

**Master of Science thesis**

**Relative importance of different coloration, smell and  
shape in the predation of wasps: field experiments on  
hunting dragonflies (Odonata: *Aeshna grandis*)**

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## TIIVISTELMÄ

Myrkyllinen tai muuten syömäkelvoton eläin voi varoittaa saalistajaa haitallisuudestaan kirkkaalla värityksellä tai muulla piirteellä. Ilmiötä kutsutaan aposematismiksi. Varoitusvärimekanismeja on aiemmin tutkittu lähinnä selkärankaisten saalistajien – etenkin lintujen – näkökulmasta. Selkärangattomien saalistajien mahdollinen vaikutus aposematismien evoluutioon on saanut vain vähän huomiota osakseen. Sudenkorennot (Insecta; Odonata) ovat hyönteislahko, jonka ravinto koostuu pääasiassa muista lentävistä hyönteisistä. Ne käyttävät saalistuksessaan hyväkseen näköaistia. Eräiden tutkimusten mukaan väritys toimii osana sudenkorentojen parinvalintamekanismeja, mutta värien mahdollista vaikutusta sudenkorentojen saaliinvalintaan ei ole aiemmin tutkittu. Tutkimuksessani selvitin, välttävätkö ruskoukonkorennot (Odonata; *Aeshna grandis*) saaliinaan ampiaisia (Hymenoptera; *Vespula norwegica*), jotka ovat myrkyllisyydessään syömäkelvottomia monille saalistajille. Tutkin myös, mitkä ampiaisen ominaisuuksista – väritys, haju ja muoto – vaikuttavat ruskoukonkorentojen saalistusreaktioihin. Tein neljä eri kenttäkoetta. Ensimmäisessä kokeessa tarjosin vapaana saalistaville sudenkorennoille neljän eri tyyppin eläviä saaliseläimiä. Saalistyypit olivat: musta kärpänen, musta-keltaraidallinen kärpänen, musta ampiainen ja musta-keltaraidallinen ampiainen. Tainnutin saaliseläimet hiilidioksidilla ja maalasin kaikkien takaruumiiseen joko mustia keltaisia raitoja. Tulosten mukaan ampiaiset olivat ukonkorennoille syömäkelvottomampia kuin kärpäset. Musta-keltaraidalliset kärpäset puolestaan joutuivat harvemmin ukonkorenon hyökkäyksen kohteeksi kuin mustat kärpäset. Tämä viittaa siihen, että aposemaattinen väritys on vaarattomalle kärpäselälle valintaetu hyönteissaalistajaa vastaan. Kiinnostavaa kyllä, mustat ampiaiset eivät kärsineet varoitusvärin puuttumisesta vaan joutuivat ukonkorentojen hyökkäysten kohteeksi yhtä harvoin kuin musta-keltaiset ampiaiset. Ensimmäisen kokeen perusteella vaikutti siis siltä, että värityksen ohella ampiaisella täytyy olla jokin muu piirre, jonka perusteella korento tunnistaa sen haitalliseksi. Seuraavissa kokeissa tutkin ampiaisten mahdollisten aposemaattisten signaalien – väritys, muoto ja haju – suhteellista merkitystä saalistavan ruskoukonkorenon saaliinvalintaan. Tutkin, reagoivatko saalistavat ukonkorennot eri tavalla keinotekoisiiin mustiin, musta-keltaraidallisiin ja keltaisiin saaliisiin. Kuten ensimmäisessä kokeessa, myös tässä kokeessa ukonkorennot välttivät musta-keltaista saalistyyppiä verrattuna kahteen muuhun saaliiseen. Seuraavassa kokeessa käytin saaliina kahta keinotekoisista saalistyyppiä; vaahtomuovin palasia, jotka oli kastettu joko survotuista ampiaisista tai kärpäseläimistä tehtyyn

liemeen. Kokeen avulla selvitin, vaikuttaako haju yksinään saalistajan reaktioihin. Tulosten mukaan hajulla ei ole merkitystä ukonkorentojen saaliinvalinnassa. Viimeisessä kokeessa tarjosin mustia, hajuttomia karpäsen ja ampiaisen muotoisia saaliita saalistaville ukonkorennoille. Tulosten mukaan sudenkorennot välttivät ampiaisen muotoisia saaliita verrattuna karpäsen muotoisiin saaliisiin. Tulos ei kuitenkaan ollut merkitsevä. Neljän kokeen tulokset viittaavat siihen, että kelta-mustaraidallinen väritys yhdistettynä muotoon tekee ampiaisesta ukonkorennonle huonon saaliin. Koska myös musta-keltainen väritys yksinään vähensi hyökkäystodennäköisyyttä saalista kohtaan, syötäväksi kelpaavat saalistyyppit voivat käyttää hyväkseen ukonkorennon taipumusta välttää tätä väriyhdistelmää (Batesin mimikry). Tulevaisuudessa olisi kiinnostavaa selvittää, onko välttämistäipumusten taustalla opittu reaktio vai onko välttäminen geneettistä.

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## ABSTRACT

Aposematism is a phenomenon, where poisonous or acrid prey signal their unprofitability to potential predators with conspicuous colouration or some other feature. The mechanisms of aposematism have – excluding a small number of exceptions – been studied from the vertebrate predators' (especially birds) point of view. In contrast, the possible impact of invertebrate predators to the evolution of aposematism has gained only little interest. Dragonflies (Insecta; Odonata) are a numerous group of invertebrate predators that feed mainly on flying insects and exercise prey selection by vision. Although it has been reported that colours do work as cues in the mating systems of dragonflies no studies have been carried out tackling the question whether dragonflies use their colour vision when exercising prey selection. In the present field experiments I investigated whether hunting dragonflies (Odonata: *Aeshna grandis*) avoid attacking on wasps (Hymenoptera: *Vespula norvegica*), that are a highly unprofitable group of prey for most predators. Further, I was interested in finding out which features of the wasp – colouration, smell and shape – affect the attack rates of hunting dragonflies. To tackle these questions I carried out four field experiments. In the wasp avoidance experiment I offered four different types of living prey (black flies, black-and-yellow flies, black wasps and black-and-yellow wasps) to the hunting dragonflies. I stunned the prey items with carbon dioxide and manipulated all of them either with black or yellow paint. According to the results, the dragonflies avoided the wasps over the flies. Within the flies the black-and-yellow coloured individuals were significantly avoided over the black ones suggesting that aposematic colouration on harmless fly provided a selective advantage against invertebrate predators. Interestingly, there was no significant difference in the reactions towards the black and the black-and-yellow wasps indicating that some other feature in wasps might work as well as an aposematic signal. In the next three experiments I studied the relative importance of the possible aposematic signals: coloration, shape and smell in wasps. First, I tested whether hunting dragonflies react differently on solid black, black-and-yellow striped and solid yellow artificial prey items. In accordance with the wasp experiment dragonflies clearly avoided the black-and-yellow artificial prey items over the solid black or solid yellow ones. In the second experiment I used two artificial prey types (prey item soaked in mashed wasps and prey item soaked in mashed flies) to test if the smell of the prey alone causes different reactions against the prey. The results suggest that smell of the prey did not

have any influence on the dragonfly hunting reactions. In the third experiment I offered artificial wasp shaped and a fly shaped prey item to free flying dragonflies. According to the results there was a slight but not significant difference suggesting that dragonflies were more reluctant in attacking the wasp shaped items than the fly shaped ones. My results suggest that most likely the typical black-and-yellow striping combined with shape makes wasps highly intimidating to dragonflies. Since black-and-yellow striping alone significantly decreased attack rate, even profitable prey species (Batesian mimics) are able to exploit the dragonflies' avoidance to certain colours and colour patterns. It is a task for future work to investigate whether these avoidances are learned or whether they have a genetical basis.

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## 1. INTRODUCTION

Unpalatable or otherwise unprofitable prey species sometimes advertise their unsuitability for predators with bright coloration or some other feature (Poulton 1890, Cott 1940, Edmunds 1974). This phenomenon, aposematism, is along with Batesian and Mullerian mimicry generally regarded as an example of post-Darwinian application of natural selection.

Warning colours are assumed to directly function in enhancing the effectiveness of warning signals by exploiting predator learning mechanisms (Gittleman & Harvery 1980, Gittleman et al. 1980, Roper & Wistow 1986, Roper & Redston 1987, Schuler & Roper 1992). Conspicuous warning colouration is reported to both facilitate the process of predator learning and prolong the time that the predator remembers the conspicuous colour pattern informing the unprofitability of the prey (Guilfoird 1986, 1990). If the predator is able to learn the signal of unprofitability an opportunity opens for a cheater to exploit the avoidance. This phenomenon where an unpalatable species mimics a palatable one is called Batesian mimicry (Bates 1862, Brower 1960, Papageorgis 1974, Mallet & Singer 1987, Brown 1988, Mappes & Alatalo 1997b).

Originally, it was assumed that the avoidance of the predator towards an aposematic prey type can only be due to learning that takes place after unpleasant encounters (e.g. Cott 1940). However, in the recent studies it has been found out that predators may also have an innate (Smith 1975, Schuler & Hesse 1985, Rowe & Guilford 1996), or unlearned (Roper & Cook 1989) ability to avoid conspicuously coloured prey types. The predators may for example show an avoidance behaviour towards black/yellow/red prey types presented for the first time to the predator and a simultaneous preference towards green/blue/brown prey (Fisher et al. 1975, Schuler & Hesse 1985, Kovach 1987, Roper & Cook 1989, Mastrola & Mench 1994, Rowe & Guilford 1996, Lindström et al. 1999b). Innate avoidance also often takes place in situations where the prey is highly poisonous for the predator as in the case of lethally toxic coral snakes avoided innately by herons and egrets (Caldwell & Rubinoff 1983).

Both the ecological and the evolutionary determinants of aposematism have been repeatedly studied using vertebrate predators (great tit, e.g. Alatalo & Mappes 1996; chicken, e.g. Gittleman & Harvery 1980; toad, Brower et al. 1970; lizard, Boyden 1976; fish, Tullroth 1998; snake, Terrick et al. 1995). There are at least two obvious reasons for this. First, as the feature to advertise the aposematic nature of the prey type is in many cases colour pattern, the potential predators are required to have colour vision or at least the ability to see colour contrasts. Secondly, in order to actually work as a protection mechanism to the prey, aposematism requires a predator that is able to learn and remember. Vertebrates, especially birds, most often meet both of these requirements.

Along with vertebrate predators there are also certain groups of invertebrates that actively forage on other invertebrates and also regularly exercise prey selection by vision (mantids, Berenbaum & Milizcky 1983, Bowdish & Bultman 1993; dragonflies, Baird 1991, Frye & Oldberg 1995). The first study to investigate the interaction between an invertebrate predator and an aposematic prey animal was an experiment carried out by Berenbaum and Milizcky (1984) with mantids and milkweed bugs. The results of their experiment suggest that an invertebrate predator (a mantid) can actually learn to avoid an aposematic animal (a milkweed bug) as prey and that this avoidance is beneficial for the predator.

Dragonflies (*Odonata*) are a numerous group of medium sized or large, visually hunting insects (Askew 1988). A hunting dragonfly bases its decision to attack on the visual features of the prey (Mokrushov 1972, Hatto 1994, Rowe 1987, Edman & Haeger 1974, Baird 1991). The first stimulus to trigger the hunting mode of a dragonfly

potential prey item actually meets the criteria of acceptability, dragonflies often either come close to or even physically encounter the possible, flying prey item (Pajunen 1964, Parr 1983). After locating the moving prey the dragonfly is supposed to estimate its size. Certain species are reported to reject too large prey items (Corbet 1999) whereas some actually prefer relatively large items (Edman & Haeger 1974) or avoid potential prey items sized below a certain limit (Baird 1991). A few cases are also reported where dragonflies attack on objects larger than themselves, e.g. individuals of larger dragonfly species and even birds (Stearns 1961). Along with prey size the prey shape is also known to play a part in the prey selection of dragonflies (Corbet 1999).

It is known that females of certain dragonfly species choose their mates by using colour cues (Corbet 1999). It has also been shown that dragonflies are able to perceive ultraviolet reflections (Horvath 1995). Although it seems self evident that dragonflies have both colour vision and ultraviolet vision, no experiments have been carried out tackling the question of whether dragonflies use these abilities in their prey selection.

Different interactions can be found among the studies concerning dragonflies and aposematic prey animals. Field observations of O'Donnell (1996) suggest that dragonflies (*Gynacantha nervosa rambur*) avoid wasps (*Polybia aequatorialis* Zavattari and *Mischocyttarus* sp.) as prey. According to O'Donnell's observations dragonflies follow the wasps for a short instant but eventually reject them for an unknown reason. As a total contrary to the observations of O'Donnell, Rowe (1987) reports that some large species actively forage on bees and wasps. Moreover, the results of an experiment made by White & Sexton (1989) suggest that dragonflies (Gomphidae, *Hagenius brevistylus*) actively prey on aposematically coloured monarch butterflies (Lepidoptera: Danaidae) using a feeding mode that is virtually identical to that of some birds (i.e. leaving the most poisonous part of the butterfly, the wings, untouched). The remarkable differences in the interactions of dragonflies and their potential aposematic prey items are most likely due to the variety of poisonousness in the prey animals and also to the local counter adaptations of dragonflies and their prey. The observations of O'Donnell (1996), Rowe (1987) and White & Sexton (1989) offer valuable information about the interactions between dragonflies and aposematic prey animals, however, they do not provide any convincing evidence suggesting that the aposematic signals actually have any effect on the hunting behaviour of the dragonflies.

The aim of the present study was to find out whether dragonflies (brown hawker, *Aeshna grandis*) avoid wasps (*Vespula norvegica*) as prey, and further, which cues do they use in decision making while hunting. To tackle these questions I carried out four separate field experiments using free flying dragonflies as predators. First, I tested whether dragonflies avoid wasps over flies, and then what is the relative importance of different signals (coloration, shape and smell) of wasps that might cause the observed avoidance.

## 2. MATERIALS AND METHODS

### 2.1. Study area and the predator species

The present experiments were carried out on the surroundings of Konnevesi research station (62°37'N, 26°20'E), in central Finland during August 1999 and June-August 2000.

I used brown hawker (Odonata: *Aeshna grandis*) as the predator in all four experiments. Brown hawker lives its larval stage in water and after emerging disperses away from the water until it is mature (Askew 1988). Hawkers – like most species of dragonflies – spend their 'maiden flight' time mainly by feeding on other flying insects on shiny, warm spots in the woodlands. The experiments were carried out on these swarming



spots. Because – along with immature individuals – also mature dragonflies are reported to forage on these swarming spots (Askew 1988), the set of the predators used in my studies most likely included both mature and immature dragonflies. Brown hawker was a suitable species to be used as the predator because it is a fairly common in Central Finland. It is also large enough (69-76mm in total body length) to attack and forage on the wasp sized prey insects. Moreover, the flight period of brown hawkers is quite long lasting from the end of June to the early October.

## 2.2. Study design of the wasp avoidance experiment

The wasp avoidance experiment was carried out during the daytime hours (11am - 17pm) of seven sunny days in August 1999 (the 3rd, the 4th, the 5th, the 6th, the 9th, the 17th and the 18th of August).

The wasps (*Vespula norvegica*) and the flies (*Sargophagidae sp*) used in the wasp avoidance experiment were caught with a butterfly net from the surrounding meadows of the Konnevesi research station. I caught the animals during the previous or the same day as they were used in the experiment to ensure that they were still alive and fresh. Meantime, I stored them in a refrigerator (+7-10 degrees).

I divided the prey items into four treatments that were: I = black flies, II = black-and-yellow flies, III = black wasps and IV = black-and-yellow wasps. I then painted all the animals of the four treatments to ensure that the paint itself – for example its smell – did not cause any bias to the results. I did the painting with a thin brush after stunning the prey items with carbon dioxide in a test-tube. I painted three black stripes on the normally dark abdomen of the flies in the treatment I (black flies) and three yellow stripes on the abdomen of the flies in the treatment II (black-and-yellow flies). The normally yellow parts of the wasps in the treatment III were painted black. Thus, they turned out as ‘black wasps’ losing their characteristic black-and-yellow colouration that is generally considered to be aposematic. In contrast, the wasps of the treatment IV (black-and-yellow wasps) got three black stripes on the naturally black parts of their abdomen. Hence, they ended up carrying a colouration fairly close to that of their natural one.

I recorded the reflectances of the black and the yellow paints used in painting the prey animals and compared them to the natural reflectances of the black and the yellow parts of the prey animals. I did this to ensure that the paint spectral reflectance curves and the natural colouration spectral reflectance curves were roughly of the same shape, in other words that the yellow paint used in the experiment was really yellow and eg. no unexpected ultraviolet peaks would affect the results. All of the reflectances were recorded in the range 360-700 nm at 5 nm intervals using a spectroradiometer (EG&G Gamma Scientific GS3100 Radiometer, Light Touch Software 1.04a). I measured the reflectances as a proportion of the light reflected from a calibrated 98 % white standard (LabSphere™).

I weighed the prey items before using them in the experiment. Only the ones that weighed more than 45 mg and less than 70 mg were used. Others were released immediately after catching and weighing. The individuals that met the size criteria were systematically divided accordingly their weight into four treatments. The mean weights of the four treatments were: black flies  $63.8 \pm 7.6$ ; black-and-yellow flies  $66.7 \pm 8.0$ ; black wasps  $71.8 \pm 19.7$ ; black-and-yellow wasps  $69.5 \pm 5.9$ , mean $\pm$ SD respectively. There was no significant difference between the sizes of these four groups ( $F_{3,78} = 1.070$ ,  $p = 0.367$ ). Neither did the variances of the groups differ significantly ( $p = 0.370$ ).

The total number of the prey items was 79 (black flies: 23; black-and-yellow flies: 20; black wasps: 19; black-and-yellow wasps: 17). If the dragonfly attacked and damaged the prey it was replaced with a fresh one. However, one prey individual was never presented to not more than three dragonfly individuals.

I offered the prey items one by one attached to a two meter long piece of thin fishing line. I attached the prey items to the fishing line with a drop of instant glue after the painting when the prey item was still unconscious and easy to handle. A tiny piece of black paper (about 2mm x 2mm) was first glued on the tip of the fishing line to facilitate the attaching of the line to the ventral side of the thorax of prey animal. The other tip of the fishing line was bound to a 2,5 m long fishing rod. The prey items attached to the fishing line flew properly for a while but soon got exhausted and did not make any flight movements when later offered to the swarming brown hawkers. The only movements that took place were the side-to-side swings and the little nods that were (when offering the prey) made to provoke the dragonflies. Since the prey items were practically immobile when offered to the predator the characteristic way of flying or the flying sounds did not affect the hunting reactions of the dragonflies.

I recorded five different degrees of attack intensity of dragonflies: 1) encounters where the dragonfly clearly stopped and noticed the prey but did not touch it; 2) encounters where the dragonfly slightly touched the prey but did not grab it in to its forefeet like to forage it; 3) encounters where the dragonfly grabbed the prey into its forefeet for an instant shorter than two seconds and then let go of it; 4) encounters where the dragonfly grabbed the prey into its forefeet for an instant longer than two seconds but did not kill it; 5) encounters where the dragonfly grabbed the prey and killed it (in these cases the hawkers also usually foraged at least part of the prey).

During the experiment, I registered a total of 27 encounters of brown hawkers and black flies, 24 encounters of hawkers and black-and-yellow flies, 24 encounters of hawkers and black wasps and 25 encounters of hawkers and black-and-yellow wasps.

As soon as one of the swarming brown hawkers noticed and encountered the prey item, I observed the reactions of the hawkers and switched to another swarming spot at least 500 meters away from the previous one (except when I could tell for sure that two different dragonfly individuals encountered the prey at the same spot). Moreover, I never visited the same swarming spot twice. Thus, I made sure that each hawkers was only used once in my experiment and no pseudo-replication took place.

### **2.3. Study design of the colouration experiment**

The colouration experiment was carried out during the daytime hours (11am - 17pm) of ten sunny days in June and August 2000 (between the 11<sup>th</sup> June and the 31<sup>st</sup> of August). In this experiment I investigated the possible effects of the prey colouration alone on the decision making of a hunting dragonfly. As prey in the experiment I used cylinder shaped pieces of rubber that were painted black, yellow or black-and-yellow striped with odourless paints. The diameter of one artificial prey item was 0,5 centimeters and the length 0,4 centimeters. I attached the artificial prey items to the fishing line with a 1,5 cm piece of iron wire.

The procedure of the prey offering was the same as in the wasp avoidance experiment. In this experiment I recorded three different degrees of attack intensity of dragonflies: 1) encounters where the dragonfly clearly stopped and noticed the prey but did not touch it; 2) encounters where the dragonfly slightly touched the prey item but did not grab it in to its forefeet like to forage it; 3) encounters where the dragonfly grabbed the prey item into its forefeet.

During the colouration experiment, I registered a total of 33 encounters of brown hawkers and black, rubber prey items, 35 encounters of hawkers and yellow prey items and 32 encounters of hawkers and black-and-yellow striped prey items. (Fig. 2a).

## 2.4. Study design of the smell experiment

The smell experiment was carried out during the daytime hours (11am - 17pm) of five sunny days in June and August 2000 (between the 11<sup>th</sup> June and the 31<sup>st</sup> of August). The aim of this experiment was to investigate whether the smell of the prey has an effect on the hunting behaviour of a dragonfly. The prey items in this experiment were artificial: 0,5 x 0,5 x 0,5 cm pieces of foamy plastic. I smashed five insects (wasps or flies) and a drop of water in a test-tube with a rod made of glass. I then dipped a piece of foamy plastic to the wasp or fly liquid to make the smell stick to the foamy plastic prey. The prey items were attached to the fishing line with a 1,5 cm piece of iron wire. The procedure of the prey offering was equal to the one that I used in the wasp avoidance experiment.

In this experiment I recorded three different degrees of attack intensity of dragonflies: 1) encounters where the dragonfly clearly stopped and noticed the prey but did not touch it; 2) encounters where the dragonfly slightly touched the prey item but did not grab it in to its forefeet like to forage it; 3) encounters where the dragonfly grabbed the prey item into its forefeet.

In the smell experiment I registered a total of 17 encounters of hawkers and foamy plastic prey items that smelled like fly and 20 encounters of hawkers and prey items that smelled like wasps. (Fig. 2b).

## 2.5. Study design of the shape experiment

The shape experiment was carried out during the daytime hours (11am - 17pm) of five sunny days in June and August 2000 (between the 11<sup>th</sup> June and the 31<sup>st</sup> of August). In this experiment I investigated whether the shape of the prey item alone affects the hunting behaviour of a dragonfly. As prey items I used dried wasps and flies that were painted solid black with odourless paint. Thus, no colouration or smell could affect the predator reactions towards the prey. Before drying and painting I weighed the prey items. Thus, I was able to choose wasps and flies that were of the same size (67.0mg–68.0mg) and the size of the prey item would not affect the results.

In this experiment, again, I recorded three different degrees of attack intensity of dragonflies: 1) encounters where the dragonfly clearly stopped and noticed the prey but did not touch it; 2) encounters where the dragonfly slightly touched the prey item but did not grab it in to its forefeet like to forage it; 3) encounters where the dragonfly grabbed the prey item into its forefeet.

During the shape experiment I registered a total of 30 encounters of brown hawkers and fly shaped prey items and 30 encounters of hawkers and wasp shaped prey items. (Fig. 2c).

## 2.6. Data analysis

The experimental data of the wasp avoidance study were analysed by using an ANOVA model, where the reactions of the predators (after ln-transformation) were the dependent factors and the colour (black, black-and-yellow) and the species (fly, wasp) of the prey the categorical factors. All p-values are for two-tailed tests. The data of the colouration experiment and the smell experiment were analysed by using Kruskal-Wallis one-way ANOVA. The data of the shape experiment was analysed by using Mann-Whitney U-test.

Sex of the dragonflies did not affect their attack intensity in any of the experiments, neither did the day or the time of the day (all p-values > 0.40) affect the results. Thus, no regard was paid for these variables in the further analysis.

### 3. RESULTS

#### 3.1. The wasp avoidance experiment

There was a significant interaction in the wasp avoidance experiment between the colouration and the species of the prey ( $F_{3,79} = 4.52$ ,  $p = 0.037$ ). Thus, the effect of the colouration on the attack intensity of the predators depended on whether the prey was a wasp or a fly. Generally, the attack intensity against wasps was very low and did not differ between the two colour groups of wasps ( $t = -0.25$ ,  $df = 34$ ,  $p = 0.805$ ). However, in flies the black-and-yellow striping significantly decreased the attack intensity ( $t = 2.60$ ,  $df = 41$ ,  $p = 0.013$ ). (Fig. 1.).

To rule out the possibility that the time of the day would affect the results I divided it into three categories: 1) 11 a.m.-13 p.m., 2) 13-14 p.m., 3) 14 p.m.-. I did not find any effect of time of the day on the predator reactions ( $F_{2,78} = 0.290$ ,  $0.749$ ). Finally, I tested the effects of the prey mass on the predator reactions finding no significance here either (in flies:  $r_s = -0.38$ ,  $N = 43$ ,  $p = 0.811$ ; in wasps:  $r_s = -0.16$ ,  $N = 36$ ,  $p = 0.359$ ).

#### 3.2. The colouration experiment

In this experiment, the colouration of the artificial prey item significantly affected the reactions of the dragonflies ( $H = 6.77$ ,  $n = 100$ ,  $p = 0.034$ ). In pair-wise comparison there was a significant difference between the predator reactions against the black and the black-and-yellow prey items ( $z = 2.417$ ,  $n_1 = 32$ ,  $n_2 = 33$ ,  $p = 0.048$ ; after sequential Bonferroni correction). However, I found no significant difference when comparing the predator reactions towards the black items and the yellow items or the yellow items and the black-and-yellow items ( $z = -1.756$ ,  $n_1 = 33$ ,  $n_2 = 35$ ,  $p = 0.158$  and  $z = -0.925$ ,  $n_1 = 32$ ,  $n_2 = 35$ ,  $p = 0.355$ ; after sequential Bonferroni correction), respectively. (Fig.2). Thus, the results are in accordance with the wasp avoidance experiment but the colouration experiment shows that the coloration per se has significant effect on the predator reaction. (Fig. 2a).

#### 3.3. The smell experiment

I found no difference between the reactions of dragonflies against wasp-smelling and fly-smelling prey items ( $z = -0.130$ ,  $n_1 = 17$ ,  $n_2 = 20$ ,  $p = 0.916$ ). (Fig. 3.). This suggests that hawkers do not use smell as a cue when making the decision to attack. (Fig. 2b).

#### 3.4. The shape experiment

In the present experiment I found a tendency but not significant, that dragonflies are more reluctant to attack against wasp shaped prey items compared to the fly-shaped ones ( $z = 1.816$ ,  $n_1 = 30$ ,  $n_2 = 30$ ,  $p = 0.069$ ). (Fig 2c.).

### 4. DISCUSSION

In the wasp avoidance experiment the brown hawkers significantly avoided the wasps over the flies (Fig. 1.). Initially, the possible features to cause this avoidance are: colouration, shape, smell, taste, sound, the way of moving. Also a combination of two or more of these features (i.e. multiplicity signal) is possible.

The sound and the way of moving were eliminated in the present experiments by offering the prey items to the predator attached to the fishing line. The taste of the prey could not have had any effects on the attack intensity either because in the wasp avoidance experiment majority of the dragonflies rejected the wasps before ever touching them

[ $x(\text{attack rate}) = 1,3$ ]. The role of these three features in the wasp signalling system remains vague. Further, the results of the smell experiment suggest that the smell of the prey does not have any effects on the attack intensity when the predator is a brown hawkker (Fig. 2b). Thus, the possible features to cause the wasp avoidance in the wasp avoidance experiment could have been the colouration or the shape of the wasps or both of these.

In the wasp avoidance experiment, there was a significant interaction between the colouration and the species of the prey. In other words the black-and-yellow colouration significantly decreased the attack intensity of the dragonflies towards the flies but in wasps the colouration did not affect the hunting behaviour of the brown hawkkers (Fig. 1). Moreover, the dragonflies of the colouration experiment significantly avoided the black-and-yellow prey items over the black ones (Fig. 2a). First of all, these results suggest that hawkkers actually see colours which is in line with some earlier studies (Corbet 1999). Secondly, it seems that the colouration of the potential prey item can be used as an aposematic cue by a hunting dragonfly. In wasps, the attack intensity of the hawkkers was generally low despite the fact that their colouration was solid black or black-and-yellow. In other words the possible effects of the wasp colouration were diluted by some other feature. This inevitably leads one to think about the role of the wasp shape in the aposematic signalling. Although in the shape experiment neither the shape of a wasp nor the shape of a fly gave the prey item significant protection against a dragonfly there was a tendency ( $p = 0.069$ ) that dragonflies did not attack the wasp shaped prey items as openly as the fly shaped ones. (Fig. 2c). Thus, it could have been the shape that gave the wasps protection in the wasp avoidance experiment in the situation where they did not carry their characteristic black-and-yellow warning colouration (Fig. 1).

Aposematic signals of potential prey animals are sometimes multicomponent, compounding of several cues (e.g. colour, taste and smell, Marples et al. 1994; smell and colouration, Rowe & Guilford 1996). For example certain *Coccinellidae* -beetles carry a multiplicity signal consisting of colour, taste and smell (Marples et al. 1994). The results of the present experiments suggest that wasps carry a multicomponent signalling system where at least colouration and shape work as aposematic cues. When other one of these components (colouration) is ruled out the other one (shape) still causes the avoidance (Fig. 2c) and vice versa (Fig. 2a). Whether the sound, the way of moving or the taste of the wasp plays a role in this system needs further investigation. Also the combination of the features signalling the unprofitability of the wasps against some more developed predators should be tested.

Almost all of the previous studies tackling aposematism have been carried out using vertebrate predators (see e.g. Alatalo & Mappes 1996, Gittleman & Harvery 1980, Brower et al. 1970). The present set of experiments, however, supports the results of a few earlier studies (see experiments with mantids and milkweed bugs by Berenbaum & Milizcky 1983 and Bowdish & Bultman 1993) to show that aposematic cues might give some protection also against an invertebrate predator.

A point that should be taken into consideration, is the possible advantage that the black-and-yellow coloured flies might have gained in the experiment due to the novelty effect. A great deal of authors have suggested that a novel, brightly coloured prey gains from its novelty in a form of decreased predation risk (Coppinger, R. P. 1969, 1970; Götmark 1994; Mappes & Alatalo 1997a; Schlenoff 1984) This is reported to be due to the quite conservative nature of many predators when selecting their prey and also to the innate bias of predators against certain colour combinations (e.g. yellow or red combined with black). On the other hand, it has also been suggested that novel, aposematic prey items actually suffer from increased predation risk posed upon the prey because of the increasing visibility (Harvey et al. 1982, Guilford 1990). However, though the black-and-yellow flies in the wasp avoidance experiment can, to some sense, be considered as a novel prey type (there are no animals that are naturally coloured *exactly* like them), their actual

novelty is strongly opened to question because of the vast variety of dipteras (Insecta: *Diptera*) and hymenopteras (Insecta: *Hymenoptera*) that naturally carry a black-and-yellow colouration. Moreover, if the black-and-yellow flies in the experiment actually gained protection from their novelty against the dragonflies, this have then merely resembled the situation of the natural novel prey types that are, as mentioned earlier, in some cases suggested to gain from their novelty.

#### 4.1. Implications to Batesian mimicry

In Batesian mimicry a palatable prey type mimics (in colour pattern, in shape etc.) an unpalatable prey type and, thus, gains protection against predation (Bates 1862, Brower 1960, Papageorgis 1974, Mallet & Singer 1987, Brown 1988, Mappes & Alatalo 1997b). A classic case of Batesian mimicry is the mimic-model -relationship between certain hoverfly (Insecta: Syrphidae) and wasp species (eg. Howarth & Edmunds 2000, Edmunds 2000). There seems to be very strong variation in the accuracy of the mimicing in the hoverfly-wasp -relationships (Edmunds 2000). The black-and-yellow flies of the avoidance experiment were kind of artificial, hoverfly-like Batesian mimics for the naturally coloured wasps. It seems that this mimicing was fairly effective even though the degree of the signal was quite imperfect (the artificial colour pattern of the black-and-yellow fly did not specifically resemble the natural one of the wasp) and the colouration alone did not seem to be that an important feature for the model (the wasp) (Fig.1). This supports the results of some earlier studies suggesting that when the model species is highly unprofitable for the predator, even quite imperfect mimic offers significant protection against predation (Dittrich et al. 1993).

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APPENDICES

Predator reaction

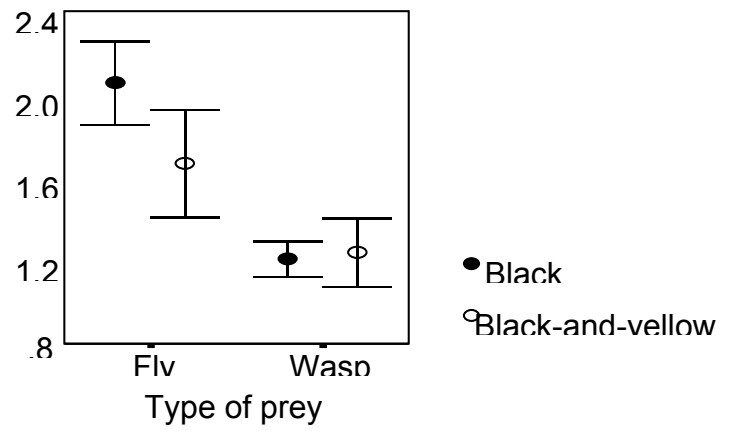


Figure 1.

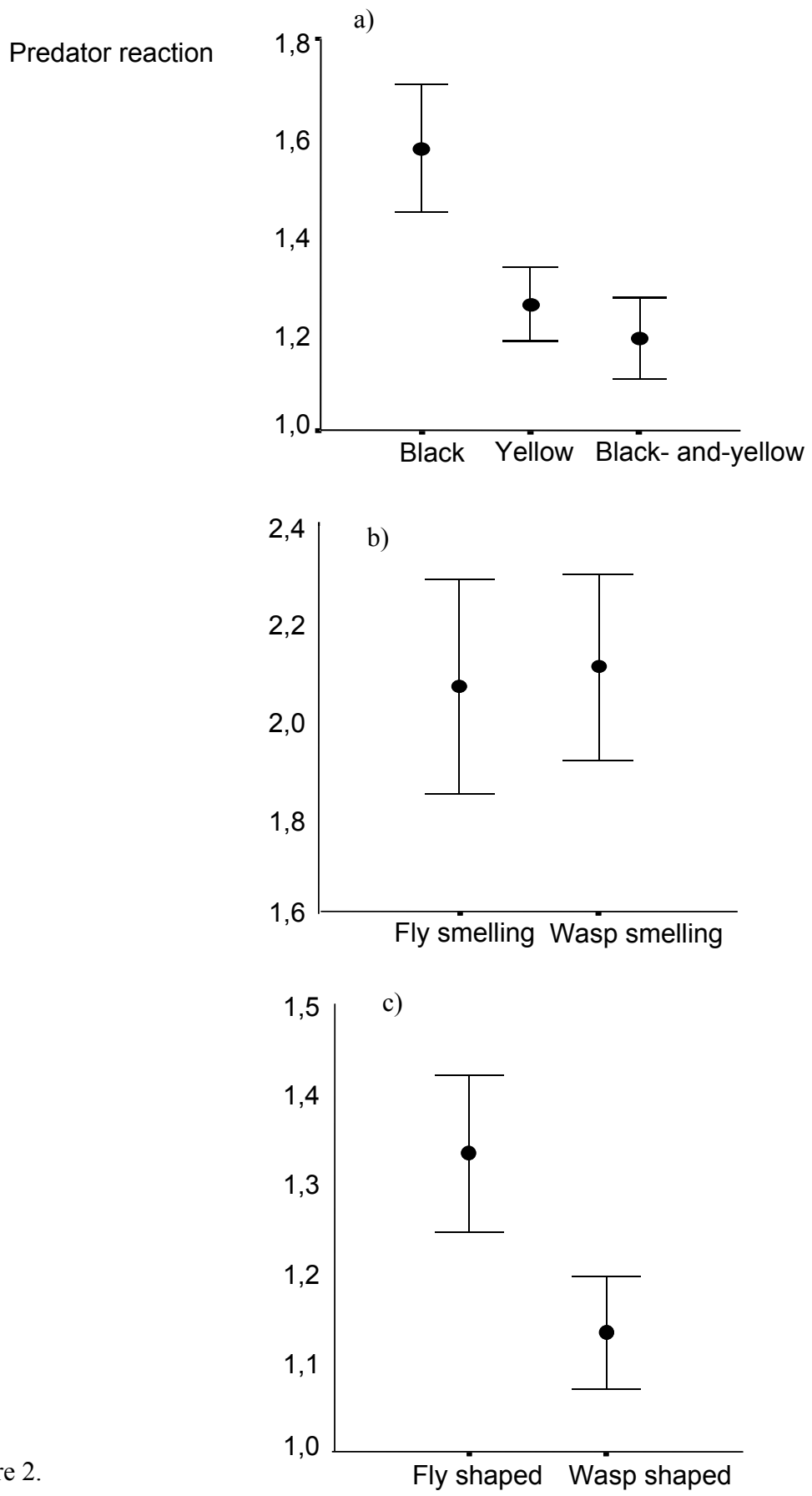


Figure 2.