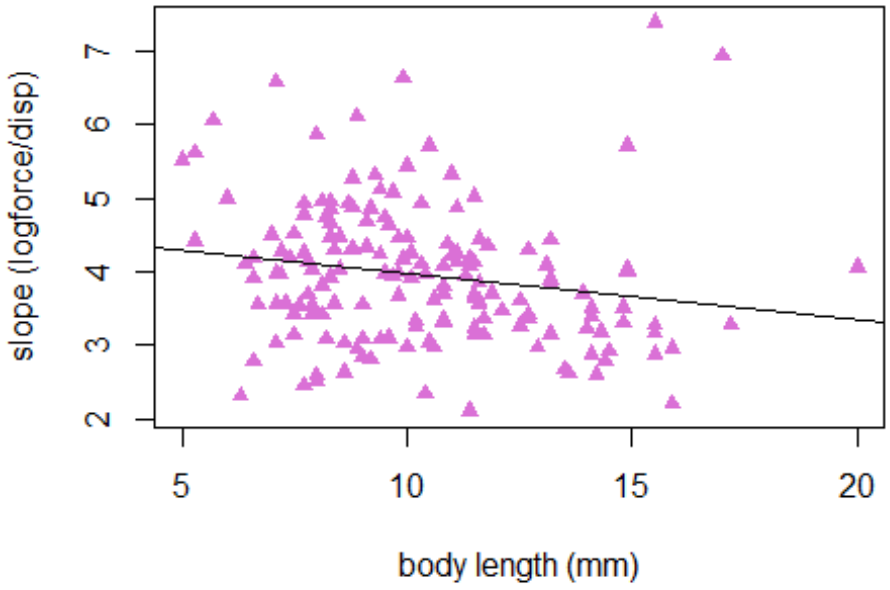
D.4 Justify exclusion of slope in analysis

```
dy <- read_excel("C:/Users/statistics & data/micrm4R5.xlsx",
                 sheet = "y values")
dy<- subset(dy, dy$mkillr != "NA")
dy$slope<-coef(model4)$ID[,2]
slopsz<-(lm(slope~bodyL,data=dy))
summary(slopsz)
```

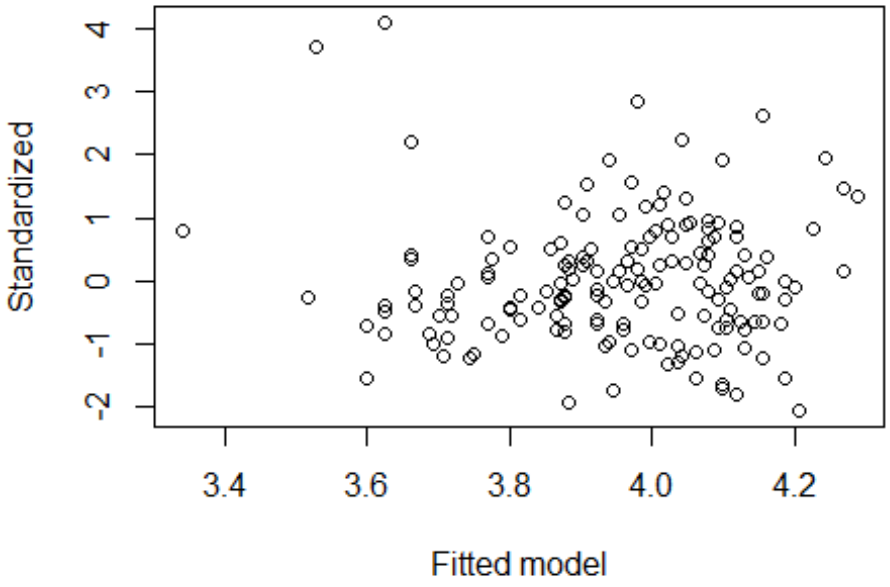
```
##
## Call:
## lm(formula = slope ~ bodyL, data = dy)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -1.8918 -0.6177 -0.0877  0.4679  3.7665
##
## Coefficients:
```

```
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  4.60316    0.28489  16.158   <2e-16 ***
## bodyL       -0.06312    0.02668  -2.366   0.0192 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.9327 on 161 degrees of freedom
## Multiple R-squared:  0.03359,    Adjusted R-squared:  0.02759
## F-statistic: 5.596 on 1 and 161 DF,  p-value: 0.01919
```

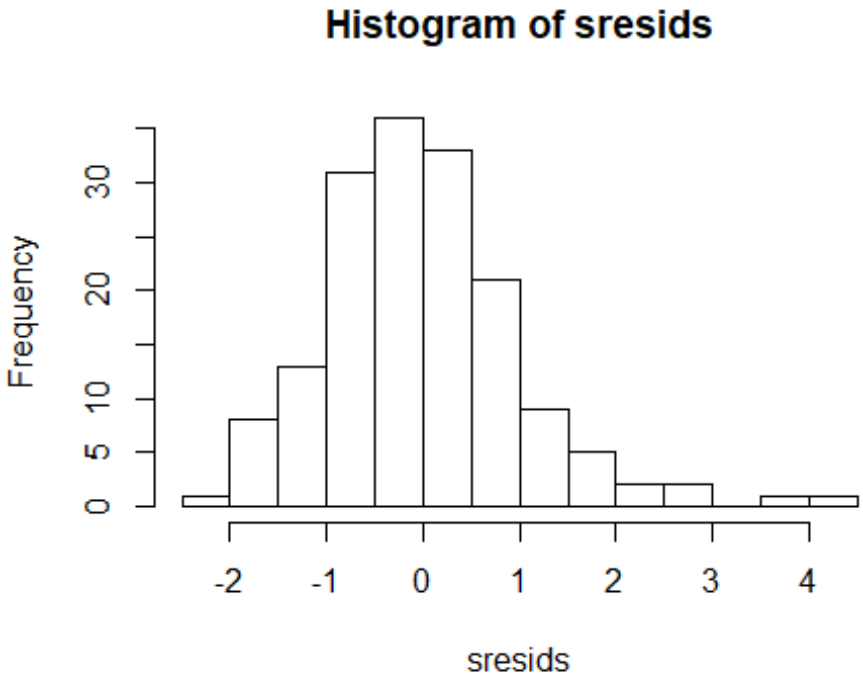
```
plot(dy$slope~dy$bodyL, pch=17, col="orchid",
     xlab="body length (mm)",ylab="slope (logforce/disp)")
abline(slopsz)
```




```
sresids<- rstandard(slopsz)
fittedv<- fitted(slopsz)
plot(fittedv,sresids, xlab = "Fitted model", ylab = "Standardized")
```

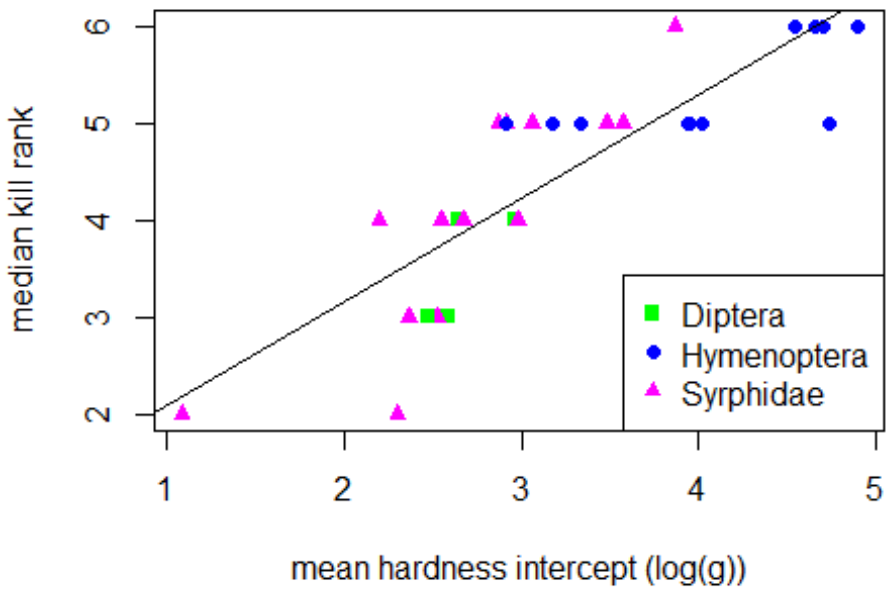



```
hist(sresids)
```



D.5 Micrometre aggregate data & visualise

```
dy <- read_excel("C:/Users/statistics & data/micrm4R5.xlsx",
                 sheet = "y values")
#narrow down to averages/ medians for subgroups
dy<- subset(dy, dy$mkillr != "NA")
dy$rani<- coef(model3)$ID[,1]
dy$Group<-as.factor(dy$Group)
rdata1<- aggregate(rani~subgroup,  data = dy, FUN = mean, na.action = n
a.pass )
rdata2<- aggregate(mkillr~subgroup,  data = dy, FUN = median,na.action
= na.pass)
rdata3<- aggregate(bodyL~subgroup, data = dy, FUN = mean, na.action = n
a.pass)
group<-c("Hym","Hym","Dip","Dip","Syr","Syr","Syr","Syr","Hym","Syr","H
ym","Hym","Hym","Hym","Hym","Syr","Hym","Dip","Syr","Syr","Syr","Syr","
Syr","Syr","Dip","Syr","Syr","Hym","Hym")
rdata2$Group<-as.factor(group)
finald<- merge(rdata1,rdata2)
finald<-merge(finald,rdata3)

plot(finald$rani, finald$mkillr, pch=c(15:17)[finald$Group], col=(c("gr
een", "blue","magenta")[finald$Group]),
       ylab="median kill rank",xlab="mean hardness intercept (log(g))")
legend("bottomright",legend=c("Diptera", "Hymenoptera","Syrphidae"),
       pch=c(15:17),col=c("green", "blue","magenta"))

groupmodel<- glm(mkillr~rani, data = finald)
abline((coef(summary(groupmodel))[1]),(coef(summary(groupmodel))[2]))
```



D.6 Micrometre linear model summary

```
groupmodel<- glm(mkillr~rani, data = finald)
summary(groupmodel)

##
## Call:
## glm(formula = mkillr ~ rani, data = finald)
##
## Deviance Residuals:
##      Min        1Q    Median        3Q       Max
## -1.47878  -0.24945   0.00848   0.41413   0.91193
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)   1.0146     0.4277   2.372   0.0251 *
## rani          1.0697     0.1271   8.417 4.99e-09 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for gaussian family taken to be 0.3806407)
##
##     Null deviance: 37.241  on 28  degrees of freedom
## Residual deviance: 10.277  on 27  degrees of freedom
## AIC: 58.215
```

```
##
## Number of Fisher Scoring iterations: 2
```

```
confint(groupmodel)
```

```
## Waiting for profiling to be done...
```

```
##                 2.5 %   97.5 %
## (Intercept) 0.1763540 1.852875
## rani        0.8206299 1.318850
```

D.7 OLR Resistance magnitude and kill rank

```
library(MASS)
```

```
finald$mkillr1<-factor(finald$mkillr,levels=c("1","2","3","4","5","6"))
ordhard<-polr(mkillr1~rani, data=finald, Hess=TRUE)
summary(ordhard)
```

```
## Call:
## polr(formula = mkillr1 ~ rani, data = finald, Hess = TRUE)
##
## Coefficients:
##      Value Std. Error t value
## rani 5.738      1.644   3.491
##
## Intercepts:
##     Value   Std. Error t value
## 1|2 -1.8923 51.7463    -0.0366
## 2|3 11.9034  4.2346     2.8110
## 3|4 14.2574  4.3195     3.3007
## 4|5 16.7395  4.7471     3.5263
## 5|6 24.6835  7.1194     3.4671
##
## Residual Deviance: 40.29806
## AIC: 52.29806
```

```
st <- coef(summary(ordhard))
pval <- pnorm(abs(st[, "t value"]),lower.tail = FALSE)* 2
st <- cbind(st, "p value" = round(pval,9))
st
```

```
##          Value Std. Error     t value     p value
## rani  5.737957   1.643804  3.49065749 0.000481834
## 1|2  -1.892341  51.746279 -0.03656961 0.970828172
## 2|3  11.903393   4.234631  2.81096359 0.004939337
## 3|4  14.257445   4.319544  3.30068329 0.000964497
## 4|5  16.739535   4.747105  3.52626200 0.000421470
## 5|6  24.683522   7.119411  3.46707358 0.000526158
```

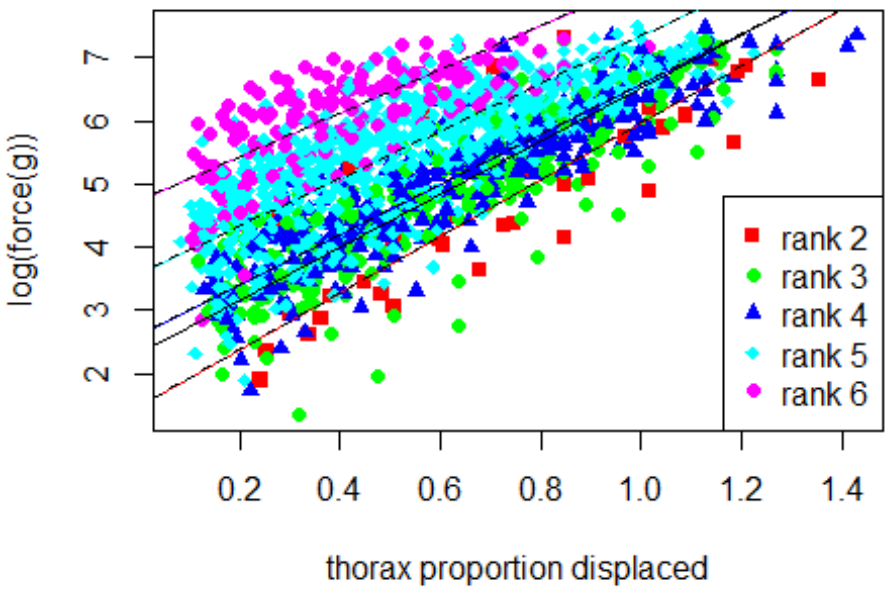
```
ci<-confint(ordhard)
```

```
## Waiting for profiling to be done...

ci

##    2.5 %   97.5 %
## 3.161710 9.677514
```

D.8 Micrometre Trendline Visualisation

```
plot(togxy$disp,togxy$logforce, pch=c(14:19)[togxy$mkillr], col=c("blac
k","red","green","blue","cyan","magenta")[togxy$mkillr],xlab="thorax pr
oportion displaced",ylab="log(force(g))")
legend("bottomright",legend=c("rank 2", "rank 3","rank 4","rank 5","ran
k 6"),
       pch=c(15:19),col=c("red","green","blue","cyan","magenta"))
model0<- lmer(logforce~disp*as.factor(mkillr) + (1|subgroup/ID), data =
togxy) # interaction model
summary(model0)

## Linear mixed model fit by REML ['lmerMod']
## Formula: logforce ~ disp * as.factor(mkillr) + (1 | subgroup/ID)
##    Data: togxy
##
## REML criterion at convergence: 1194.5
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -7.3241 -0.5083  0.0401  0.5421  3.3639
##
## Random effects:
##  Groups      Name        Variance Std.Dev.
##  ID:subgroup (Intercept) 0.2435   0.4935
##  subgroup    (Intercept) 0.1064   0.3261
##  Residual                0.0867   0.2945
## Number of obs: 1559, groups:  ID:subgroup, 163; subgroup, 29
##
## Fixed effects:
##                          Estimate Std. Error t value
## (Intercept)                1.4581     0.3614   4.034
## disp                       4.5063     0.1648  27.348
## as.factor(mkillr)3         0.8400     0.4092   2.053
## as.factor(mkillr)4         1.1437     0.4001   2.859
## as.factor(mkillr)5         2.1038     0.3821   5.506
## as.factor(mkillr)6         3.2792     0.4107   7.985
## disp:as.factor(mkillr)3   -0.2932     0.1770  -1.656
## disp:as.factor(mkillr)4   -0.5324     0.1750  -3.042
## disp:as.factor(mkillr)5   -0.7407     0.1708  -4.337
```

```
## disp:as.factor(mkillr)6  -1.0542     0.2051  -5.140
##
## Correlation of Fixed Effects:
##             (Intr) disp   as.()3 as.()4 as.()5 as.()6 d:.()3 d:.()4
d:.()5
## disp        -0.336
## as.fctr(m)3 -0.883  0.297
## as.fctr(m)4 -0.903  0.303  0.798
## as.fctr(m)5 -0.946  0.318  0.835  0.854
## as.fctr(m)6 -0.880  0.296  0.777  0.795  0.832
## dsp:s.fc()3  0.313 -0.931 -0.311 -0.282 -0.296 -0.275
## dsp:s.fc()4  0.316 -0.942 -0.279 -0.316 -0.299 -0.278  0.877
## dsp:s.fc()5  0.324 -0.965 -0.286 -0.293 -0.323 -0.285  0.898  0.909
## dsp:s.fc()6  0.270 -0.803 -0.238 -0.244 -0.255 -0.309  0.748  0.757
0.775

abline(coef(summary(model0))[1], coef(summary(model0))[2],col="red") #
for rank 2
abline(coef(summary(model0))[1] + coef(summary(model0))[3], coef(summar
y(model0))[2] + coef(summary(model0))[7],col="green") # for rank 3
abline(coef(summary(model0))[1] + coef(summary(model0))[4], coef(summar
y(model0))[2] + coef(summary(model0))[8],col="blue") # for rank 4
abline(coef(summary(model0))[1] + coef(summary(model0))[5], coef(summar
y(model0))[2] + coef(summary(model0))[9],col="cyan") # for rank 5
abline(coef(summary(model0))[1] + coef(summary(model0))[6], coef(summar
y(model0))[2] + coef(summary(model0))[10],col="magenta") # for rank 6
abline(coef(summary(model0))[1], coef(summary(model0))[2],col="black",l
ty=2) # for rank 2
abline(coef(summary(model0))[1] + coef(summary(model0))[3], coef(summar
y(model0))[2] + coef(summary(model0))[7],col="black",lty=1) # for rank
3
abline(coef(summary(model0))[1] + coef(summary(model0))[4], coef(summar
y(model0))[2] + coef(summary(model0))[8],col="black",lty=2) # for rank
4
abline(coef(summary(model0))[1] + coef(summary(model0))[5], coef(summar
y(model0))[2] + coef(summary(model0))[9],col="black",lty=2) # for rank
5
abline(coef(summary(model0))[1] + coef(summary(model0))[6], coef(summar
y(model0))[2] + coef(summary(model0))[10],col="black",lty=2) # for rank
6
```


log(force(g))
2
3
4
5
6
7
0.2
0.4
0.6
0.8
1.0
1.2
1.4
thorax proportion displaced
rank 2
rank 3
rank 4
rank 5
rank 6