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3 Methods in Studying Insect Behaviour

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3.1. Introduction

At its simplest, behaviour is what animals do – in particular their actions and reactions. Behaviour is central to the ecology of insects and thus underlies most questions in entomology. It may be an explicit part of a project but even with the most applied question, ultimately, the behaviour of insects determines if and how they become pests, whether it is how they get to the crop or what they feed on.

Where the focus of projects can differ is in the type of question being asked or addressed. The same behaviour can be looked at in complementary ways – the 'four whys' (Tinbergen, 1963): its immediate cause (or control); its development during the life of the individual (ontogeny); its function, and how it evolved. While your current question might be the mechanism or immediate stimulus for the behaviour, it may be that looking at another 'why' might provide new insight. For example, the proximate question of host plant selection by an insect might be partly explained by its evolutionary history as shown by patterns among related species. Much of the most successful behavioural work combines these approaches.

This chapter is designed for two types of researcher – those interested in behaviour for its own sake; and those whose initial question is not behavioural but who find they need to understand more about how their insects behave in a particular system. Behavioural approaches have proved useful for a wide variety of applied problems in entomology, including assessing potential biocontrol agents (see this volume, Chapter 11), studies of resistant plant varieties (see this volume, Chapter 6) and of pesticide resistance (see this volume, Chapter 10). As an example, consider the mode of action for the increased pest resistance of glossy leafed cabbage, *Brassica oleracea*, varieties over normal-wax varieties (see Eigenbrode *et al.*, 1995; this volume, Chapter 6). The resistance is not based on toxicity – indeed there was no resistance to diamondback moth, *Plutella xylostella*, in greenhouse experiments without predators. The key to resistance in the field might then be predation but was the effect due to the behaviour of the caterpillar, predators, or both? Behavioural experiments on neonate *P. xylostella* larvae showed that they spent more time searching and walking on glossy leaves, and their wax extracts, and mined into the leaves less, behaviours that left them more exposed. Predator behaviour was also important and the effects may be partly due to greater mobility of the arthropod predators on glossy leaves. By establishing the effects of the glossy waxes these behavioural studies can inform conventional plant breeding and potential genetic manipulation of surface lipids for greater pest resistance.

The study of insect behaviour should not be viewed in isolation of the behaviour of other organisms. Some of the most interesting work comes from cross-fertilization of ideas from vertebrate studies, say of food choice, to insects (and back!). The present times are characterized by an increasing specialization of both journals and scientists and there is a tendency for entomologists to look only at entomology journals, missing ideas from, for example, more general behavioural ecology (see, e.g. Alcock, 1993; Krebs and Davies, 1993). Within entomology, the division into pure and applied can be short-sighted. Applied projects can yield valuable data for wider behavioural questions. For example, the intensive study of moth pheromones, largely prompted by their importance as pests, has provided an excellent resource for studies of speciation and the evolution of mate choice (McNeil, 1991, 1992; Phelan, 1992). Equally, understanding moth mating systems better can improve the success of pest control by mating disruption with synthetic pheromone.

Insects as subjects for studies of behaviour have a number of advantages. Their small size and rapid rates of reproduction mean that one can work with statistically satisfactory numbers of animals, but at the same time most are large enough to be marked and treated as individuals. As a result, insects offer good model systems for studying many fundamental questions in animal behaviour, from foraging theory to sexual selection.

2. Observing and Recording Insect Behaviour

Martin and Bateson (1993) give an excellent introduction to measuring behaviour and this book would be a good starting point for any researcher. As they emphasize, preliminary observations offer the chance to become familiar with your animals and their behaviour. The best way initially is to take simple notes while watching your animals. As you watch, think about your questions and develop hypotheses. The more competing hypotheses the better. For example, is it host egg size, or just host chemicals, or both, that are important to an ovipositing parasitoid? Make specific predictions based on these hypotheses and design your experiments to distinguish between them.

3.2.1. Choosing the focus

Ideally, keep an open mind about the apparent functions of the behaviours or 'purpose' of the animal you are observing. A teleological assumption about the 'purpose' of the behaviour can blind you to underlying mechanisms (Kennedy, 1992); how the animal actually accomplishes the task, for example, of navigation from flower to nest. Yet at the same time teleological shorthand can help in thinking about a problem initially. The skill is to remain vigorously critical of hidden assumptions.

To make sense of the behaviour you will need to break the continuous stream of activity into units in some way. For example if watching a searching parasitoid wasp you might divide the sequence into: fly, land, walk, antennate the larva, probe with ovipositor, withdraw the ovipositor, drag it on the larva, walk, take off. When attempting to divide the behaviour into units to record, choose ones large enough to make recording possible (rather than, for example, the movement of each leg, when your focus is really on the searching path of the parasitoid). Choosing the appropriate units or categories of behaviour is a matter of experience. It is often possible to collapse data categories upwards but you cannot create detail afterwards if you have not recorded it. However, if your categories are too complex or fine, the patterns will be lost in the detail. Among the kinds of things your units could include are: (i) movements; (ii) the effects on other individuals; or (iii) spatial measures such as changes in the distance from a host plant or potential mate. Make the definitions of your behaviours as explicit as you can and write them down, giving them neutral terms. Even well defined units may be graded rather than all or nothing (Harris and Foster, 1995).

From the beginning, make your observations quantitative: put numbers to your observations (but be prepared not to use your preliminary data). Without quantitative data you will not be able to make useful comparisons.

3.2.2. Structuring your observations

For many experiments there may be a relatively simple end point – for example the numbers of insects landing on targets of different colours. Similarly, in experiments where only one animal is tested at a time, no decision is needed about which animal to watch. However, in other experiments sequences of behaviour may be of interest, or their frequency of occurrence, or the behaviour of individuals in groups. Martin and Bateson (1993) review some of the principal methods for sampling rules (who to watch and when) and recording rules (how the behaviours are recorded). Much work in insect behaviour involves recording all instances of a certain behaviour, such as biting a leaf, during a time period. However, instantaneous recording of the behaviour happening at regular intervals (prompted by a bleep from your timer or event recorder) can allow more individuals to be observed simultaneously. Recording the presence or absence of a behaviour within a time interval (one-zero recording) may have a role in some situations.

3.2.3. Recording the data

Having made your preliminary observations it will be time to begin collecting your data. In some cases dictating an account of the behaviours on to audiotape as you watch may be useful, in particular at the beginning of a study or if you are following elusive insects in the field. However, given the time to transcribe the tapes you may want to move to, or , most likely, start with, other ways of recording data.

CHECK SHEETS

When you have established the behavioural units to record, a simple check sheet system of columns down a page, with each line being a time unit (e.g. 1 min) for example, can help standardize your record taking. It can save time as you put ticks, frequencies or code letters in a box rather than writing out the behaviours in full, and so it may allow you to record more behaviours. Analysis is also quicker, especially if each behaviour is in a separate column. Labelled boxes at the top of each sheet can ensure that you remember to include all the important background details for each experiment including the observer, time, temperature, stimulus and other information. Such prompts are especially important if many people are making the observations. When designing your form ensure you leave some space so you can note rare events or other behaviours for which you may not have allocated codes. Of course check sheets can also be used if transcribing video/audio tape.

COMPUTER EVENT RECORDERS

When the behaviours of interest have been defined, a computer with event recorder software which allows you to tap observations directly into the machine may be useful. Small portable computers, and larger personal organizers, can be used for data collection in the field. Among the advantages of computers over paper check sheets are the greater accuracy of recording durations of behaviour, the ability to record faster and more complex sequences of behaviour, the elimination of the transcription stage from paper to computer for analysis, and the ability to store large quantities of data (Martin and Bateson, 1993). The disadvantages include the temptation to collect too much information or to proceed to analysis without inspecting the data. Much depends on the reliability of the hardware and software (losing the data can be a real danger). The ability to export the data on disc for analysis and manipulation by other software is an important feature. To be worth while, the computer event recorder needs to save you time, make difficult observations possible, or make analysis easier. Depending on your experiment a pen and check sheet may be more practical.

One of the best known commercial packages for recording animal behaviour is The Observer ™ Noldus IT (info@noldus.nl; Costerweg 5, PO Box 628, 6700 AG Wageningen, Netherlands) which will cope with different experimental situations/designs, some data analysis, and in addition other software modules can integrate with video playback equipment. However, the cost of commercial software may put it out of the reach of many researchers who instead have written their own.

PHOTOGRAPHY AND VIDEO

Video can be very useful but a good guiding principle may be: watch first, do not video. It can be very tempting to move rapidly to videoing or filming. The advantages of using video include the ability to record behaviours that might happen rarely, too quickly, or too slowly, or under difficult observation conditions, where an observer might change the behaviour of the subject, for complex social interactions, or in the dark (where infrared sensitive video cameras can offer night vision). A video recorder can be more patient than a human observer – useful when there are long periods of inactivity between behaviours of interest. However, among the disadvantages of video or cine-film are that crucial actions can happen just off-camera, focus, depth of field and (for video especially) resolution may be limited, and analysis can be very time-consuming indeed. Ultimately the behaviour will need to be scored in much the same way as above.

Contributors in Wratten (1994), on the use of video in ecological and behavioural research, review many practical aspects including use for studying flying insects in the field (Riley, 1994) and laboratory (Young *et al.*, 1994), as well as walking insects (Varley *et al.*, 1994). One tip is to try out particular video machines yourself for the clarity of image when using single frame advance, a common use in playback.

Keeping track of time within video sequences need not be difficult. Many video cameras can create a digital clock in one corner of the image allowing you to easily return to particular sequences or help in the analysis. Young *et al.* (1994) and Varley *et al.* (1994) give other methods for adding timing signals to help synchronize cameras or enable computer handling of images. The Noldus package for video analysis exploits the hidden timing code, laid down on the tape by the video recorder itself, to keep track of time if for example you fast forward between behaviours. Other systems may be available.

If you label the sequences on the video tape by videoing the equivalent of a clapper board title sequence at the start of each experiment (with its number, stimuli details, and date) sorting out tapes will be easier and you will be able to spot the start of experiments when searching on fast forward (index marks also help). You may be able to use the sound track to record your comments during the experiment.

Analyse the tapes as you go along. Do not leave the analysis until the end of the experiments on the assumption that the tape has recorded all the details you need. You may find, for example, that the contrast is not sufficient to allow you to distinguish the detail you need.

ANALYSING VIDEOS TO TRACK MOVEMENT

Video is often used to record the paths of insects, for example towards or away from an odour source. However, it is worth asking if knowing the detailed path is needed to answer the question being studied. Are there simpler measures that could be observed at the time, for example distance of the insect from the target, or time spent stationary? Where the focus *is* on the underlying search mechanisms used by the animal or an explanation of how observed differences in response occur, track measurements are needed. For many other studies video tracking is a distraction.

Going from the video to tracking insect movement is time-consuming. The simplest, and most laborious, way is to go through the tape a few frames at a time, marking the position of the insects on an acetate sheet laid over the video monitor screen. These positions can later be digitized manually. Young *et al.* (1994) provide formulae for calculation of track parameters (see also Bell, 1991). Different stages can be computerized culminating in automatic tracking systems in which the computer tracks the path itself.

Automated analysis offers significant savings in time, especially when the alternative is going through a film or video manually, frame by frame. However, people are much better than a computer at following an insect on screen, especially in real time. A good compromise is to link the video player to a computer which can combine the video and computer image on the same monitor and allow hand digitizing with a mouse on screen. Varley *et al.* (1994) give details of one such system which also doubles as an event recorder.

Tele-tracking, an early system for automatic tracking, compares the incoming signal from a video, as it scans across the screen a line at a time, with a brightness threshold and gives the *x–y* coordinates for the first bright dot encountered in each frame. It gets confused by groups of insects or fussy backgrounds (Young *et al.*, 1994). A more sophisticated technique is image analysis, which turns the image into pixels on the computer screen, and uses software to find the insect by, for example, subtracting the previous image to detect movement (Young *et al.*, 1994). Image analysis can also be used to give the spatial distribution of activity, rather than tracking individual insects, for example, within an ant nest (Strickland and Franks, 1994).

ACTOGRAPHS AND OTHER AUTOMATIC RECORDING METHODS

For some kinds of questions, such as those on activity rhythms, automatic data collection offers major advantages, not least that it makes the investigation of circadian rhythms possible without sleep deprivation for the experimenter. The behaviour usually needs to be a fairly simple one, such as an increase or decrease in movement in a cage, which can be detected easily. By careful design of the sensors it may be possible to allow the system to distinguish different behaviours. One disadvantage of using an actograph is that one cannot see what is happening and this may limit one's scope for thinking of the next experiment.

An important feature of whatever method is used is that it should not be detectable by, or change the behaviour of, the insects themselves. The technique needs to be validated by observation to confirm the effectiveness of the recording mechanism and that it accurately reflects the insects' behaviour.

There are many different mechanisms but basically all actographs detect movement by breaking a light beam or a change of some other sort detected electronically (early actographs used levers to exaggerate the scale of movement and traced a track on a smoked drum) and are limited only by your ingenuity. New techniques are likely to be developed as new transducers are invented and electronic devices get cheaper. Among the methods used to detect movement so far are rocker-cages, infrared, ultrasound, wing beat sound, vibration, changes in capacitance, temperature, Doppler shift radar (e.g. Kyorku and Brady, 1994; Snowball and Holmqvist, 1994). As the output is often an electrical signal, most systems can now be linked to a data logger or a computer via an analog/digital converter card which allows for easier analysis (e.g. Beerwinkle *et al.*, 1995, for a flight mill, but the same principles apply).

RECORDING ENVIRONMENTAL VARIABLES

The small size of insects means that they are usually more affected by the environment than larger animals. Temperature is a major factor influencing their behaviour. It can thus be very important to be able to measure this, together with relative humidity. Meteorological station data may be inappropriate since it is not what the insects are experiencing. Rather, one needs microclimate data taken on a scale relevant to the insect. Unwin and Corbet (1991) and Unwin (1980) consider a range of methods. Data loggers may be useful for recording the data over a period of time.

Some insects are able to maintain internal temperatures well above ambient and thermoregulatory behaviour has become a new field of study (Heinrich, 1993). The standard way of recording internal temperatures, stabbing the thorax with a thermocouple for example, has been criticized by Stone and Willmer (1989). Jones (1982) used a blue pigment, which faded in sunlight, mixed in yellow paint and painted on to snail shells. Suitably calibrated, the fading from green allowed estimation of how much time the animals had spent in the sun (it works equally well with beetles).

3.2.4. *Statistics and experimental design*

Determining preferences, for example for one plant species over another, is a common aim of experiments in insect behaviour. In a thoughtful essay, Singer (1986) discusses the definition of preference (which is not straightforward) and the merits of a range of experimental designs: no choice, sequential choice, and simultaneous choice (where all the alternatives are offered at the same time). Different results can be obtained depending on the preference testing technique. Although Singer discusses oviposition behaviour, his conclusions have wide relevance to anyone using preference tests.

In no choice tests, ceiling effects (in which all stimuli get high responses) or, conversely, floor effects (where almost none respond) may be resolved by trying another behavioural measure, for example latency (Martin and Bateson, 1993).

Vaillant and Derridj (1992) offer an alternative, based on the Monte Carlo procedure, to traditional non-parametric analysis of two-choice experiments. In the special case of choice experiments where living food grows (e.g. plants), or dries out (e.g. evaporation from insect prey being eaten by liquid-feeding predators), during the course of the experiment you may need particular methods for the analysis (Peterson and Renaud, 1989). They also make the point that there are rarely enough replicates of controls to allow clear differentiation between treatments.

The importance of good controls cannot be overstressed. Solvent controls are needed whenever chemicals are presented in solution. Control insects need to be 'sham operated', that is handled as much and in the same way as much as possible as the experimentals. In some experiments it may be possible to use an animal as its own control (analysed as a matched pair) but there can be problems of order effects, especially in the context of the wider occurrence of experience effects in insects (see learning, Section 3.3.4).

Running experiments blind, so the observer is not aware of which treatment is being tested, is not often done in entomology but can be strongly recommended (Martin and Bateson, 1993). Assigning subject animals to treatments should be rigorously randomized, especially if it is difficult (because the treatments are obvious to the observer, for example if testing leaf shapes) to run the experiments blind.

Multiple recording of the same insects, leading to pseudo-replication, can be a problem in both field and laboratory experiments. It may be possible to design the experiment so insects can only be counted once, for example falling into a pitfall (White and Birch, 1987).

Where differences in behaviour between two types of animal may be subtle, any one variable by itself may not show a difference. While the behaviour of solitary and gregarious locusts is very different there is a continuous gradation between the two states. In 'gregarization', changes in behaviour occur more rapidly than in morphology or colour so behavioural analysis allows finer resolution of the changes. By observing the behavioural responses of individual solitary or crowd-reared nymphs when presented with a group of conspecifics, Roessingh *et al.* (1993) were able to use logistic regression to derive a predictive model based on 11 simple behavioural measures. The model distinguishes the two extreme classes of insect but, more important, gives a single index of the 'behavioural phase status' of an insect anywhere between. Analogous methods might be useful for studying other primer effects with a long time scale, for example caste determination in termites.

In some cases discovering the sequential relationship between behaviours may be of interest, for example, whether behaviour B follows behaviour A or whether the sequence of behaviours occurs at random. A well established method to test this is Markov analysis (Martin and Bateson, 1993, and references therein). If the probability of a behaviour occurring is determined by the behaviour immediately before, then the process is first

order; if determined by the two behavioural events happening before, second order, and so on. Assumptions made in this analysis, and often violated, are that the transition probabilities do not differ between individuals or change over time. New 'hidden Markov models' allow such changes in transition probabilities over time to be estimated, for example in the likelihood of foraging behaviour with increasing time since the last meal (MacDonald and Raubenheimer, 1995). Simpson and Ludlow (1986) suggest a different approach using 'hazard analysis' which also provides an example of the use of GLIM in behavioural analysis.

3.3. Factors Influencing Behaviour

Any investigation of insect behaviour needs to be informed by an understanding of the factors (perhaps beyond the immediate concerns of the experiment) that influence their behaviour.

3.3.1. Internal state

The response of an insect is determined not only by the stimulus but also by changes in the internal state of the insect such as those driven by circadian rhythms. For example, in many species there are particular periods each day when activity or responsiveness to stimuli such as pheromones is greatest (for review see McNeil, 1991). Underlying such daily rhythms are other physiological changes centred on factors such as the length of time since the last meal. On a longer time scale, mating history, age, nutrition, and effects of experience (q.v.) can affect behaviour in profound ways (Barton Brown, 1993). Another potential internal factor influencing behaviour is the presence of parasites (see Bell *et al.*, 1995).

Internal stimuli may be difficult to manipulate except at a gross level, for example mated or not mated, or by testing the insect at a particular age, or by controlling access to some resource such as food or oviposition sites (Harris and Foster, 1995). It may also be useful to pre-test your insects to see if they will respond appropriately. For example, if you are investigating orientation in flight, it may make sense to include only insects which take off in a pre-test, if preliminary experiments show this is both a good predictor of later takeoff behaviour and that this selection does not bias the results (similar tests have been used to test viability of biocontrol agents before release (see Mills, this volume, Chapter 11)).

Circadian rhythms in responsiveness and day-to-day variation make it essential to carry out experiments during the active period and to use fully randomized complete blocks to ensure that all treatments are treated equally within the period. Frequently, factors such as size, age or date can be controlled for statistically in the analysis.

Experimental difficulties aside, the recognition that there might be individual variation in behaviour *within* a species, and that this variation can be interesting rather than simply a nuisance, may be one of the most important developments in modern animal behaviour. Differences between individuals could be based on differences in size, for example major and minor males of the solitary bee *Centris pallida* (Alcock *et al.*, 1977), or conditionally on the behaviour of other conspecifics at the time. Individuals could also be influenced by prior experience as well as a host of other variables affecting internal state (see, e.g. Borden *et al.*, 1986).

3.3.2. Genetics and insect behaviour

Many of the differences in behaviour between individuals may have some genetic basis as well as a developmental component (Hoffmann, 1994). Host plant selection behaviour again provides many examples, of both variation within a population, among host races in the apple maggot fly, *Rhagolitis pomonella*, and between populations of the same species in oviposition behaviour in British and Australian populations of the cabbage butterfly, *Pieris rapae* (see Bernays and Chapman, 1994; also this volume, Chapter 6).

All aspects of insect behaviour can have a genetic component, from circadian rhythms to courtship. Butlin and Ritchie (1994) give examples of the genetic basis of courtship signals in the context of speciation, and Bell *et al.* (1995) and McNeil (1991) review the genetics of pheromone production and reception in moths. Earlier work on the behaviour genetics, typically on laboratory animals such as *Drosophila*, tended to use behaviours that were easily scored in the laboratory but an increasing number of studies concern ecologically relevant behaviours (Hoffman, 1994).

Whether or not it is the focus of your project, the genetic basis of the behaviour(s) may nonetheless be important. For example, for laboratory studies you will need to be sure of the strain or biotype you are using as these can differ (and may even be defined on the basis of behaviour). Field workers will need to be aware of potential inter-population differences.

In applied entomology, selection for behavioural resistance can be important for vector control and IPM programmes. Genetic bottlenecks and inadvertent selection can have a significant influence on programmes to laboratory rear biocontrol agents and sterile males (and in routine laboratory cultures) (Prokopy and Lewis, 1993).

3.3.3. Developmental and transgenerational effects

Many insects show lasting changes in adult behaviour according to the conditions during development as a nymph or larva. The effect of crowding leading to phase change to the gregarious form in locusts is one of the most impressive examples of this type of phenotypic plasticity (Roessingh *et al.*, 1993, and references therein). In the locust, *Schistocerca gregaria*, the conditions the mother was reared under, and indeed population density at the time of mating or oviposition, can also affect the behavioural phase state of the offspring (Islam *et al.*, 1994). Such effects are an example of maternal

inheritance. Maternal inheritance, where the mother influences traits of her offspring by means other than by transmission of nuclear alleles, is known in a wide range of insect taxa (reviewed by Mousseau and Dingle, 1991). In most cases the mechanisms are not known but effects mediated via egg size or egg concentrations of maternal hormones are among those demonstrated in various species so far. Maternal effects may provide adaptive phenotypic plasticity across the generations so that offspring develop appropriate phenotypes for the conditions likely to follow the conditions the mother experiences (Mousseau and Dingle, 1991). Maternal effects can be another cause of individual variation in behaviour.

3.3.4. Prior experience and learning

While von Frisch's early experiments on bee vision exploited associative learning to give clues to the colours bees could distinguish, learning has until recently not been thought important in other insects. Recent progress across a wide range of taxa and ecological situations has been reviewed by Papaj and Lewis (1993). There are many definitions of learning but perhaps the simplest is a change in behaviour with experience, excluding factors like changes in egg load over time. Some of the best studied examples are learning by parasitoid wasps (Turlings *et al.*, 1993; Vet *et al.*, 1995), bees (Gould, 1993) and, increasingly, phytophagous insects (Bernays and Chapman, 1994; Bernays, 1995).

Parasitoid wasps have evolved searching strategies to solve the difficult task of finding cryptic prey and many species seem able to modify their responses to foraging cues based on experience. This ability to learn profitable cues has now been demonstrated for almost 20 different species (Turlings *et al.*, 1993). Most of the learning is associative: the wasps innately recognize host-derived stimuli (unconditioned stimuli, US) on contact and associate these stimuli with surrounding stimuli (conditioned stimuli, CS), previously of little interest (Turlings *et al.*, 1993). The conditioned stimuli could be olfactory such as host frass, but may be visual, such as visual characteristics of plants, mechanosensory, or perhaps most effectively, a combination. Such learning allows the wasps to use, for example, characteristic volatiles released in relatively large quantities by host-damaged leaves as a cue to find hidden host larvae. The behaviour of a female wasp can be changed by a plant-host contact of as little as 20 s (Turlings *et al.*, 1989) but unless reinforced by successful ovipositions the preference behaviour wanes, giving a flexible response to foraging outcomes.

Changes in behaviour with experience have only been studied in a small range of situations, among them the topics above. There may be similar effects occurring in other aspects of insect behaviour, for example mate choice, which currently are not recognized. There are two points of practical relevance to all researchers. First, experience, only a small part of which may be known to the experimenter, before the observation or experiment starts will have an influence on the behaviour of the subjects. Second, insects should not be used more than once in an experiment if possible as later behaviour may be influenced by a first treatment.

The implications of insect learning to pest management are examined by Prokopy and Lewis (1993) who make the point that both beneficial insects and pests show learning of various kinds. A well established phenomenon is the effect of artificial rearing conditions on the behaviour of parasitoids for release, which may reduce their effectiveness in the field. Learning could also affect other phenomena such as pest population estimates based on mark–release–recapture, if handled insects behave differently. Pest habituation, or conversely sensitization, could affect the effectiveness of plant resistance.

3.3.5. *External stimuli*

External stimuli such as visual, auditory or olfactory stimuli are some of the most obvious influences on behaviour and are the ones that can most easily be manipulated by the experimenter. Most behavioural experiments involve changing these external stimuli (see below). While external stimuli presented to the animal might be kept constant, they will not always produce the same inputs as peripheral receptor responses are influenced by physiological variables – for example, locust taste chemoreceptors are influenced by haemolymph levels of nutrients such as amino acids, being less responsive when levels of that nutrient are high (see Simpson *et al.*, 1995).

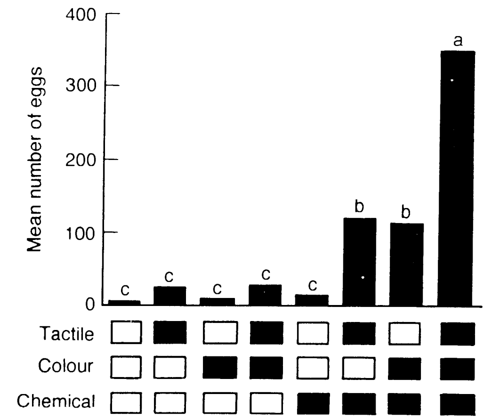
Taking account of the way animal perceptual worlds differ from ours is always important in behaviour but perhaps especially for work on insects. Olfaction and taste are much more important in insects and even apparently familiar senses such as vision are different from our own both in spectral sensitivity and acuity.

3.3.6. *Integration*

The theme developed by Harris and Foster (1995), in an excellent review, is the integration of internal and external sensory inputs in behaviour. Internal factors include, for example, those that change with age, prior experience, and mating status. Moreover, despite the desire of the experimenter to concentrate on one external stimulus, for example host odours, the majority of insect behaviours may be driven by *combined* inputs from many senses – from vision as well as chemoreception (see also Prokopy, 1986).

While single sensory inputs, such as a chemical stimulus, can be investigated by themselves, multifactorial (and possibly multi-level) tests can tease out the stimuli involved in behaviours, and may sometimes show interactive effects of stimuli that would be missed had they each been tested alone (Harris and Foster, 1995). A good example is that of the oviposition behaviour of Hessian flies, *Mayetiola destructor*, a pest of wheat which lays its eggs on the leaves. In a three-way factorial experiment testing colour, chemical, and tactile stimuli, Harris and Rose (1990) found all three stimuli had a

significant effect on numbers of eggs laid (and all possible first and second order interactions were also significant; see Fig. 3.1). From this one might conclude that the three stimuli were all integrated in the response. However, as Harris and Foster (1995) point out, a second experiment (Harris *et al.*, 1993), which investigated the behaviours leading to egg laying, as well as number of eggs laid, showed that although colour influenced approach and landing behaviour it did not affect the number of eggs laid once the female had landed, whereas chemical stimuli were important at each stage. There are some disadvantages of more complex factorial experiments: logistic problems of carrying out enough replicates and interpretation of interactions are among these.



Tests	Treatment	F value	Significance
Main effect	chemicals	89.4	<0.0001
Main effect	colour	40.1	<0.0001
Main effect	tactile	47 6	<0.0001
First order interaction	chemicals × colour	37.7	<0.0001
First order interaction	chemicals × tactile	34.3	<0.0001
First order interaction	colour × tactile	8.6	<0.006
Second order interaction	chemicals × colour × tactile	8.6	<0.006

Fig. 3.1. Integration of stimuli: the role of colour, chemical, and tactile cues in oviposition by Hessian flies, *Mayetiola destructor*, on plant models. A 2 × 2 × 2 factorial gave eight combinations of stimuli. Wax coated filter-paper leaves were coloured green (compared with undyed white), dosed with wheat cuticular wax extract (compared with control solvent alone), and given vertical grooves in the wax to simulate leaf veins (or left without grooves). One of each stimulus combination was present in the simultaneous arena test with a single female. Black boxes indicate the first cue of each pair is present. The main effects of each stimulus and the higher order interactions were all significant. (From Harris and Foster, 1995; data from Harris and Rose, 1990; reproduced with permission from Entomological Society of America.) However, later experiments showed the effect of colour (green) was due to its effect in increasing landing (Harris *et al.*, 1993).

In some situations it may be difficult to separate stimuli for analysis. For example, many studies showing a response of flying insects to visual targets in the presence of pheromone may at the same time be offering a barrier to the air flow, as the target is solid. In trying to separate out visual stimuli and the effects of a wind barrier on the response of insects to an odour plume, Wyatt *et al.* (1993, 1997) used a silhouette drawn on the upwind mesh of the wind tunnel to offer a visual stimulus with no effect on the air flow. A barrier could be placed upwind of the mesh when required, and when used with an unmarked mesh screen could test the effect of a barrier without a visual stimulus (Fig. 3.2). The results with male woodworm beetles, *Anobium punctatum*, responding to their sex pheromone were surprising (Wyatt *et al.*, 1997): the barrier alone was as effective as a visual stimulus with barrier, although without the barrier a visual target induced quicker landing than no visual stimulus or barrier, giving a significant interaction of visual target and hidden barrier in the analysis. The challenge for the experimenter dealing with multiple stimuli is to devise a soluble problem which nonetheless helps understanding of a more complex real world.

A notable feature of insects, and other animals, is the ability to use not only a multiplicity of stimuli but to use one sense when another is not available. Such redundancy allows an insect to function despite loss of input from one of its senses through old age, injury, or vagaries of the environment in the case of insect navigation when, for example, cloud obscures the

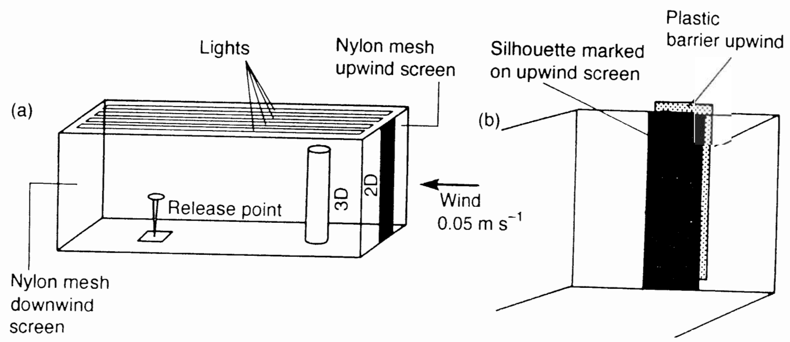


Fig. 3.2. Separating the effects of visual and barrier effects of trees on upwind orientation of insects to odour sources. (a) Diagram of the wind tunnel (not to scale) showing the position of the three-dimensional black cylinder or two-dimensional silhouette. In each experiment, the release point of single male beetles was from a take-off platform 20 cm up from the floor and 100 cm downwind of the pheromone source (also 20 cm up from the floor). (b) Separating the visual and barrier effects offered by a two way factorial experiment. The visual stimulus when present was a black shape marked on the upwind mesh and the wind barrier was a plastic shape placed immediately upwind of the mesh. (After Wyatt *et al.*, 1997.)

sun. The advantage to the insect is clear but since most experiments involve either depriving an insect of a sense at a time or providing limited controlled stimuli, drawing conclusions can be difficult. If insects can switch to another sense then the ability of the insect to continue to do the task successfully is not an indication that it did not normally use these cues. Studies on animal navigation for example, including that of bees, have been bedevilled by this problem (Dusenbery, 1992).

3.4. Investigating Behaviour

The techniques for investigating behaviour are similar whatever the system. Among the major decisions are whether to use laboratory or field experiments and observations, or often best, a combination of both.

3.4.1. Laboratory experiments

Laboratory experiments can be a very effective way of studying the behaviour of insects. When developing your set-up try to create conditions which elicit as natural behaviour as possible. Temperature, relative humidity and lighting can be important variables. For example, intriguing experiments by Kamm (1990) showed that seed chalcid wasps would not make orientated flights to host olfactory stimuli in the laboratory but would under natural light in a greenhouse (it turned out polarized light was required). Often the development of laboratory set-ups is a matter of luck. For example, use of agar as a substitute for mud for a burrowing beetle in the laboratory would not have worked if the beetle had needed to close its jaws on sand grains (Wyatt, 1986).

It is possible to observe nocturnal insects under lights with 'far-red' filters (e.g. Ilford 609, passing wavelengths light > 680 nm). This can work well. However, recent work suggests infrared light-emitting diode (LED) light sources might be better (mosquitoes can respond to backgrounds illuminated in red light (> 700 nm) but not the > 900 nm light produced by LEDs (Gibson, 1995)). Arenas lit with such LEDs can be viewed with modern solid state cameras which are very sensitive to long-wavelength light (>900 nm) (Young *et al.*, 1994).

Subtle changes in laboratory insect cultures can have important effects on insect behaviour. These often result from inadvertent artificial selection pressures. For example, Birch *et al.* (1989) were unable to demonstrate a courtship role for the male hairpencils in the cabbage moth *Mamestra brassicae*. Later it was shown that this was a laboratory artefact: the hairpencils of wild males and males from fresh cultures did have a pheromone which seemed to be lost from older laboratory cultures (Poppy and Birch, 1994).

The design of laboratory experiments aims at a happy medium. While you cannot create a complete habitat, the more it matches the natural habitat the better the experiments may predict behaviour in the wild. For example, if you are investigating responses to resource stimuli it may be worth investigating factors such as habitat structure, patch size and resource distribution (Prokopy, 1986). Ultimately, the goal is to test the predictions in the field.

3.4.2. *Field observation*

Laboratory experiments may allow for easier replication of experiments but they raise the question: do animals really behave in the same way in the wild? This can only be answered by observing animals in their natural environment, in the field.

Important new subjects such as the role of learning in entomophagous parasitoids (see Section 3.3.4) can grow up without a firm base of studies showing its significance in the field (Papaj, 1993). Similarly, almost all studies on the feeding patterns of herbivorous insects have been laboratory based, with a range of plant choices determined by the experimenter. Raubenheimer and Bernays (1993) heroically followed individual marked female grasshoppers (*Taenipoda eques*) for the whole day (sunrise to sunset) in Arizona. Using a hand-held electronic event recorder (see Section 3.2.3) they recorded all the feeding bouts, their duration and marked the plants for later identification as the insects moved around the habitat. For this species, perhaps reassuringly, many parameters such as the clustering of feeding events into bouts, were very similar to laboratory studies of other acridids although there were some differences which highlighted the need for equivalent laboratory studies of this species. Similar field techniques have been applied to other insects. For example, Aluja *et al.* (1989) labelled every twig on trees to allow the 3-D movement of apple maggot flies, *Rhagoletis pomonella*, to be followed. Near-focusing binoculars to follow insects may be useful in field studies. Nocturnal insects have been observed in the field by using night vision equipment, for example looking at behaviour of moths flying to pheromone traps (see Riley 1994; also this volume, Chapter 5). Opp and Prokopy (1986) discuss other aspects of field experiments. For example, you may need to mark your animals (see Chapter 5).

Much of the classic work on ant navigation has been done in the field (see e.g. Wehner *et al.*, 1996) as was Tinbergen's pioneering work with the digger wasps. In each case experimental manipulation was combined with observation. Placing models or test arenas in the field so animals encounter them naturally can be very effective (e.g. Judd and Borden's, 1991, experiments on onion fly, *Delia antiqua*).

A major advantage of field experiments is that the internal state of the animals can be assumed to be natural but with the disadvantage that it might not be known or necessarily repeatable. Another reason for studying animal behaviour in the field is when important factors, especially stimuli, might be affected by moving the experiment into laboratory. For example, cutting plants to provide leaves for feeding choices could offer wilting leaves and initiate wound responses by the leaves, all factors now known to

affect insect feeding behaviour (Bernays and Chapman, 1994). When it is impossible to reproduce field conditions in the laboratory, for example the range and activity of predators, field experiments may be the only way of investigating topics such as the effectiveness of parental care. For example, by elegant *in situ* exclusion experiments Tallamy and Denno (1982) showed that eggplant lace bug, *Gargaphia solani*, females were able to successfully defend their broods against arthropod predators.

While there are many advantages in doing field experiments there are good reasons why many experimenters attempt to do at least part of their work through laboratory experiments. The principal problem in temperate regions is the unpredictability of the weather. On cool or rainy days there may be no insect activity. Low animal densities may also be a problem. With field behavioural work on some species (perhaps especially for pests – not chosen for their ease of study) the problem is not being able to identify species or their sex accurately at a distance (Finch, 1986). In these cases you will need to be able to catch the individual after the experiment.

Where field conditions are difficult or the animals are difficult to rear, a compromise may be to use field collected animals in laboratory experiments where you can better control the conditions.

It is possible to discover much about the field behaviour of insects by the use of traps as an indirect way of testing stimuli, for example odours or colours, rather than watching individual animals. Conversely, behavioural observations of individuals can be used to improve traps. Small differences in design can have large effects on trap catch (Phillips and Wyatt, 1992). Vale and co-workers used electrocuting grids to show the numbers of tsetse flies approaching and going into different trap designs (e.g. Vale, 1982).

Testing pheromone identifications based on laboratory bioassays is one important use of traps. Cardé and Elkinton (1984) discuss the design and interpretation of field trials, including trap interactions (Byers, 1992; see also this volume, Chapter 7). The design of field trials still presents a major practical problem (e.g. Sanders, 1989; Wyatt, 1997). It can be difficult to find matching control plots far enough away to be unaffected by the pheromone treatments on the experimental plots, but close enough to offer similar conditions. This reinforces the need to be able to do realistic tests of pheromone blends and release rates before field trials.

3.4.3. *Visual stimuli*

Visual stimuli are important in the lives of all insects. Their influence has been extensively investigated in both host-finding by phytophagous insects (see this volume, Chapter 6; Prokopy, 1986; Bernays and Chapman, 1994) and by pollinators (this volume, Chapter 9). Finch (1986) gives a good review of features to consider in bioassay of colour responses of insects. Among the most difficult problems are producing standard colours changing in hue (dominant wavelength) but not in brightness (intensity of reflected light). Colour saturation (spectral purity) is another variable. For initial experiments it is possible to use standardized colour papers to test for attraction to hues and to hues of different saturation (Finch, 1986). Neutral 'grey' papers from black to white can be used to test whether attraction apparently based on hue is really one to brightness. The shorter wavelengths have more energy (quanta) than longer ones, towards red, and thus appear brighter.

When considering brightness, an important point is that light sources, artificial and natural, do not have an even output across the spectral sensitivity of the insect eye. With a knowledge of the light source output spectrum, and the reflectance spectrophotometer readings from the pigment, it is possible to calculate the relative number of quanta reflected from a pigmented surface (Kolb and Scherer, 1982; e.g. Harris *et al.*, 1993).

Coloured lights can also be used, with the advantages that specific wavelengths can be used and one can control duration as well as intensity. For example Kolb and Scherer (1982) were able to show that the effect on drumming and egg laying behaviour for *Pieris brassicae* L. was wavelength specific rather than a response to brightness. Coloured light has been used for testing landing responses of aphids and white fly in vertical flight chambers (see Section 3.4.7).

The background on which targets are presented can also have effects. For example Harris *et al.* (1993), working with Hessian flies (*Mayetiola destructor*), found marked differences in the landing responses to targets of different reflectivities depending whether the arena walls were white or black.

3.4.4. Using physical models

Ever since Tinbergen's experiments showing stickleback male courtship responses, models have been an important way of investigating responses to stimuli. The principal advantage is the chance to allow control of the stimuli, which can be manipulated in turn, allowing experiments on the integration of behaviour (above).

A major use of models has been testing host plant selection by insects (Bernays and Chapman, 1994) (see Sections 3.3.6 and 3.4.3). Variability between plants, for example, can be eliminated by using painted models with reflectancies checked with a spectrophotometer, for example (Prokopy *et al.*, 1983) testing attraction of cabbage root flies, *Delia radicum*. A host of experiments on pollination, learning in honey bees, flower constancy, reward response have used model flowers (see this volume, Chapter 9) with or without methods for giving a nectar reward.

Visual stimuli are not limited to colour. Target shapes also have strong effects on landing. Harris *et al.* (1993) showed vertical shapes were more attractive to female Hessian flies. The 'vertical contour length' proved important, with the taller and longer the better but other things equal, the greater the area the more eggs laid (so long as the target was not horizontal), matching the predicted preferred shape as the species lays its eggs on grass stems.

Models can also be used to investigate the importance of surface texture and other tactile cues. For example, Harris and Rose (1990) used parallel vertically orientated grooves to mimic leaf venation of the host grass plants of the Hessian fly. Model eggs made from glass beads have been used to study host egg selection by parasitoid wasps (Vinson, 1985), enabling shape, size, and contact kairomones to be tested. The use of models can improve reproducibility and where the stimulus would otherwise be another animal it controls for behavioural interactions between them.

3.4.5. Chemical stimuli

Chemical stimuli are important in many aspects of insect behaviour from communication to host plant selection. This section emphasizes olfaction but techniques in contact chemoreception are very similar. While electroantennograms (EAG) or single cell recording (SCR), perhaps coupled with gas chromatography, can pinpoint potentially active compounds, behavioural analysis is still needed as EAGs or SCR cannot predict the behavioural response of the insect. Components for which there are few receptors can be missed as may synergies between components (q.v.) which are not usually observed at the peripheral level. Byers (1992) provides a concise discussion of these points and the design of bioassays.

A behavioural bioassay is a repeatable experiment for measuring response to a stimulus. In the context of semiochemicals, or equally host odours, it allows you to trace which fractions contain activity during fractionation. Baker and Cardé 's (1984) review of bioassays, although primarily for pheromones, is still useful. Depending on your objectives, simple bioassays may be the best. One of the main criteria is ease of use – in scoring as well as execution – although the crucial point is that it should be a reliable measure of the behaviour you ultimately want to assess: a laboratory bioassay, for example, should be able to identify a pheromone which works in the field (wing fanning by males has been used in this way for laboratory pheromone bioassays instead of more complicated flight orientation bioassays). You may be able to reduce the duration of the bioassay if you discover on plotting the data that the result is given more quickly than first thought.

There are many different types of walking bioassay – from still air arenas, with pheromone placed in the centre, to Y-tube olfactometers with pheromone laden air flowing down one side, clean air down the other, more elaborate four-arm olfactometers (Baker and Cardé, 1984) – and a wide variety of wind tunnels for testing in flight (Baker and Linn, 1984; Young *et al.*, 1994).

Kennedy (1977) highlights the need for discriminating bioassays as opposed to ones which do not reveal the orientation mechanisms used by responding insects. For example, animals collecting in one arm of an olfactometer could be due to insects stopping there (inverse chemoorthokinesis), or to increased or decreased random turning (direct or inverse chemoklinokinesis) or to directed turning (chemotaxis) or to odour-conditioned anemotaxis (for an explanation of terms see Table 3.1). Y-tube and four-arm olfactometers share the problem of sharp boundaries to the odour plumes which allow the insects to use chemotaxis in ways that could not be done at a distance from the source. Bioassays that allow control of the way the insect contacts the pheromone, for example by a catwalk for walking at right angles to the gradient of odour, are preferred. However, if a bioassay allows one to identify active components it can be useful even when the orientation mechanisms are not revealed.

Behavioural bioassays to assess fractions as they come direct from a gas chromatograph (GC) have been developed (e.g. Leal *et al.*, 1994; Nazzi *et al.*, 1996). A combined GC/EAG/behavioural bioassay using associative learning of a sugar reward and odour, which exploits the bee's tongue extension behaviour, has been used by Wadhams *et al.* (1994) to investigate honey bee responses to flower odours. However, searching for synergy (below) remains difficult when fractions are only tested as they come from the GC column in a linear time sequence.

Most insect pheromones appear to be composed of more than one synergistic component acting together. Each synergist alone may have little activity, but presented together at appropriate concentrations they are bioactive. This poses problems for isolating the active compounds from an insect extract as sub-fractions will not show activity unless other synergists are also present. As the number of major fractions rises the complexity of bioassaying the potential combinations increases as a power. Byers (1992) compares the efficiency of different bioassay strategies and firmly advocates 'subtractive combination' (fractions containing active synergists are revealed when removing the fraction reduces the activity of the whole extract). De Jong (1987) provides a method for efficiently determining the optimum blend if the components are known.

Apart from the design of the bioassay, additional questions for work with olfaction include the type of substrate used to release the chemicals, the strength of the stimulus, and the way in which two or more chemicals should be mixed (Baker and Cardé, 1984). Most experiments release chemical stimuli from filter paper or rubber septa. Alternative dispensers using diffusion from open tubes may be particularly appropriate for field experiments or when high release rates are required (Byers, 1988).

Bioassays for investigating glandular sources of trail substances of social insects and their chemical characterization have been critically reviewed by Traniello and Robson (1995) in particular citing the failure of many bioassays to distinguish between recruitment and orientation which are distinct behaviours in trail communication and foraging organization in both ants and termites.

The extreme sensitivities of insects to their semiochemicals dictates extreme cleanliness in the laboratory to avoid contamination (solvent controls are a safeguard). The soaking of all glassware in detergents such as Decon[R] 90 between experiments is recommended.

Table 3.1. A summary of categories of odour-induced manoeuvre resulting in movement towards an odour source. (From Kennedy, 1986.)

Name	Sensory input: Odour concentration differences detected by sampling:	Minimum receptors needed	Locomotory output = manoeuvre: Form	Locomotory output = manoeuvre: Steering
Orthokinesis	Successively along path	One	Change in linear speed or frequency of locomotion	Self-steering throughout
Klinokinesis	Successively along path	One	Change in rate or frequency of turning	Self-steering throughout
Schemakinesis ('longitudal klinotaxis')*	Successively along path	One	A particular pattern	Self-steering throughout
Klinotaxis ('transverse klinotaxis')	Successively, by small swings left & right ('wig-wagging')	One	Turning to the more or less stimulated side	By the odour gradient, but 'wig-wags' probably self-steered
Tropotaxis	Simultaneously left & right	Bilateral pair	Turning to the more or less stimulated side	By the odour gradient
	Directional cues generated by wind			
Menotaxis (now including 0° & 180° angles)	Wind direction may be sampled successively left & right by serial counterturning	Multi-directional	Maintaining a set angle to the wind direction = *anemotaxis**	By wind-generated cues: mechanical for walkers, visual for flyers

* Positive' anemotaxis = upwind; 'negative' = downwind; across-wind was called anemo-*menotaxis* in the original Kühn sense.

3.4.6. Acoustic stimuli

The first stage is recording the sounds and analysing them. Ewing (1989) and Bailey (1991) offer practical advice on methods as well as providing good introductions to the field of insect bioacoustics. Digital recording is now possible even on portable cassette recorders. Many software packages for analysing sound are available (e.g. 'Canary' for the Mac, reviewed by Wilkinson, 1994, and 'AVISOFT-SONAGRAPH' for IBM systems, reviewed by McGregor and Holland, 1995). For up-to-date information on available software, including shareware, try the Cornell Bioacoustics Research Program (web page http://www.ornith.cornell.edu/BRP).

A playback system allows you to test your hypotheses in studies of communication. Among the many decisions to be made about playback experiments are the intensity, volume, distance between sources, original volume measurement, sound quality, distortion, fidelity, which parts of the signal to use, and the problem of between-individual variation. A particular problem with insects and other poikilotherms is the effect of temperature (see Bailey, 1991).

Some software packages can also be used for manipulating the signals for playback – or creating entirely synthetic signals for playback through a loudspeaker or other device. Synthetic signals allow much greater control over the stimulus offered (e.g. for vibratory courtship signals of lacewings (Wells and Henry, 1992)). Michelsen *et al.* (1992) used a mechanical 'robot bee' to investigate communication in the bee dance.

3.4.7. Flight and locomotory behaviour

Wind tunnels have become common in laboratories researching olfactory behaviour in insects but have also been used for studies of orientation, whether or not mediated by chemicals, and of migration. Very practical advice on designing and using wind tunnels is given by Baker and Linn (1984). Wind tunnels with air pushed by a fan predominate as these give fewer problems than pull-fans at the relatively low wind speeds used.

Some insects such as flies and beetles have had a reputation for being difficult to work with in the wind tunnel but much may depend on getting the conditions, such as lighting and wind speed, right. It may be worth persevering if the experiments would be useful. Prior treatment of the insects, and choice of laboratory strain for cultures, may be particularly important given the potential causes of variability (see Section 3.3).

As part of studies on flight orientation mechanisms two groups have been examining moth response to individual pheromone puffs using different ways of producing these and different but equally ingenious methods to track the passage of odour puffs down the wind tunnel (Mafra-Neto and Cardé, 1994; Vickers and Baker, 1994). This last paper describes an in-flight electroantennogram (EAG), using an antenna (from a second male) attached

by Velcro across the head of the flying moth, to allow contacts with the pheromone puffs to be matched to behaviour.

Insects such as winged aphids can be kept in sustained flight in vertical wind tunnels, flight upwards towards a bright light countered by air flow from above. Such a wind tunnel has been successfully automated, with video-tracking feeding a computer controlling the air flow to keep the insect on station (see Young *et al.*, 1994). The computer also controls the intermittent flashing of lights which give visual stimuli from the side. Host plant odour cues have also been tested on aphids in this system, e.g. Nottingham and Hardie (1993). Vertical wind tunnels have also been used with beetles (Blackmer and Phelan, 1991) and whitefly (Blackmer and Byrne, 1993).

Locomotor compensators, which work like a multi-directional treadmill, have been used with success for walking insects in studies of both olfactory and auditory stimuli (see Bailey, 1991; Bell, 1991). The insect walks on a servosphere which is moved in the opposite direction by a feedback loop driven by a control system in response to movement detected by a video camera. One advantage of the system is that the orientation of the insect in relation to the odour air flow, for example, is always known. However, as only one animal can be tested at a time, use has been largely restricted to studies where the track of the insect was the focus. The cost of the hardware is also a major handicap.

3.4.8. Feeding behaviour

Feeding is a central part of any insect's behaviour and has important consequences for applied ecology including a potential role in plant and pesticide resistance. (The feeding behaviour of phytophagous insects is discussed in this volume, Chapter 6.) Feeding behaviour in blood sucking insects such as mosquitoes and tsetse flies has been studied mostly at the level of host finding (for reviews see Lehane, 1991; Colvin and Gibson, 1992; trapping techniques for mosquitoes are described in Service, 1993). However, biting behaviour on different hosts and, using artificial membranes, effects of phagostimulants have also been investigated (e.g. Moskalyk and Friend, 1994). Marked circadian rhythms of activity and thus feeding are found (e.g. Kyorku and Brady, 1994). The feeding behaviour of predators such as carabid beetles, important in biological control, has been studied partly in laboratory trials, which is not very satisfactory, but also by testing field caught animals with ELISA (Sopp *et al.*, 1992), and now using monoclonal antibodies (Symondson and Liddell, 1995; also this volume, Chapter 12), against potential prey items.

Feeding behaviour provides an excellent focus for investigating the integration of internal and external influences on behaviour, and its consequences such as life history effects, at both immediate and evolutionary time scales. Simpson and Raubenheimer (1995) offer a practical framework for studying feeding behaviour in the context of the multiple nutrient requirements of animals.

3.4.9. Reproductive behaviour

Over the last 25 years the emphasis of research on insect reproductive behaviour has been increasingly moving towards a focus on individual selection (Thornhill and Alcock, 1983; Alcock and Gwynne, 1991). The most active research areas, and methodologies used, tend to follow those of behavioural ecology more broadly, that is a recent focus on the evolution of mating systems and sexual selection. The specific insect techniques involved in this area of research are mostly those of catching and marking individuals. The practical advantages insect research has over vertebrates include the short generation time (which makes genetic studies possible) and the ability to continue studies with laboratory cultures to elucidate the fitness benefits of paternal investment for example. Work on reproductive behaviour contributes to studies on speciation (Butlin and Ritchie, 1994).

Arthropod studies have made important contributions to our understanding of sperm competition, including a recent reappraisal of two-male sperm precedence experiments using genetic markers or sterilization of one male with irradiation. Using single locus minisatellite profiling to check the paternity of offspring of multiple matings, Zeh and Zeh (1994) have shown that the strong last male-sperm precedence usually shown in laboratory two-male experiments breaks down when more than two males mate with a female.

3.5. Conclusions

Keep it simple: neat experimental design wins over high technology. Although it may sound imprecise, developing a 'feeling for the organism' through time spent watching is likely to reward you with better experiments. Relating questions to the ecology and evolutionary background of the animal may often provide the best answers.

3.6. Acknowledgements

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