

Oviposition preference: its definition, measurement and correlates, and its use in assessing risk of host shifts

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Summary

To predict evolution in plant–insect systems we can begin by defining potentially heritable traits of plants that describe how they interact with insects and potentially heritable traits of insects that describe how they interact with plants. Examples are “acceptability” as a plant trait and “preference” as an insect trait. Some practical applications of this approach and of dissecting preference into its components are discussed. The question: Given one population of insects and 2 categories of plant, which category of plant do the insects prefer, and is this preference adaptive? seems like a simple question, but testing it can be confounded by two problems. First, plants vary both within and among species, and we don’t know how to classify them from the insects’ perspective. Second, insects vary along axes of preference that we hadn’t imagined. An example is given from butterflies in which variation among insects in how they rank plant individuals (within species) can masquerade as variation in which species they prefer. An apparent solution to this problem would be to offer each insect a different, randomly chosen pair of plants in the two plant categories being compared. But insects don’t interact with plants at random in nature, and we show that forcing them to do so in an experiment generates misleading results.

From a practical perspective I argue that risk assessment would benefit from incorporation of the concept of “motivation” alongside “preference” and that candidate species should be tested at maximum levels of motivation. I also describe how taking advantage of detailed behavioural traits of a study insect allows the development of a preference-testing technique. The technique itself may or may not transfer to other systems; what should transfer is the approach to exploiting natural traits of the insect, whatever they may be. This approach also includes a rationale for identifying and testing the assumptions underlying the design of a preference test.

Introduction

Critiques of biocontrol procedures have been based on observed use of non-target plants as hosts by introduced insect control agents (Louda *et al.* 1997). Such critiques underline the need to estimate as precisely as possible the risks that such events will occur (Zwölfer & Harris 1971, McEvoy 1996, Simberloff & Stiling 1996, McFadyen 1998, Withers 1999, van Klinken & Edwards 2002). These efforts could involve estimating the likelihoods of acceptance of particular specified non-targets, and/or the general propensity of a candidate agent to expand its host range in a general sense. An important aspect of risk assessment is to understand the relationship between the results of preference tests performed on captive insects and the likelihood that

these insects would attack low-ranked (less-preferred) hosts in nature. Here, I discuss the definition and testing of preference and summarize prior work on the conceptual and practical separation between insect preference and plant acceptability. I also consider the ways in which the internal state of the insect may affect its motivation, or readiness to feed or oviposit, and the manner in which the concept of “motivation” might be useful in risk assessment. Finally, I suggest that we do not yet know whether a monophagous population of an oligophagous species poses a greater risk as a candidate agent than an equally monophagous population of an entirely monophagous species.

For many herbivorous insects, especially flying insects, oviposition preference is the principal mechanism by which the insect–host relationship is established. It is this trait of the insect that interacts with spatial distributions, abundances and acceptabilities of plants to generate patterns of insect–host association across the landscape. The arguments presented here,

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developed specifically from studies of Melitaeine butterflies (genera *Melitaea* and *Euphydryas*), provide a worked example of how oviposition preference might be defined, measured and related to events in the field. Readers are left to deduce the extent to which these conceptual approaches and techniques may be useful for understanding the insects with which they work.

Definitions of terms

I use the following definitions, modified slightly from those suggested earlier (Singer 1982, 1986, 1994, 2000, Singer *et al.* 1992b):

- An “encounter” occurs between plant and insect when the insect arrives at a distance from which it could perceive stimuli emanating from the plant. For example, an insect may encounter a plant visually, perceive it, approach or alight upon it and then encounter it chemically and physically.
- “Acceptance” is a positive response made by an insect to a plant that has been encountered. For example, a flying insect may accept visual stimuli by turning towards a plant and alighting on it. The insect may then accept (or reject) contact chemical stimuli by feeding or ovipositing (or not).

Insect traits:

- “Motivation” is a general tendency to feed or oviposit, without reference to any particular host. A motivated insect is sensitive and responsive to stimuli that may lead it to feed or oviposit (Singer *et al.* 1992b).
- “Perceptual ability” is the set of likelihoods of perceiving a particular specified set of plants that are encountered.
- “Preference” is the set of likelihoods of accepting a particular specified set of resources that are perceived (Singer 1986, 2000). In practice, it would normally be measured as the set of likelihoods of accepting resources that are encountered. One aspect of preference is “host range”, the set of plants that would be accepted under specified conditions. In a conservative assessment of a candidate agent we should be principally interested in the host range at maximum motivation.
- “Specificity” has been defined (Singer 1982, 1986) and used by some biologists (e.g. Courtney *et al.* 1989, Thompson 1998) to mean the strength of preference, regardless of its direction. However, this usage has not become well-established and there are several current uses of “specificity”. It is sometimes synonymous simply with “host affiliation” or with “insect diet” and sometimes has a wider meaning incorporating both preference and performance (van Klinken & Edwards 2002).

Plant traits:

- “Apparency” is the set of likelihoods that a plant will be perceived by a specified set of insects (Feeny 1976, Singer 1986).
- “Acceptability” is the set of likelihoods that a plant will be accepted after being encountered by a specified insect or set of insects (Singer 1986, 2000).

The definition given here renders “preference” a useful trait in thinking about the potential for evolutionary change because it is a trait of the insect whose variation can be measured among individuals and populations. In contrast, “preference” is often defined by ecologists as the proportion of a particular resource in the diet as a function of the availability of that resource in the habitat (Hassell & Southwood 1978, Crawley 1984). This ecologically important parameter is an emergent trait of the plant–insect interaction rather than a trait of insect or plant (Singer & Parmesan 1993, Singer 2000). A partial solution to the difficulty posed by the diversity of meanings of “preference” is to use the term “*electivity*” (Ivlev 1961, Singer 2000) for the ecological parameter and “preference” for the behavioural parameter.

With the definition of “preference” used here, experiments in which an insect is offered a single plant (no-choice tests) should not strictly be called tests of “preference,” although a series of such tests might be so called. An insect cannot have a “preference” for a single resource. We might better describe it as having an “*affinity*” for such a resource.

Sequence of events in host search by Melitaeine butterflies: responses to visual, chemical and physical stimuli

I’ll begin with a description of the oviposition behaviour of our study insects, from which our conceptual approaches have been derived. While the results of this study may not be directly applicable to different types of insect, the approach and manner of analysis could be more widely relevant. In *Euphydryas editha* at Rabbit Meadow, Sequoia National Forest, California, alighting was primarily or entirely in response to visual stimuli, as evidenced by strong relationships between fixed (non-learned) alighting bias and plant visual traits (Parmesan *et al.* 1995). After tasting a plant and finding it chemically acceptable, the Melitaeine curls its abdomen under a leaf, extrudes its ovipositor, and probes the lower surface of the leaf. This probing is clearly a response to chemistry because it can be stimulated by placing the insect on a dampened filter paper on which an ethanol wash of host leaf surface has been evaporated. There is apparently no chemical sense on the ovipositor: eggs are readily laid on non-host or even non-plant material, all that is necessary is that the tarsi of the insect contact the host; the ovipositor does not need to do so. Once the plant has been chemically

accepted, oviposition depends principally on physical features of the site such as the size, shape and orientation of the leaf and the extent to which it yields when pressed by the ovipositor.

Pre-alighting butterfly preference and host apparency

Apparency was first visualized by Feeny (1976) as a property of a plant that influenced its susceptibility to being found by herbivores. Singer (1986) defined it as the set of likelihoods that a plant will be perceived by some specified insect or set of insects. The property of the insect that interacts with plant apparency is the insect's perceptual ability, defined here as the set of likelihoods of perceiving a particular specified set of plants that are encountered. The concept of apparency has fallen into disuse. This may be because apparency is, in practice, hard to measure and/or to separate from perceptual ability. We usually cannot tell whether an insect that passes over a plant without stopping fails to perceive the plant or perceives it and decides against alighting on it. Whether or not the plant is perceived depends on an interaction between insect perceptual ability and plant apparency. Whether or not the insect alights on a plant that it has perceived depends on an interaction between the insects' pre-alighting host preference and plant acceptability.

With present knowledge and techniques these factors may be difficult or impossible to tease apart in practice. However, defining them in principle is, I think, useful to help generate the incentive to understand the mechanisms at work. In one case, our group has made progress in identifying pre-alighting preference rather than the apparency/perceptual ability relationship as a cause of observed patterns of alighting. *E. editha* at Rabbit Meadow had added to their diet a novel host, *Collinsia torreyi*, just a few (<20) generations prior to our study. The butterflies found this novel host very inefficiently: in the habitat patches where it was the principal host, the proportion of alights upon it was lower than the proportion the butterflies would have achieved by alighting on vegetation at random (Mackay 1985, Parmesan *et al.* 1995). This inefficiency of finding *Collinsia* could have resulted from two causes:

1. evolutionary lag in the insects' pre-alighting preference, such that *Collinsia* was perceived but not preferred for alighting (even though many insects accepted it readily on contact).
2. failure of the insects to perceive *Collinsia* by virtue of the interaction between its apparency and the insects' perceptual abilities.

The first explanation was suggested by Mackay (1985). At that time *Collinsia* had been used by the Rabbit Meadow butterflies for less than 20 generations. Parmesan (1991), by comparing populations that had undergone host-shifts in different directions, showed that *Collinsia* was found efficiently when it was the

traditional, rather than the novel host. She therefore attributed the inefficiency of finding *Collinsia* at Rabbit Meadow to an evolutionary lag in the response to natural selection on pre-alighting preference, rather than to an evolutionary constraint associated with the failure of the insects to perceive *Collinsia* at all.

Post-alighting oviposition preference and host acceptability

In the same manner that perceptual ability can be viewed as an insect property that interacts with plant apparency, preference can be viewed as an insect property that interacts with plant acceptability (Singer 1986, 2000). The simultaneous variation of both preference and acceptability creates a series of difficulties for experimental design and interpretation (Singer 2000, Singer & Lee 2000, Singer *et al.* 2002). Despite these difficulties, it has been possible in one case to illustrate how variation of both preference and acceptability made independent contributions to patterns of insect-plant association in the field (Singer & Parmesan 1993). Two populations of *Euphydryas editha* chose different host species, partly because of a genetic difference between the sites in acceptability of one of the two host species and partly because of a genetic difference in insect oviposition preference.

Preference-testing technique: development of the sequential choice test for Melitaeines

The most common form of preference-testing used with butterflies is to place the insect in a cage with several test plants and allow oviposition to occur for a day. At the end of the day the eggs on each plant are counted. The positions of the plants are then rotated to control for "position effects", and the experiment is repeated on the following day (Thompson 1993, Bossart & Scriber 1995, Wehling & Thompson 1997). This technique doesn't work well with Melitaeines, for several reasons. First, the insects don't duplicate natural flight behaviour in small cages. During the time-period when a caged butterfly would naturally be searching for hosts if it were at liberty, it is likely instead to sit on the walls of its cage. Therefore, by the time the butterfly does move sufficiently to encounter plants, it is highly motivated and likely to accept the first host that it finds (see below). Second, these insects each lay few, large egg clusters. This experimental design therefore produces few data from each individual butterfly when the data are numbers of egg clusters. There is insufficient statistical power to compare preferences of individuals. In response to this difficulty, our group has developed a testing technique for post-alighting preference that generates more data from each individual than the number of egg clusters that it lays. This technique is the sequential choice test (Singer 1982, 1986, Singer *et al.*

1992b), a technique that overcomes the problem of low egg cluster number by staging a series of encounters between a butterfly and test plants and using as data the results of each encounter, while preventing the insects from actually ovipositing. An insect that is not allowed to oviposit will continue to show acceptances and rejections of plants that it encounters, thereby providing more information than could have been obtained from a single oviposition.

This test takes advantage of the manipulability of Melitaeines. A female placed gently on a host, either in the field or in the greenhouse, appears to behave as though she had naturally alighted on that host. But does she? Indeed she does! Rausher *et al.* (1981) found that manipulated butterflies duplicated the choices they had made before they had been captured.

A second test of the relevance of manipulated trials to actual host use was made by testing the preferences of insects captured naturally ovipositing on different species at the same site (Singer 1983, Singer *et al.* 1993). There was a strong association between the tested preferences and the observed ovipositions. Again, this shows that a test using manipulated butterflies measures *something* that is connected with the observed variation of host use.

How to perform a sequential choice test

Manipulated tests are clearly pertinent to events in the field. What are the actual procedures involved in the testing? Each insect is offered a series of staged encounters at, say, 15-minute intervals. Each encounter lasts a maximum of three minutes. Acceptance is judged from pressing of the extruded ovipositor against the plant for a count of three. Rejection is the absence of this behaviour during the entire three-minute period. An insect that accepts is not allowed to oviposit, but is manually removed from the plant before the first egg has been laid.

The test is based on the observation that, as time passes, the probability that a particular plant would be accepted, if it were encountered, jumps from 0 to almost 1 very rapidly, in the space of just a few minutes (Singer 1982). That probability then remains close to 1, at least during the principal hours when oviposition is likely (noon to 4pm) until oviposition occurs. Suppose that an insect is offered the same plant over and over and over and over again, in repeated staged encounters, and is prevented from actual oviposition as described above. There is a rejection phase when the plant is consistently rejected, followed by an acceptance phase when the plant is accepted about 95% of the time (Singer 1982). Now suppose that the same insect is offered staged encounters with two plants, X and Y, in alternation. If we indicate a rejection by R and an acceptance by A, we may observe one of three types of sequence, shown below. Each acceptance or rejection

in these sequences is the result of an *entire* three-minute trial, with no account taken of the time to acceptance *within* any such trial:

1. RX; RY; RX; RY; RX; AY; RX; AY; RX; AY; AX; AY; AX; AY; AX
2. RX; RY; AX; RY; AX; RY; AX; RY; AX; RY; AX; RY; AX; RY; AX; AY
3. RX; RY; RX; RY; RX; RY; RX; RY; AX; AY; AX; AY; AX; AY; AX; AY

This procedure would constitute a sequential choice test. In case (1) we would say that Y is preferred because X is rejected in encounters that follow acceptance of Y. In case (2) X is preferred, and in case (3) no preference is detected.

To the extent that the behaviour of manipulated butterflies really represents what they would do if they were at liberty, then the result shown in (1) estimates the length of time that a butterfly would search in the motivational state where encounter with Y but not X would result in oviposition, before reaching the motivation at which either X or Y would be accepted, whichever were the next plant to be encountered. This length of time is called the “discrimination phase” (Figure 1). It is a measure of the strength of preference for Y over X. The discrimination phase in (1) is shorter than that in (2), so the preference for Y over X shown in (1) is weaker than the preference for X over Y in (2).

Because the insect cannot be offered continuous exposure to both plants, the length of the discrimination phase cannot be measured precisely. Its minimum length is the time difference between the first acceptance of the preferred host and the last rejection of the second-ranked host. In practice, this minimum length is the value that has been used, partly because the maximum cannot be estimated for insects that never accept the second-ranked host. Use of the minimum value gives us the freedom, if we so choose, to utilize data from butterflies that escape or die under interrogation, before they have accepted all the hosts in the test series.

It is impossible to estimate the length of a discrimination phase unless one begins the test sequence before *any* of the test plants are acceptable. If the first staged encounter with just one of the test plants results in acceptance, then the discrimination phase has already begun. If this happens, it may be possible to obtain a rank order of preference, but estimation of discrimination phase length requires allowing the insect to oviposit and recommencing the test. The test, once begun, should ideally not be interrupted. An insect that rejects both test plants before such an interruption may switch directly to accepting them both when testing recommences. In such a case the opportunity to discover which plant would have been accepted first has been lost. The insect should be allowed to oviposit and its test re-started.

Figure 1. Stylized depiction of changes in responses of insects to two plants, when repeated encounters are staged and oviposition is NOT allowed. Records are shown for three butterflies, each of which prefers plant A over plant B. Discrimination phases are indicated by “DP”. (Modified from Singer *et al.* 1992b.)

Expressing the results of the sequential choice test

The original description of the sequential choice test (Singer 1982) suggested that the test allowed preference to be described in two ways. The “rank order” of preference is the order in which the different plants are first accepted, while the strength of preference or “specificity” is estimated from the length of the discrimination phase. This distinction has been adopted by some authors who have found it useful in order to argue that rank order is more highly conserved in evolution than strength of preference (Courtney *et al.* 1989, Thompson 1993). In Melitaeines both aspects of preference can vary simultaneously, giving rise to a bell-curve of preferences (Figure 2). In this figure, specificity is depicted as the distance along the abscissa from the “no preference” point, and “rank order” is opposite on either side of this point. The minimum length of discrimination phase, again on the abscissa, is determined using only time differences during the period (11:30am to 4:30pm) when oviposition is likely. Therefore 5 hours in the figure is equivalent to 1 day, 10 hours to 2 days, etc.

Figure 2 shows that the range of plants that would be accepted, if they were encountered, expanded at different rates in different individual butterflies sampled from the same population. In *E. editha* this type of variation is heritable (Singer *et al.* 1988), and responds rapidly to natural selection (Singer *et al.* 1993).

Assumptions of the sequential choice test

Any preference test carries baggage in the form of assumptions of varying testability, and ours, alas, is no exception! The following section identifies some of our assumptions and discusses the extent to which they have been tested.

Assumption 1. There is a precise time at which an insect switches from a “rejection phase”, during which a particular plant would be consistently rejected if encountered, to an “acceptance phase”, during which that plant would be consistently accepted. The timing of the switch from rejection to acceptance differs in responses to different plant categories. If this assumption were not true, the “discrimination phase” would not be real. How true is it? This can be tested by asking what is the frequency of rejection of plants that have been previously accepted, when no oviposition has intervened. For plants that were moderately or highly acceptable to the insects, the frequency of such rejections was typically 5% or less, but in one case a plant that was first accepted several days after the highest-ranked host was never consistently accepted (Singer 1982). For most hosts, a plant that had been accepted was accepted again with about 95% probability, provided that no oviposition had occurred, that there was no adverse change in the weather and that the end of the day was not at hand.

Figure 2. Distribution of measured discrimination phases at Rabbit Meadow in 1981. Lumped data from two adjacent habitat patches, one where *Collinsia* was used and one where *Pedicularis* was used. (Modified from Singer 1983.)

Assumption 2. When a test covers more than one day, we assume that the motivational state of the butterfly at the beginning of the second day's test is the same as its motivational level at the end of the first day's test. This is not easy to test, and we have not explicitly tested it, but the behaviour of *E. editha* is consistent with the assumption in the following manner. When we commence testing at some time between 11:30 and noon, we usually observe that the range of plants that are accepted resembles the range that had been accepted at 4pm the previous afternoon. It is unusual for additional plants to be accepted at this time, or for plants to be rejected that had been accepted the day before. We tentatively conclude that the passage of time between 4:30pm and 11:30am has little effect, and time during this period is not included in the calculation of the discrimination phases.

Assumption 3. We assume that an encounter with plant A at time 1 has no effect on the insect's responses to either plant A or plant B at some subsequent time. This assumption is obviously violated when a butterfly has just accepted a host and is transferred quickly to another one. There is a clear "carry-over" effect making the second host much more likely to be accepted than if the butterfly were made to fly or allowed to rest in a cage with no hosts for a few minutes. Therefore, when we observe an acceptance, we allow the insect at least five minutes' rest before testing another plant.

Apart from this effect, experiments consistently fail to show any effect of manipulated experience on host acceptance. Two such experiments are described below:

- a) We collected wild females each morning at Rabbit Meadow and split them into two groups. One group was offered 9–10 repeated encounters with C (*Collinsia*), the other with P (*Pedicularis*). In the afternoon of the same day all butterflies were offered the same test plant species. This test plant was sometimes C and sometimes P, on alternate days. So, each day's experiment asked whether butterflies with different recent experience of host encounter differed in their responses to a single test plant. No such effects were found (Thomas & Singer 1987)
- b) We collected teneral females (as mating pairs with no prior host encounter) at Rabbit Meadow and offered some of them alternating encounters with P and C, while others were offered only C or only P. We then offered each insect a single test with either C or P on the afternoon of the second day of its adult life. Again, no effects were detected (Singer 1986).

Assumption 4. Handling the butterflies does not affect their responses. In fact, handling does have a clear effect: it increases the likelihood of oviposition. A butterfly can be "encouraged" to oviposit by being

picked up and quickly replaced on the test plant. Perhaps picking the insects up and replacing them makes them respond as though they were encountering plants more frequently, and they may be sensitive to plant density. We don't know. Our method of dealing with this violation of assumption 4 is that, whenever an insect appears to be rejecting a plant, she is picked up and replaced at least three times during each three-minute staged "encounter", before the result of the test is recorded as rejection. By this means we attempt to "encourage" oviposition equally in all of our test subjects.

It is clear from this account that the assumptions of our technique are to some extent violated, and that subjectivity cannot be totally eliminated from these sequential choice trials. We cannot be sure of the exact relationship between the test results and the behaviour of the butterflies in the field. However, several tests have shown that variation among individuals or populations in natural behavior in the field is paralleled by variation in the results of preference tests administered subsequently (Rausher *et al.* 1981, Singer *et al.* 1993).

Distinguishing in practice between "preference" and "motivation"

Starting with Dethier's (1959) paper on "mistakes" made by ovipositing butterflies and continuing to the present day, there has been continued discussion in the literature about the frequency with which insects oviposit on hosts that are suboptimal, hosts that are not preferred, or even on non-hosts that are toxic (Chew & Robbins 1984, Feldman & Haber 1998). These discussions have often involved questions about the roles played by unusual oviposition events in evolution of diet (Thomas *et al.* 1987). Perhaps such events are preludes to host-shifts? Whether or not this is true depends on the behavioural mechanisms that cause unusual ovipositions and on the likelihood that insects performing such unusual acts do so because of heritable preferences (Karowe 1990, van Klinken 2000).

In this context our ability to test preferences of freshly-captured Melitaeines in the field has enabled us to investigate the behavioural mechanisms that underlie observations of natural oviposition on low-ranked hosts. Why might a Melitaeine be found ovipositing on a plant other than its preferred host? There are two possibilities. First, the insect has been searching for a long time without finding its preferred host. Second, its discrimination phases are short and it does not search for long before it would accept a second or third-ranked host. In the first case, we could describe the butterfly as highly motivated to oviposit. In the second, we could say that its preference is weak or its specificity is low. Why should we bother to make this distinction? The evolutionary consequences are different in the two cases. Differences among individuals in motivation caused by differences in length of search are not likely

to be heritable, while differences in length of discrimination phase could be heritable, and indeed, are likely to be so (Singer *et al.* 1988, 1992b). This argument is pertinent to questions about the consequences of a single event in which an introduced agent feeds on a non-target plant.

To clarify the distinction between preference and motivation, I have depicted in Figure 1 (taken from Singer *et al.* 1992b) stylized records for three butterflies, two of which (#2 and #3) differ in motivation but not in preference and two of which (#1 and #2) differ in strength of preference but not in motivation. The figure indicates that, at 13.50h, butterfly 3 would accept plant A if that plant were encountered, but butterfly 2 would reject it. This would be ascribed to the difference in motivation. At 14.50h, butterfly 2 would reject plant B while butterfly 1 would accept it. This would be ascribed to their difference in strength of preference. We have shown experimentally that variation of motivation and of preference occur simultaneously in the field and that these variables can be teased apart (Singer *et al.* 1992b).

Correlates of preference

1. Relationship of preference to fecundity

Preference is often thought to be driven by "eggload". An insect that feels increasing "egg pressure" might be increasingly motivated to oviposit. Differences among individuals in fecundity or rate of egg maturation would then generate differences in strength of oviposition preference (Courtney & Hard 1990). However, we (Agnew & Singer 2000) suspected that several of these conclusions had been derived from incorrect attributions of cause to observed correlations in the field. In our own study insects, the individuals that matured eggs fastest were *not* the individuals with the fastest increase in their range of accepted hosts.

2. Relationship of maternal preference to offspring performance

Discussion of relationships between preference and performance typically confounds several different questions. Three of the most important ones are:

1. Is preference correlated with performance among populations? In other words, is preference variation among populations associated with performance variation in the same set of populations?
2. Is preference correlated with performance within populations? In other words, do individual mothers with particular preferences produce offspring with particular performances?
3. Is host choice adaptive at the population level? To what extent is the rank order of plants in the insects' preference hierarchy concordant with the rank order of the same plants in their ability to support larval

growth and survival (cf Wiklund 1975, Jaenike 1990, Mayhew 1997)?

All three of these types of correlation occur in *E. editha* (Rauscher *et al.* 1981, Ng 1988, Singer *et al.* 1988, 1994). The second type even occurs with respect to variation among individual host plants (Ng 1988).

3. Correlations among preferences

Preference for A versus B may not be independent of preference for C versus D (Courtney *et al.* 1989). Then again, it may be! (Singer *et al.* 1992a).

Novel axes of variation revealed by preference-testing of Melitaeines

Melitaeines make substantial discriminations within as well as among host species. This process generates complexity because discrimination within species is not nested within discrimination among species, as one might reasonably expect. Preference-testing of insects on conspecific and heterospecific plants has revealed novel axes of variation, the existence of which threatens many standard and apparently sensible experimental designs. Three examples are discussed below.

1. Individual *Melitaea cinxia* butterflies varied in the relative importance they assigned to variation within and among host species (Singer & Lee 2000). Singer & Lee showed how variation in discrimination within plant species might falsely appear as variation in discrimination among species. This could be an important, general and overlooked problem in experimental design.
2. When *Euphydryas aurinia* butterflies and their hosts were sampled randomly, we obtained the odd result that insects from populations feeding on *Gentiana*, *Lonicera* and *Cephalaria* all preferred over their own hosts a plant species, *Succisa pratensis*, that they never encountered in the field (Singer *et al.* 2002). This appearance of maladaptation was an artefact of sampling host populations at random. It disappeared when the populations were sampled differently, using naturally-accepted plants (Singer *et al.* 2002). This result casts doubt on experiments that ask whether host choice is adaptive by manipulating insects to feed on randomly-chosen members of different host species. Alas, this category includes many of our own experiments (e.g. Singer *et al.* 1994).
3. *E. editha* at Rabbit Meadow were offered *Pedicularis* plants in sequential choice trials. Newly hatched larvae were then placed on the plants to ask whether plants that were generally preferred supported higher offspring survival. They did not (Ng 1988). However, this apparently simple result, that discrimination is NOT adaptive, disguised an unexpected complexity. Individuals that discriminated among *Pedicularis* plants produced offspring

that survived better on plants preferred by discriminating individuals. Offspring of insects that did not discriminate survived equally well on plants accepted or rejected by discriminators (Ng 1988). If we put the question in the form: “are the plants that are most acceptable in a general sense also the most suitable in some general sense?” we get a misleading result!

These three effects create considerable difficulties for the design of experiments that manipulate plants and insects into specific interactions and then examine the consequences of those interactions for either or both partners.

Conclusions

Changes in oviposition preference are intimately involved in observed diet shifts in nature (Singer *et al.* 1993, Singer & Thomas 1996). These natural observations validate the approach of incorporating detailed studies of preference in risk-assessment (e.g. Heard & van Klinken 1998, Barton-Browne & Withers 2002). Here, I have described the approach to defining and measuring preference that our group has developed in working with populations of Melitaeine butterflies. This approach first introduced the role of time since last oviposition (TSLO) as an important cause of changes in host acceptance (Singer 1982, 1986). For reasons detailed earlier in the report, we have chosen to describe these time-dependent changes in observed host range as driven by changes in motivation, and to define “preference” independently of motivation. So, when an insect that is deprived of opportunity to feed undergoes physiological changes that cause it to accept a wider range of hosts, this is a change in motivation. Its preference is measured by the manner in which these changes occur. Learning (Cunningham *et al.* 1999) influences preference and thereby affects the relationship between motivation and acceptance.

While the detail of this study may not be broadly applicable, what are the more general messages of this approach for risk assessment? First, that candidate insects should be tested at the maximum levels of motivation that they are likely to attain in the field. In many cases we don't know what conditions maximize motivation, so this requires study. Time of day, insect age and body temperature may be important. In insects whose motivation varies with TSLO, as do our study insects, maximizing motivation entails prolonged testing with deprivation of opportunity to feed or oviposit, as suggested by Barton-Browne & Withers (2002). No-choice tests are useful in this context since allowing feeding/oviposition on a preferred resource may hold motivation to low levels at which low-ranked resources are not accepted. In any case, insects normally encounter plants sequentially rather than simultaneously. What may appear to the experimenter to be a choice situation may from the insects' perspec-

tive be a series of no-choice situations. Even when choice tests in which test plants are juxtaposed resemble natural situations that occur frequently, they don't represent all the natural conditions. Some individual insects are sure to wander away from the hosts on which they developed, encounter habitats that don't contain those hosts, and experiment with hosts that they would not have attacked if they had stayed at home (Thomas *et al.* 1987; Singer *et al.* 1992b). Situations are bound to occur where the control agent is exposed to the non-target plants and not to the target. The important question then becomes, not which plant is preferred, but whether the non-target is acceptable to the insect in a no-choice situation. For this purpose no-choice tests are the tests of choice (cf Hill 1999).

A second question that arises from our work is this: do we have any use for species such as *E. editha*, in which populations may be either oligophagous or monophagous? In other words, is a monophagous population of an oligophagous species useless as a candidate agent, despite its population-level monophagy? Our preference testing shows that a sample taken from a monophagous population may comprise entirely insects that would search for several days for their preferred host species before accepting a second choice. These are specialized insects that might indeed be suitable candidate agents. But would such a population be more likely to indulge in a host shift because it is sampled from an oligophagous species rather than from a monophagous one? The answer to this question isn't known, but is susceptible to analysis by molecular phylogenetic techniques. Pending such analysis, all we can say is that when these insects have undertaken host shifts in the past they haven't necessarily speciated. We can't say that host shifts have occurred with higher frequency over time than in other groups of insects that have speciated with each host shift. If I continue this line of reasoning further, I'll get into discussion of the definition of "species", which lies far outside the purview of this paper. However, as several recent works (e.g. Hoffman *et al.* 2002) indicate, we should not worry too much about how to define species, host races and biotypes, but we SHOULD worry that the candidate agents that are introduced belong to EXACTLY the same entity, be it a species, a biotype or a host race, that has been subjected to specificity testing. Insects vary among populations in their host adaptations, and we should never assume that a sample originating from one population will behave in the same way as the same "species" sampled elsewhere.

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