The structure of a patch
– Within and between patch variation and the oviposition of
  the small white butterfly

Tove Maria Nilsson

The structure of a patch

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Abstract

The selection of a host plant for egg-laying is an important element in insect-plant relationships, the survival of the offspring often depends on the choices made by gravid females. Plants are diverse and not evenly distributed in space or time and insect females search for host plants at gradually finer spatial scales. To choose a host plant they use a combination of olfaction, vision, contact chemoreception and tactile senses and the resolution of those senses are important. Cruciferous crops are of economic importance and have a well known chemistry. Consequently the insects associated with them have received particular attention over the years. One such insect is the small white butterfly, *Pieris rapae*, a well known and worldwide pest of cruciferous crops. The objective of this MSc thesis was to gain a greater insight in the short range host selection by females of the small white butterfly and their use of senses at different spatial scales. This was done in the laboratory using three genotypes of *Brassica oleracea,* leaf models sprayed with extracts from those genotypes, and leaf models with different shape and colour. Three experiments were performed: one with plants, one with models differing in form sprayed with the same extract and one with identical models sprayed with different extracts. The genotypes or different models were distributed either evenly between three patches or concentrated in a single patch keeping the density constant. I found that the distribution of eggs on plants was not significantly affected by how the host plants were arranged but the egg distribution on the leaf models was affected. With the models differing in form the contrast increased with proximity and with the models differing in chemistry the contrast decreased with proximity. It is clear from this study that females of the small white butterfly are able to distinguish between different host plants based on both visual appearance and chemistry, but that their relative importance varies with scale. Chemistry has more impact on a slightly larger scale while the visual aspects of the leaves have the most impact at a very fine scale when the contrasting leaves can be perceived simultaneously.

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Introduction

All plants are not equal; orders, families, species, genotypes and even individual plants and leaves differ in characteristics like form (visual appearance) and chemical composition depending on both environmental and genetic factors. The heterogeneity of plants affects the host plant choices that herbivorous insects make. In general herbivorous insects show a high degree of food specialization and this is an important factor shaping insect-plant relationships (Schoonhoven et al., 1998). Oligophagous insects, such as the Colorado potato beetle (*Leptinotarsa decemlineata*) and the small white butterfly (*Pieris rapae*), feed on a number of plant species, though all belonging to the same family. Insects locate and select suitable host plants using a combination of olfaction, vision, contact chemoreception and tactile senses, these senses work differently at different scales (Jones, 1992). Processes used by insects to find and select host plants for feeding or oviposition has been well studied over the years, particular interest has been shown the insects associated with the plant family Brassicaceae. The reason for this interest is partly due to the economic importance of brassicaceous crops and partly due to the relatively well known and distinctive chemistry identified quite early. The link between plant chemistry and specialist herbivores of brassicaceous plants was first established by Verschaffelt (1910).

The small white butterfly, *Pieris rapae*, is a well known pest of brassicaceous crops. It is native to Europe and North Africa, but spread by European settlers to North America, New Zeeland and Australia (Elton, 1958). Over the years it has become the subject of a large number of scientific studies. In order to find alternative ways to reduce the damages made by *P. rapae* caterpillars it is important to understand how the host plants are chosen by the female butterflies and there are still questions to be answered. Patch spatial scale and heterogeneity are factors that could be manipulated in order to reduce the attack rate of herbivorous insects.

The objective of this study is to gain a greater insight in the short range host selection by females of the small white butterfly and their use of senses at different spatial scales. This is done by using three genotypes of *Brassica oleracea* and arranging them in homogeneous or heterogeneous patches while keeping a constant density of the genotypes. The experiments are performed in a turntable to minimize the impact of

plant position. Will the *P. rapae* females differentiate between different genotypes or models with different characteristics and will their choices be affected by how the plants are arranged? How do form and chemistry affect the oviposition choices at different spatial scales? In addition to real plants the same setup is used for leaf models sprayed with extracts derived from the different genotypes. How is the selection affected by differences in form when the plant chemistry is identical? What will be the result when the leaf models are identical in form but differing in chemistry? The small white butterfly is a predominantly visual searcher showed to respond primarily to colour (Renwick & Radke, 1988) which will likely result in a preference hierarchy among the visually different models. If the genotypes differ in chemical composition there might also be a preference hierarchy for the visually identical leaf models since Städler et al. (1995) found that different glucosinolates stimulated oviposition in *P. rapae* in a varying degree when applied to non-hosts. If the females differentiate between leaf models based on their chemistry, will the preference hierarchy be the same as for real plants? Will homogenous patches reinforce choice or will the nearness to contrasting plants or models make choices more distinct in the heterogeneous setup?

Host selection in flying insects

Plants in nature are diverse and not evenly distributed; on the contrary heterogeneity exists at several spatial scales and creates patches with a large variance in size, shape and species composition. The patch is a fundamental concept in ecology and can be defined as a relatively homogenous area that differs from the surrounding landscape (Forman, 1995). A patch can consist of a clearing in a forested area, an agricultural field or a patch of moss on a rock. According to the "resource concentration hypothesis" proposed by Root (1973) it is more likely for phytophagous insects with a narrow host range to find, and remain in, a large concentrated stand of host plants. The reverse pattern is called "Edge effect" (Jones, 1992) and applies to many butterflies including *Pieris rapae* (Cromartie, 1975).

Newly hatched insect larvae are often relatively immobile and thus oviposition decisions made by females represent a crucial link in the life cycle of many insect species. The complexity of the landscape leads to insect females making decisions on an increasingly fine spatial scale. Jones (1992) states that for flying insects it is useful

to divide the process of host selection into pre- and post-alighting phases but recognizes that five stages in the process are also commonly discussed in the literature: habitat finding, host-finding, host recognition, host acceptance and host suitability. For *P. rapae* the behavioural events leading up to oviposition can be divided into a searching flight, landing and contact evaluation of potential host plants (Renwick & Radke, 1988). Host plant selection can be viewed as a sequence of decisions made based on sensory inputs, in the initial stages insects use visual and olfactory signals to locate a patch of potential host plants. From a distance a potential host plant may be seen by insects as a silhouette against the sky (Prokopy & Owens, 1983) but olfaction can be used from an even greater distance. An insect responds to positive plant cues by moving closer. Once an insect has arrived in a patch of host plants the volatiles may elicit landing but they are vague in directionality. Landing is probably guided by visual cues (Finch & Collier, 2000) such as colour, shape and size, which are precise in directionality but vague in terms of host specificity. After landing on a potential host plant a gravid female must assess its suitability using physical and chemical cues (e.g. Renwick & Chew, 1994). If the plant is accepted, one or several eggs may be laid; the female may also leave the plant, making trivial movements within the patch, landing on several plants, before making a decision. During the whole sequence of host plant finding positive and negative cues are weighed in the central nervous system of the insect and the search may be aborted or restarted at any stage of the sequence (Schoonhoven et al., 1998).

Olfaction

In insects the olfactory receptors are primarily located in the antennae but can also be present in the maxillary and labial palpi (Schoonhoven, 1968). In Lepidoptera the main olfactory organ is the antenna (Hansson, 1995). Located on the antennae are several types of sensilla consisting of neurons, a cuticular structure and accessory cells. Signals run from the neurons through their axons to the central nervous system (Schoonhoven et al., 1998). Substances with a molecular weight between 100 and about 200, such as many alcohols, aldehydes, aromatic phenols and terpenoids easily volatilize when exposed to air. Volatiles can be released from plants in two ways: from damaged plants and, at lower rates, through open stomata, leaf cuticles and gland walls. (Schoonhoven et al., 1998). Plant volatiles can be classified into general and specific volatiles (Schoonhoven et al., 1998). The general volatiles are called

green leaf volatiles, consisting of aldehydes and six-carbon alcohols and responsible for the characteristic smell of 'cut grass' from damaged plant tissues, the relative amounts of these volatiles varies between plant species. In addition to the general volatiles most plants also emit specific volatiles characteristic of their taxon (Schoonhoven et al., 1998). Intact and damaged plants differ in emitted volatiles, for example headspace volatiles from whole plants and macerated plant parts of 6 species of crucifers were analysed by Tollsten and Bergström (1988). They identified in total 34 volatiles in the headspace of whole plants, and the composition differed between species. The same number of volatiles where identified for macerated plant parts but they were mainly other compounds than in the analysis of whole plants. Several isothiocyanates, volatile breakdown products of the glucosinolates characteristic for brassicaceous plants (Hopkins et al., 2009), were identified in macerated plants but not in intact ones.

Both visual and olfactory plant cues can be used by insects to find their host plants and they are often used in an integrated way with the relative importance differing between species and especially between nocturnal and diurnal insects (Renwick & Chew, 1994). Even if diurnal insects may rely more on their vision to locate their hosts, the plant odour may still be important, especially from a distance. Plant odours are carried away from a plant by the wind and sufficient amounts of that odour will stimulate the insect to start flying upwind in search of its source. Two contrasting hypotheses have been proposed on the role of volatiles in host plant finding (Visser, 1986). The first is that species recognise their host based on species-specific volatiles. The second is that host plants are recognized based on the specific ratio of general volatiles. According to Bruce et al. (2005) the evidence to support the first hypothesis is scarce while there is "overwhelming evidence" to support the second. An example is the Colorado potato beetle that shows a positive reaction to a blend of green leaf volatiles in a ratio natural for potato plants (Visser & Avé, 1978). When the ratio is altered so that it becomes 'unnatural' the attraction is switched off and none of the volatiles acts as an attractant if presented alone. Van Loon et al. (1992) measured electroantennogram responses of *Pieris rapae* and *Pieris brassicae* and found selective responses to several compounds naturally occurring in their host plants, both common green leaf volatiles (most effective) and other more specific volatiles. It has been established that female *P. rapae* can detect host plants based on olfaction only

(Ikeura et al. 2010). Volatiles do not only attract insects, they can also act as deterrents eliciting avoidance behaviour in the insect; while isothiocyanates mediate host location in the cabbage aphid *Brevicoryne brassicae* they repel the black bean aphid, *Apis fabae*, for which brassicas are non-hosts (Nottingham et al. 1991). Once an insect has followed the odour of the host plant to within a close range, the odour can elicit landing. Renwick & Radke (1988) found that the involvement of olfaction in landing by *P. rapae* appears to be restricted to an avoidance response to non-hosts and the absence of negative signals from potentially acceptable plants. Olfaction although it can elicit landing is unlikely to give sufficient direction and at this stage vision is very important.

Vision

There are two basic types of insect eyes: multifaceted compound eyes and single chambered simple eyes (Land, 2009). Simple eyes are common in immature insects while compound eyes are almost exclusively the principal organ of sight in adults (Land, 2009). Compound eyes consists of 5-10 thousands visual units called ommatidia oriented in a way that makes the total field of vision approximate a sphere (Mazokhin-Porshnyakov, 1969). There are two basic types of compound eyes; apposition eyes, where each receptor cluster has its own lens (common in diurnal insects) and superposition eyes were the image at any point of the retina is the product of multiple lenses (Land, 2009). Superposition eyes are more sensitive to light and common in nocturnal insects (Land, 2009). Compound eyes show a huge variation both internally and externally due to selective adaptation to different life strategies (Prokopy & Owens, 1983). Compared to the retina of vertebrates the visual system of insects is quite coarse (Mazokhin-Porshnyakov, 1969) and the resolution is poor due to the small lenses (Land, 2009). Another difference compared to vertebrate eyes is that insects have a fixed focus (Prokopy & Owens, 1983). Just as humans, most insects have trichromatic colour vision, but their visual spectrum is shifted toward shorter wavelengths (Land, 2009). Dragonflies and some butterflies have four-colour vision (Land, 2009). Insects are able to see light in a spectrum ranging from ultraviolet (approximately 300-400 nm) to red (approximately 650 nm) (Prokopy & Owens, 1983).

Plants have spectral quality (i.e. colour and "reflectance of surface"), dimensions (size and shape) and patterns that can serve as visual cues for insects (Prokopy & Owens, 1983). The green hue of plant leaves is due to the chlorophyll and seldom varies but saturation may differ slightly due to other foliar pigments (e.g. carotenes) and nitrogen content. The reflectance of light from leaves and thus their appearance can be affected by the surface pubescence, glaucouseness (waxy bloom, common in brassicas), glare, cellular water content and lack of chlorophyll (Prokopy & Owens, 1983). The size, shape and pattern of plants vary far more between and even within species than the spectral quality of the leaves. If an insect is at all able to visually locate a host plant from a distance it is probably just seen as a silhouette against the horizon. Plant cues such as colour, leaf shape and pattern cannot be distinguished until the insect gets within a few meters or less (Prokopy & Owens, 1983). It is widely believed that while plant odour guides the insect to a plant the final decision to land is stimulated by the colour and shape of the plant (e.g. Renwick & Chew, 1994). Finch and Collier (2000) proposed in their 'appropriate/inappropriate landings' theory that once an insect flying over a host plant is arrested and stimulated to land by the volatiles emitted, landing is guided by vision and the insect will land on any green surface. If host plants and non-hosts are mixed the insect may land on either. Cabbage root fly (*Delia radicum*) females will after being guided by odour to the close vicinity of a host most likely land on the most visually stimulating plant (Prokopy et al., 1983). The reaction to visual stimuli varies between insects. Some insects, like Pierid butterflies, respond primarily to colour and show little or no response to shape or size (Renwick & Radke, 1988; Kolb & Scherer, 1982). Carrot fly (*Psila rosae*) females respond to leaf colour, shape and stem length (Degen & Städler, 1997) while colour, shape and size is important to apple maggot (*Rhagoletis pomonella*) flies that lay their eggs on apples (Prokopy, 1968).

Contact evaluation

After landing or otherwise touching a potential host plant an insect must assess its suitability using a range of physical and chemical plant cues (e.g. Renwick & Chew, 1994). Contact chemoreceptors may be located on some, or often all, of the tarsi, in the maxillae and the labium, and also on the antennae and ovipositor of an insect (Schoonhoven, 1968). In lepidopterans the involved sensory receptors are present on the tarsi, antennae, proboscis and ovipositor (Renwick & Chew, 1994). The physical

plant cues important for an insect can be the "waxiness" of the leaves, presence and amount of trichomes (hairs) and the surface texture (Schoonhoven et al., 1998). The surface waxes, or resin, of a plant is its first line of defence against insect herbivores. Surface waxes differ between species in both micromorphology and chemical composition and toxic components are often mixed in the wax. Sometimes the wax can make it difficult for an insect to hold on to the plant and often, but not in all cases, glossy crop genotypes suffer less insect damage than genotypes with normal surface wax (Eigenbrode & Espelie, 1995). Surfaces of plants are often covered with hairs that can be either glandular (with glands on the top) or non-glandular (Schoonhoven et al., 1998). Hair can sometimes be a hinder, especially to small insects, in performing their natural feeding or oviposition behaviour. In contrast hair can also in some cases be of assistance to an insect providing it with a better grip, shelter or an improved micro-climate. When searching for an oviposition site the texture of the plant surface can be important: females of the diamondback moth, *Plutella xylostella*, prefer to lay their eggs in concavities and along veins of leaves of their host plant rather than on a smooth surface (Talekar & Shelton, 1993).

Chemical plant cues perceived by an insect on the plant surface can deter the insect from feeding or laying eggs on the surface but can also serve as an attractant used by insects to recognize the plant as a host. Pierid butterflies are known to flutter their wings and drum their forelegs against the plant surface after landing (Terofal, 1965). It has been long established that tarsi of female *Pieris brassicae* are sensitive to mustard oil glycosides (Chun & Schoonhoven, 1973), or glucosinolates, characteristic of the plant family Brassicaceae (Hopkins et al., 2009). Glucosinolates are a class of non- volatile plant secondary metabolites, consisting of three functional groups; a β-thioglucose group, a sulfonated oxime group and a variable side chain (Hopkins et al., 2009). Over the years more than 120 different glucosinolates have been identified and the glucosinolate profile differs between species and even between different genotypes. When the plant tissue is damaged glucosinolates can be converted into volatile compounds such as isothiocyanates, nitriles, and oxazolidinethiones, by the enzyme myrosinase stored in specialized plant cells (Hopkins et al., 2009). Even though there is overwhelming evidence that several insects can get chemical information and perceive glucosinolates by contacting the leaf surface of Brassicas, how this is done is still largely unknown. Numerous possibilities have been proposed

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but several studies on the wax layer covering Brassica leaves (reviewed by Städler & Reifenrath, 2009) show no traces of glucosinolates in that layer. It is clear that a wide range of plants chemicals can act as both oviposition stimulants and deterrents to an insect and a potential host plant may contain one or several of both (Schoonhoven et al., 1998). An insect in contact with the plant surface must weigh the positive and negative inputs in the central nervous system and the result may be rejection of the plant or some level of acceptance (Dethier, 1982). The presence of a deterrent is not required for a plant to be rejected, Renwick and Radke (1987) showed that the lack of a stimulant is enough for *P. rapae* to reject a plant. Städler et al. (1995) found ten different glucosinolates that stimulated oviposition in *P. rapae* to a varying degree when applied to non-hosts and four deterrent compounds powerful enough for the butterfly to reject a host plant.

The small white butterfly

The small white butterfly, *Pieris rapae,* has a wingspan of 40-56 mm (Eliasson, 2005). The upper surface of the wings is white to pale yellow, with a black dot on the front edge of the hind wing and black tips on the forewings. Females (figure 1a) have two and males (figure 1b) have one black dot on the forewing, but males can also have all white wings without black scales (Finch & Thompson 1992). The undersides of the wings are yellow dusted in grey. Eggs are yellow and bottle shaped with ridges (figure 1c). Caterpillars are green and covered with fine hairs with a yellow dorsal line and sidelines of small yellow dots (figure 1d). Pupae are initially green and turn beige (figure 1e); most often they match the colour of the surroundings (Eliasson, 2005; Finch & Thompson, 1992).
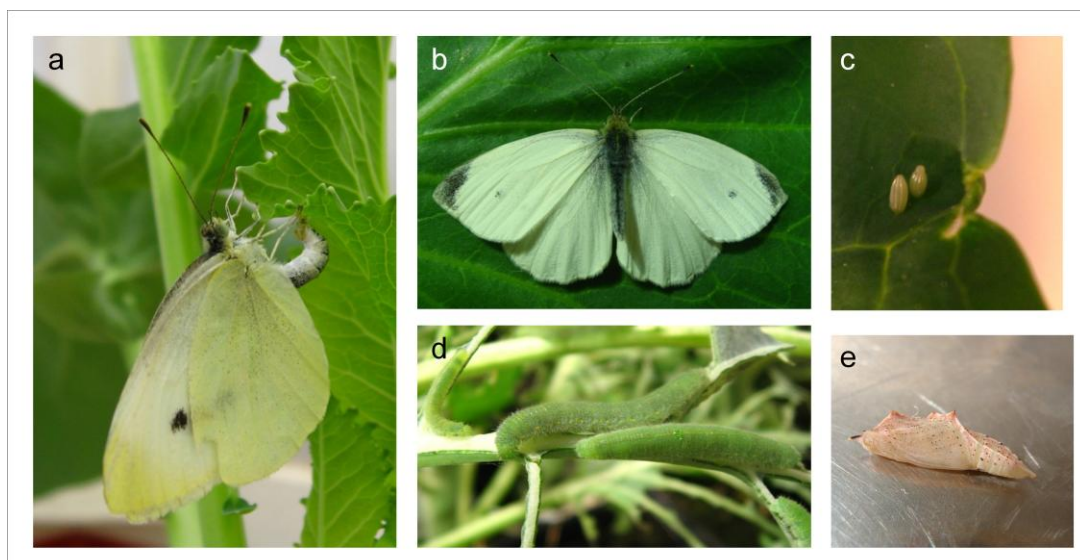

Figure 1. Ovipositing *P. rapae* female (a), *P. rapae* male (b), eggs (c), larvae (d) and pupae (e)

Adults appear in May in much of Europe and females lay their eggs singly on cultivated and wild brassicaceous plants (Finch & Thompson, 1992) on warm and sunny days (Gossard & Jones, 1977). Eggs are most often laid on the underside of leaves with the female landing on the upper side or margin of the leaf and bending her abdomen around the leaf edge to deposit an egg (Finch & Thompson, 1992; Richards, 1940). Females usually fly between the laying of each individual egg and are known to have long linear flight paths (Jones, 1977); often the eggs are spread over many different plants. In warm weather development can be fast and eggs hatch in 3-7 days (Eliasson, 2005; Richards, 1940). The solitary feeding (Jones & Jones 1984) caterpillars go through 5 instars in 11-22 days before pupating on plants, walls or another sheltered place for 7-13 days or longer (Eliasson, 2005; Finch & Thompson, 1992; Richards, 1940), winter is always spent in the pupal stage (Eliasson, 2005).

Pieris rapae is found everywhere in the agricultural landscape, in the parks and gardens of cities and in other areas where the ground is often disturbed (Eliasson, 2005). It is common in all European countries and the distribution worldwide stretches from Northern Africa, temperate areas of Asia north of the Himalayas, Australia, New Zeeland, North America and Hawaii (Eliasson, 2005). Reproduction in Sweden occurs every year only in the southern parts with the first generation appearing in May-June, further north reproduction is limited to warm summers (Eliasson, 2005). *P. rapae* can have 2-3 generations up to Mid-Sweden but under years of mass reproduction it can be found much further north (Eliasson, 2005). It is a migratory butterfly that can fly long distances during its lifetime (Eliasson, 2005; Finch & Thompson, 1992). In the warmest parts of its range *P. rapae* can have as much as 8 generations per year (Finch & Thompson, 1992).

Pieris rapae has a wide range of host plants in the Brassicaceae family including the cultivated Brassicas in gardens and fields (Eliasson, 2005). They often lay eggs on many plants in a crop and are not restricted to sheltered areas (Finch & Thompson, 1992). Caterpillars can be hard to spot when small because of their colour and their habit of feeding close to the centre of the plant, but they are revealed by their frass and damage to the leaves. If there are many caterpillars damage to foliage can be severe, but frass is usually a greater problem than the leaf area destroyed. In plants close to harvest the frass lodged between the leaves can make the crop unmarketable.

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Chemicals can be difficult to use since they have to be applied just after hatching before the caterpillars enter the heart of the plant, more than one spraying can be necessary. In small gardens it is better in the long run to avoid chemical treatment because of the negative impact on natural enemies of the small white butterfly such as hymenopterous parasitoids and beetles (Eliasson, 2005).

Material and Methods

Butterflies

Pupae of *Pieris rapae* were shipped from *Plant Sciences Group*, Wageningen University, Netherlands. After eclosion the butterflies were kept in culture in a climate chamber set to 21°C, 80% humidity and 16L: 8D photoperiod. Adult butterflies were fed sugar water (1 lump of sugar, approx. 3.6 grams, dissolved in 30 ml of water). Caterpillars were raised on a mix of three *Brassica oleracea* genotypes and on occasion some bought cabbage.

Plants

Three genotypes of *Brassica oleracea* (figure 2) and extracts thereof were used; cabbage (*B. oleracea* subs. *capitata,* cv. Consul), cauliflower (*B. oleracea* subs. *botrytis,* cv. Nautilus) and broccoli (*B. oleracea* subs. *cymosa,* cv. Marathon). Seeds were provided by Olssons Frö AB, planted in 10x10x10 cm pots with soil from Hasselfors Garden (SLU mix) and grown in glasshouses with supplement lights. Nutrients were provided with the water: nutrients from Cederroth International AB (Wallco växtnäring 51-10-43 + micro) were diluted with de-ionized water to a conductivity of 1.0 mS/cm which corresponds to a nitrogen concentration of 100 mg/l.

Figure 2 Leaf shapes of cabbage, broccoli and cauliflower in that order.

Figure 3 The tree different leaf models: diamond-shaped (diamond) with vertical folds, dark hogweed (darkh) and light hogweed (ligth).

Leaf models

The leaf models (figure 3) consisted of paper models covered with paraffin wax left over from a study on carrot flies performed by Degen and Städler (1997). To make extracts for the leaf models 200 gram of chopped leaves were added to one litre of 99% ethanol, the mixture were shaken every five minutes and filtered after one hour. The leaf models were placed in 10x10x10 cm pots filled with sand and extract equivalent to 1-2 gle (gram leaf extract) were airbrushed onto the leaves. To cut down on spraying time extracts were concentrated using a rotary evaporator.

Experimental setup

Three experiments were performed: one with actual plants and two with leaf models. The experiments using leaf models were done to separately investigate the effect of plant form (shape and colour) and the effect of plant chemistry. To investigate the effect of plant chemistry visually identical diamond-shaped models (figure 3, to the left) sprayed with extracts from the three genotypes were used. To investigate the effect of plant form three different models were used (figure 3) all sprayed with cabbage extract. In the experimental setup a patch consisted of three "plants". Two arrangements were used; homogenous (simple) with all "plants" in the patch being the same genotype/form/extract, one patch of each; and heterogeneous (complex) with all the three patches having all three genotypes/forms/extracts (figure 4). Plants were chosen with the aim to have as similar size as possible; in general cabbage had 1-2 more leaves than broccoli and cauliflower.
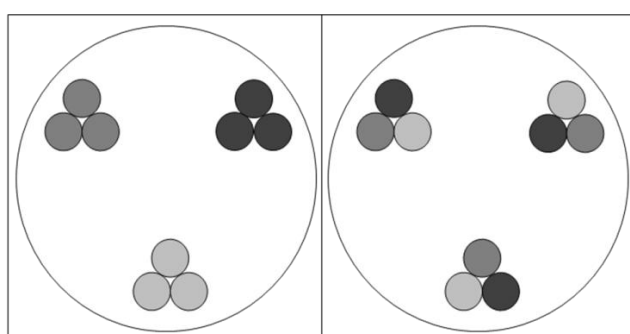

Figure 4 Experimental setup in the two levels of the turntable: the simple setup to the left and the complex setup to the right.

The experiments were carried out in a turntable that consists of a cage with two levels, each with a disk turning once every 3 minutes (figure 5). A fine mesh fabric hangs inside all of the doors to prevent escapes when the doors were open to release butterflies or to remove plants. The turntable were lit with three 36 W fluorescent

lamps in addition to those providing the light in the room. The temperature was kept at 20-24°C with the aid of a 3 kW heating fan. Before the start of a replicate the plants were arranged in complex and simple patches on the turning disks (figure 4), arrangement was alternated between the two levels. At the start of a replicate ten butterflies were placed on the mesh fabric inside the doors at each level and checked for ability to fly away. Each replicate lasted four hours. At the end of a replicate first the plants and then the butterflies were removed and eggs counted.
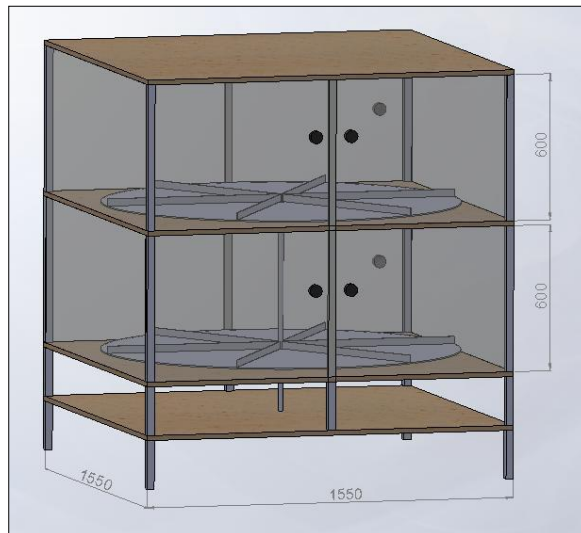

Figure 5 Sketch of the turntable used, measurements in millimetres.

For unknown reasons the females laid very few eggs on leaf models in the upper level of the turntable, consequently the majority of the replicates had to be done in the lower level. In both experiments seven replicates from the lower level and one from the upper level were analysed. Due to problems with the turntable four replicates with real plants were done in 160 by 60 by 110 cm cages in a glasshouse with supplement lighting. Two cages were used, ten butterflies were kept in the cages for the duration of all four replicates and arrangements were alternated between the cages. In the simple arrangement the position of the patches within the cage were alternated. At the start of a replicate dead or flightless butterflies were replaced and food removed.

Statistical analysis

The results from the experiment using real plants was previously analysed by Peter Hambäck for an article by Hopkins et al. (in review). In the trials he found that much fewer eggs were laid in the turntable than in the cages and thus the results from the turntable were excluded. I used that same model to model the effect of form and chemistry in complex vs. simple arrangements for the experiments with leaf models. The model was a generalized linear mixed effects model with poisson error distribution where egg number per "plant" was modelled with genotype, arrangement and interactions among variables as fixed effects. The random factors included were replicate and plant group within replicate. All models were implemented using the lmer command in the lme4-package (Bates et al. 2011) of R 2.13.2.

Results

Females of the small white butterfly responded to some degree to the scale of spatial heterogeneity in all three experiments. Analysis of the caged plants, made by Peter Hambäck, revealed a marginally significant trend that the eggs were differently distributed among the *B. oleracea* genotypes in the simple and complex arrangements (log-likelihood ratio [LR] = 2.4, $\Delta df = 2$, $p<0.1$) (Hopkins et al., in review). The ranking of genotypes differed between the simple (cauliflower = broccoli > cabbage), and the complex (broccoli > cauliflower = cabbage) arrangement (figure 6a). When *P. rapae* females were faced with visually identical leaf models sprayed with extracts of the three *B. oleracea* genotypes the distribution of eggs differed significantly with arrangement (LR = 11.8, $\Delta df = 2$, $p < 0.0001$). Visual inspection suggests that relatively more eggs were laid on cauliflower in the simple arrangement, however the ranking is the same in both arrangements; cauliflower > broccoli = cabbage (figure 6b). When leaf models where sprayed with the same extract but differed in form the egg distribution was significantly different between the two arrangements (LR = 3.1, $\Delta df = 2$, $p < 0.05$). The ranking in the simple arrangement was ligth > diamond > darkh while the ranking was ligth > diamond = darkh in the complex arrangement (figure 6c).
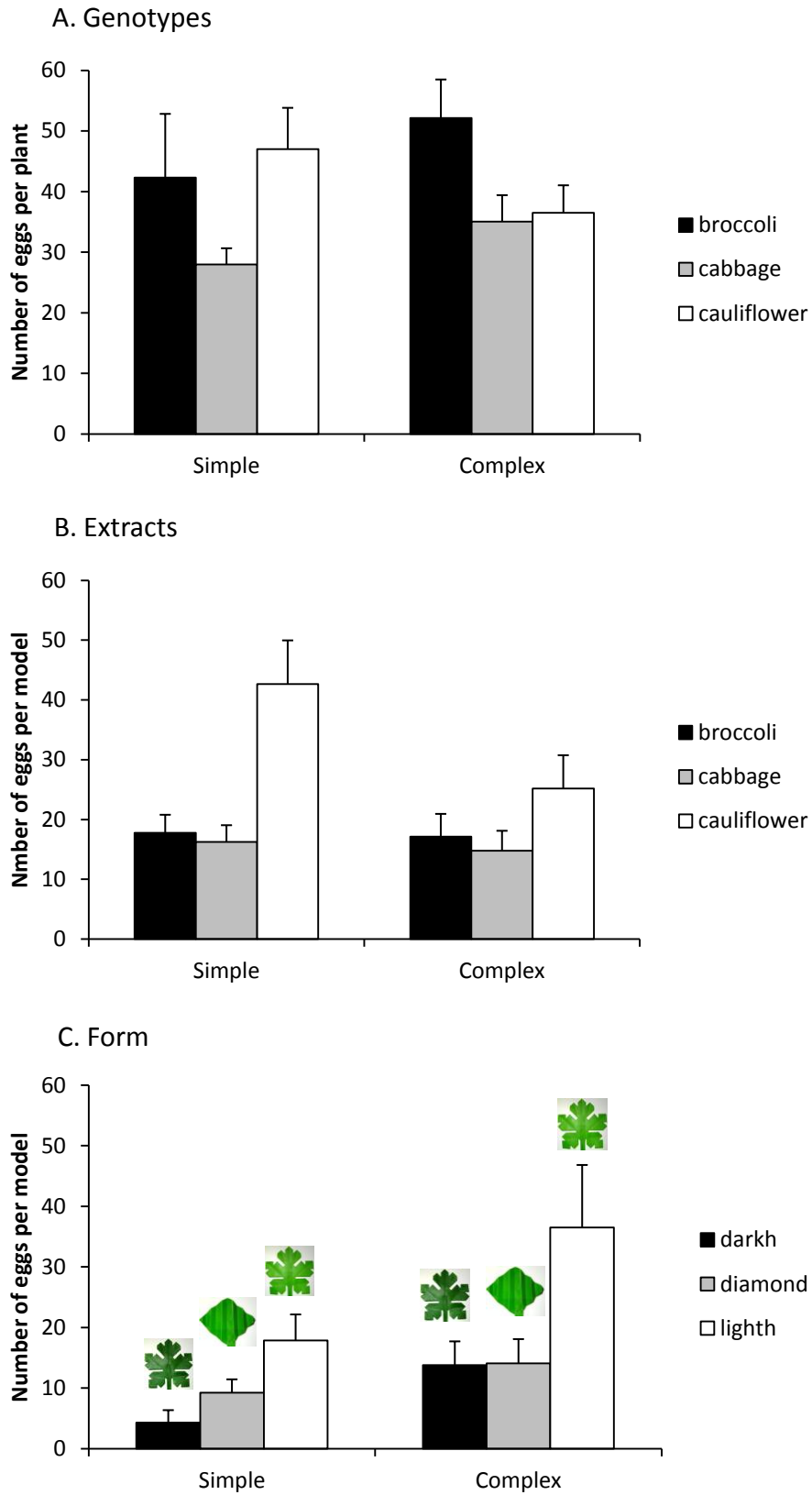
A. Genotypes

B. Extracts

C. Form

Figure 6 Number of eggs (mean ±Std) per plant or model. A. Genotypes, B. Extracts, C. Form with plants or models arranged in simple and complex patches.

Discussion

The objective of this study was to gain a greater insight in the short range host selection by females of the small white butterfly and their use of senses at different spatial scales. When real plants were used the egg distribution among genotypes did not differ significantly with arrangement, however there was a visual difference in the ranking of genotypes. *Pieris rapae* could differentiate between visually identical plants based on their chemistry alone and their choices seemed to be reinforced when plants models were placed in homogenous patches. In this case the choices were most likely based on contact chemoreception alone since virtually all volatiles vanished in the preparation and application of the extracts. *P. rapae* is a visual searcher and indeed the females differentiated between the different forms and the egg distribution among the forms differed significantly with arrangement. In contrast to the experiment with different extracts the nearness of contrasting plants seemed to make the choices more distinct in the heterogeneous setup.

Very little scientific work has been done on mixing plants within host range. Field scale studies has been carried out by Cantelo and Sanford (1984) and Hambäck et al. (2009 & 2010) with results differing between species. To my knowledge Hopkins et al. (in review) is the only study done at this small scale. The data for *P. rapae* is the same as in my study but in addition the same experiment was performed using another pierid butterfly, *Pieris brassicae*, and the nocturnal diamond-back moth, *Plutella xylostella* with diverging results; *P. brassicae* were significantly affected by the genotype-arrangement interaction in the turntable while no such interaction could be seen for *P. xylostella*.

In contrast to the few studies done on the effect of within host range patch heterogeneity an overwhelming number of studies has been done on the effects of plant visual appearance and plant chemistry on the choices made by insects. After locating an area with host plants using olfactory cues landing is widely believed to be mediated by visual cues, at least in diurnal insects. Most insects have trichromatic colour vision and some butterflies and dragonflies even have four-colour vision (Land, 2009), so there is no surprise that colour is an important factor in the choice of a host plant. Several insects have been shown to be able to discriminate between different geometrical shapes (Mazokhin-Porshnyakov, 1969) but the importance of

shape and size when landing on a potential host plant varies between insects. Degen and Städler (1997) found that ovipositing carrot flies were strongly influenced by physical plant traits in the presence of host plant extracts and preferred green, yellow and orange models with a shape similar to their host plant. *Pieris rapae* responded to colour (e.g. Hovanitz & Chang, 1964; Renwick & Radke, 1988) but showed little or no preference for shape and size when spectral qualities were the same (Renwick & Radke, 1988). Mazokhin-Porshnyakov (1969) hypothesizes that insects react by reflex to the totality of an object's characteristics and not to individual visual parameters. After landing on a plant the final decision to accept or reject the plant as a host depends on the chemical and tactile properties of the plant surface, and positive and negative plant cues are weighed (e.g. Dethier, 1982). In *Pieris rapae* the sensory inputs primarily come from the tarsi that are drummed against the plant surface, and both Renwick and Radke (1987) and Renwick et al. (1992) have identified several plant chemicals that act as oviposition stimulants or deterrents.

Form

The butterflies differentiated between the visually different models regardless of how they were placed with the lightest model being the most popular. However the preference for the lighter model were greater in the complex arrangement with the contrasting models close together, the contrast between the models increased with proximity. As mentioned the most important visual cue in host selection is likely to be colour (Renwick & Radke, 1988) and Hovanitz and Chang (1964) found green to be greatly preferred with blue second and a combination of green-blue was more favourable than green alone. Field observation shows more frequent landing on larger/older plants and this might be due to their bluish hue (Renwick and Radke, 1988) or because they make a larger target for landing. Leaves of a lighter colour are often associated to younger plants and it might be more "favourable" for the butterfly to lay eggs on young plants. While rearing the butterflies I noticed that newly hatched larvae seemed to perform less well when feeding on older plants, growth rate seemed slower and mortality rate higher than for larvae feeding on younger plants. There might be a mismatch in nature with *Pieris rapae* females preferentially landing on green-blue (older) plants and their larva performing better on younger (lighter coloured) plants.

The reason for the increasing contrast with proximity might be found in the structure of butterfly eyes. The compound eyes of insects are not uniform across the whole field of vision; the distribution of resolution is asymmetric and flying insects have a "forward focussed" "acute zone", adapted to flying through vegetation (Land, 2009). This means that although butterflies may have almost 360 degrees of vision the forward facing part of the eyes is primarily allocated to the search for nectar sources and host plants. That in turn means that while the *P. rapae* females may be able to perceive all the patches in the turntable at any one time they mainly distinguish between the models right in front of them.

Chemistry

In contrast to the visual aspects the differences between the extracts decreased with proximity. The cauliflower stand out from the two other extracts as the most preferred in both arrangements but the difference is much larger when the different extracts are kept apart as in the simple arrangement. As mentioned this differentiation is most likely an effect of contact chemoreception since virtually all volatiles evaporated during the preparation of extracts and leaf models. Mozūraitis et al. (in prep) analysed the volatiles emitted from the three *Brassica* genotypes used here and found a difference in both composition and quantity, this probably reflects a difference in non-volatile compounds. The difference in egg distribution between the simple and complex arrangements is likely to be a result of butterfly behaviour. In addition to drumming their legs against the leaf surface *P. rapae* females are known to flutter their wings and move over the surface of one or several leaves, while acquiring chemical information from the plant. Having several models sprayed with the same extract close together the butterfly may make trivial movements between them and accumulate chemosensory input. A stronger positive input may result in more eggs laid. In contrast when genotypes are mixed the positive sensory input might be weakened or the mixed chemosensory cues could confuse the females resulting in fewer eggs laid. Alternatively it is possible that being in contact with the cauliflower extract is enough to make the female lay eggs on any leaf model in the patch.

Plants

In the experiment with actual plants the difference in egg distribution between genotypes did not differ significantly with arrangement although the preference hierarchy were visually different in the simple and complex arrangement. In the

simple arrangement broccoli and cauliflower seem to be preferred over cabbage while in the complex arrangement broccoli seems to be preferred over both cauliflower and cabbage. Compared to the experiments with models butterflies are faced with many more sensory inputs when making a decision about oviposition based on real plants. In addition to the visual appearance and contact chemoreception there is a difference in olfactory and tactile input and the multi leaved plants are also more visually complex with leaves intermingling in the patches. *Pieris rapae* can make host plant decisions based on olfactory cues (Ikeura et al., 2010), but it is unclear how well their sense of olfaction work in cages this small. Maybe close range olfactory can give some guidance in the homogeneous arrangement but with the leaves of the genotypes intermingling in the heterogeneous patches it is unlikely to be directional enough. Comparing these results with the extracts; cauliflower has a high number of eggs in the homogenous patches in both experiments, this could be an effect of butterfly preference for the genotype chemistry, while broccoli being highly accepted as a plant in both arrangements but not as an extract indicates that the reason for this is something other than chemistry. The blue-green hue of the broccoli leaves may be the reason (Hovanitz & Chang, 1964). It is possible that in the heterogeneous setup the chemical cues are too muddled by the proximity of the plants and the intermingling leaves leading to decisions being made on the basis of colour. The reason for cabbage standing out as least accepted in the simple arrangement may depend both on chemistry and the glossiness of the leaves, it has been showed (Eigenbrode & Espelie, 1995) that plants with glossy leaves generally receive less eggs.

The patch

In general the acceptability of plant patches to phytophagous insects decreases with increased patch heterogeneity both at the interspecific (e.g. Bukovinszky et al. 2005) and the intraspecific level (e.g. Hambäck et al. 2010, Poelman et al. 2009,). The study by Hopkins et al. (in review) showed that *P. brassicae* and *P. rapae* may differentiate more between the *Brassica oleracea* genotypes when they are separated in homogenous patches. In contrast when Hambäck et al. (2009) used the same genotypes and compared large patches with more than 300 plants, differing in internal heterogeneity, the butterfly females differentiated **less** among genotypes in homogenous patches than when genotypes grew in close proximity. Hopkins et al. (in

review) suggests that "the ability of female butterflies to select among genotypes is highest in intermediately complex patches". The resolution of the sensory system may make it more difficult for females to make decisions in highly complex patches (Bernays, 2001), while memory constraints or inability to relocate a more suitable plant previously visited may make host selection difficult in patches with low internal heterogeneity (Hopkins et al. in review). This study shows that the relative importance or resolution of different senses may vary with scale and heterogeneity; the butterflies differentiated more between the different extracts when they were kept in homogenous patches while the case was the opposite with the different forms.

After an insect has encountered a patch of potential host plants, trivial movements within the patch expose the insect to sensory information that increases with plant diversity in the patch (Hopkins et al. in review). According to the "appropriate/inappropriate landings" theory developed by Finch & Collier (2000) insects may land indiscriminately on any green object when faced with a patch of mixed vegetation. But landing is not really indiscriminate; the plant colours play an important role even if it may not make the insect land on a preferred host plant. Females of the cabbage root fly (*Delia radicum*) will land on the most visually stimulating plant whether it is a host plant or not (Prokopy et al., 1983) and in this study the butterfly females laid eggs preferentially on the lightest model, especially in the complex arrangement. Insect females should optimize their choice of host plants, and this optimization becomes increasingly complex as the number of factors involved rises. Comparing the choices made by *P. rapae* females on the plants with choices made on models reveals a potential compromise between visual and chemical plant cues.

Challenges

Doing a study at this fine scale is met with a few challenges; the nature of those challenges is of course depending on the species used and the natural behaviour of that species. *Pieris rapae* are known to perform poorly in lab environments and seem have problems when crowded, both as larvae and adults. *P. rapae* lay their eggs singly and larvae are thus not likely to be adapted to compete with a large number of conspecifics. Crowded larvae seem to go into pupation at a smaller size and in turn become smaller and weaker adults. Crowded adults confined in cages seem to be

disturbed in their mating and several times females in the experiment had to be replaced because they displayed mating behaviour instead of egg laying behaviour. In addition to rearing difficulties *P. rapae* females are known to only lay their eggs in sunny weather and not in overcast or even in the shadow (e.g. Gossard & Jones, 1977) they are also known to have long linear flight paths when in search for host plants (Root & Kareiva, 1984). These two factors may have impacted their behaviour in the turntable.

Future work

To complete the information on the use of different senses in host plant selection the use of olfaction remains to be tested. The methods of this study was not suitable to do that since the preparation of the extracts eliminated virtually all volatiles, the use of a turntable is not ideal either. To test the effect of arrangement when olfaction is used I suggest a method similar to Ikeura et al. (2010), covering whole plants with perforated (preferably green) plastic bags. Experiments should then be carried out in large, well lit cages with one to a few females. Instead of counting eggs the females should be observed and every contact with a plastic bag recorded.

Conclusion

It is clear from this study that females of the small white butterfly are able to distinguish between different host plants based on both their chemistry and visual appearance. Vision and contact chemoreception works best at slightly different scales; the females differentiated more between the different coloured models when they were close together in complex patches while the difference between the models sprayed with different extracts were greater when they were separated in simple patches. The result of this study gives a clue about the resolution of vision and contact chemoreception in the small white butterfly.

Acknowledgements

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