Michael Singer and Camille Parmesan

Annual Review of Entomology

Preference Provides a Plethora of Problems (Don't Panic)

Michael C. Singer

Station d'Écologie Théorique et Expérimentale, CNRS et Université Paul Sabatier, 09200 Moulis, France; email: michael.singer@sete.cnrs.fr

Annu. Rev. Entomol. 2021. 66:1–22

First published as a Review in Advance on September 14, 2020

The *Annual Review of Entomology* is online at ento.annualreviews.org

https://doi.org/10.1146/annurev-ento-022720-061725

Keywords

plant–insect interactions, insect preference, plant acceptability, discrimination within host species, discrimination among host species

Abstract

This review was solicited as an autobiography. The "problems" in my title have two meanings. First, they were professional difficulties caused by my decision to study oviposition preferences of butterflies that were not susceptible to traditional preference-testing designs. Until I provided video, my claim that the butterflies duplicate natural post-alighting host-assessment behavior when placed on hosts by hand was not credible, and the preference-testing technique that I had developed elicited skepticism, anger, and derision. The second meaning of "problems" is scientific. Insect preference comes with complex dimensionality that interacts with host acceptability. Part Two of this review describes how my group's work in this area has revealed unexpected axes of variation in plant–insect interactions—axes capable of frustrating attempts to derive unequivocal conclusions from apparently sensible experimental designs. The possibility that these complexities are lurking should be kept in mind as preference and performance experiments are devised.

INTRODUCTION

If we survive long enough, we graduate, in British terminology, from "wrinklies" to "crumblies." By requesting this "autobiography," my third (see 18, 62), the *Annual Review of Entomology* cements my crumbliness. However, I don't need Annual Reviews to do that; my interactions with young scientists are more than sufficient. Here's one from the 2015 British Ecological Society annual meeting: "You're Mike SINGER?! I'm SO privileged to sit next to you! The 1980s was so much the HEYDAY of plant–insect interactions." I sat there thinking: "Heyday?! Huh? Was it? Did I do my best work three decades ago?"

Despite the temptation to respond by elaborating on my current work, I concentrate the scientific part of this review on my persistent, one might say unreasonable, fixation on oviposition preferences of Melitaeine butterflies (26, 45, 47, 50–70, 79). I disinter my prior definitions of insect preference and host acceptability (48, 49) and draw attention to the conceptual and practical difficulties in measuring these traits.

I've implied that there is an UNscientific section of this essay; indeed, I'm responding to a solicitation for an autobiography, so the section titled Part One: Personal History is an account of how a series of accidental events and encounters guided me to an interest in host shifts and to transforming myself into the "Human Melitaeine," as Chris Thomas describes me. The scientific section of the review is the part about preference. Readers interested in the science can skip from here straight to the section titled Part Two: Preference with no loss of continuity.

PART ONE: PERSONAL HISTORY

I had the good luck to be born in Yorkshire in 1944—lucky for two reasons. First, undergraduates in the 1960s were sufficiently funded in both tuition and living expenses to be independent. In consequence I was able, by answering a few deceptively simple questions in St. John's College's entrance exam (see sidebar titled Exam), to spend five years in Oxford failing to entertain the upper classes and being taught ecology by Charles Elton, behavior by Niko Tinbergen, ornithology by David Lack, and entomology by George Varley. Why did it take five years to do a three-year UK BA degree? Cold 1964–1965 winter, no heat source in my St. John's College bedroom, and freezing blast through broken bedroom window led to tuberculous pleurisy.

My second stroke of luck was that my fellow Yorkshireman Harold Wilson, then Prime Minister, declined to send troops to Vietnam, despite repeated "invitations" from the United States. While my fellow graduate students at Stanford were being issued lottery numbers for the draft, I could study without fear of being sent to fight.

How did a Yorkshire boy even get to Stanford? Butterflies! Forced to walk to school in shorts irrespective of the weather, I half-froze in the winter mornings. Around the end of March, I would

EXAM

1. Describe the kinds of enjoyment that people get from opera, rock music and jazz.
2. If the aim of man is the pursuit of happiness, why are drugs that make you happy illegal?
3. "TWO cheers for democracy." Is two the right number?
4. Can the American Way of Life be exported?
5. A man claims to have removed a plague of ants from his house by clearing his mind of anger against them and asking them to leave him alone. Give your comments.
6. Is successful forgery of an Old Master great art?

Figure 1

Edith's checkerspot butterfly.

pass the first butterfly of spring, a tortoiseshell, sitting on a rock and shivering just like me, a beautiful harbinger of coming partial relief from cold and damp. My empathy with the shivering tortoiseshells fired an interest in butterflies from the age of nine and steered me to applying to university as a biologist. Grandma Dodgson was puzzled: "Butterflies?! Very pretty, but what are they FOR?"

E.B. Ford answered Gran's anthropocentric question in his population genetics lectures, explaining how Lepidoptera could be useful in "ecological genetics" (22). I believed him, and read papers about butterflies, including one about Edith's checkerspot (*Euphydryas editha*) (**Figure 1**) by Patricia Labine from Stanford. I wrote to the Stanford Biology chair, Don Kennedy, asking about the possibility of a PhD on the larval ecology of this species. Don passed my letter to Paul Ehrlich, who sent me this message: "Dear Mr. Singer, welcome to Stanford! We will waive the GRE requirement, find a job for your wife, pay a fellowship to support you, and smooth every obstacle in your path. We look forward to your arrival."

My immediate thought was, "How much nicer Americans are than the stuffy British, but what's a GRE?" followed by: "This Stanford place must be no good at all, if you can get admitted by writing a one-page letter." I asked my tutor, who had never heard of Stanford. Neither had the next three Oxford faculty members whom I asked. Fortunately, Glynis, the librarian, directed me to Larry Gilbert, a Texan spending a one-year Fulbright fellowship in Ford's lab prior to starting a PhD at Stanford with Ehrlich himself. What a happy coincidence! Larry assured me that, despite being unheard of in Oxford in 1966, Stanford was an institution of acceptable quality. Still, the studiedly austere Linacre Professor of Zoology, J.W.S. Pringle, had no doubt that I would forget Stanford when he offered me a place to stay cosseted in Oxford. "You have an offer from AMERICANS?! Oh dear, oh dear, oh DEAR! They make you work VERY hard, don't you know?"

I didn't have to think hard to accept Paul's offer, discovering only later that he had been trying for years, without success, to persuade students and postdocs to work on the larval ecology of

Castilleja *Pedicularis* *Penstemon* *Plantago* *Collinsia*

Figure 2

Edith's checkerspot principal host genera. Photo of *Penstemon* taken by Sheri Hagwood, used with permission from the USDA-NRCS PLANTS Database.

Edith's checkerspot. The reason for his difficulty was that the subspecies with which he worked, the Bay Checkerspot (*E. editha bayensis*), has an obligate larval diapause, which normally lasts 8–10 months, cannot readily be reduced in the lab to less than four or five, and is repeated whenever larvae disapprove of their treatment by the experimenter. In consequence, the folk whom Paul pressured into working on *Euphydryas* became disillusioned and switched to other projects. I heard later that, when Paul got my letter, he charged into the coffee room sounding less nice than in his message to me: "Jesus Christ! There's a nutcase in Oxford WANTS to do that project! Boy, am I going to TIE him to that!" (Peter Raven, personal communication).

But he didn't tie me very hard. Although his main interest at the time was fending off ecological disaster [see *The Population Bomb* (21)], and his butterfly work was focused on population dynamics (19, 20), he didn't object to my wandering around California looking at *E. editha* populations in different habitats, marveling at their ability to ignore plant species that acted as their principal hosts just a few miles away, and wondering why and how this complex spatial mosaic of insect–host association had come about (**Figures 2** and **3**).

By 1970, this wondering had led me to do experiments that brought together *E. editha* from populations with different diets and offered them all the same set of hosts for oviposition. Despite the inappropriate design of the preference tests that I used (see the section titled Part Two: Preference) I found striking variation among populations in both rank order and strength of preference. Insects in some populations were highly specialized and host specific; others were much less choosy. At some sites, larvae often defoliated their oviposition hosts and migrated to secondary host species that did not receive eggs in nature. In the greenhouse, these secondary hosts were refused by butterflies that had fed on them as larvae, arguing against the then-popular hypothesis due to Hopkins (28, 77) that oviposition preferences of herbivorous insects were learned in the larval stage, with adults preferring to oviposit on plants that they remembered from youthful feeding.

In 1970, Vince Dethier, known for his interest in host shifts (14, 15), oviposition "mistakes," and butterfly population dynamics (16), visited Stanford. He and I had this conversation:

> Dethier: And what do you do, young man?
>
> Mike: I've been examining how Paul's butterfly uses different hosts at different sites, and I'm beginning to think that there's genetic variation among populations in oviposition preference.
>
> Dethier: Young man, you really should start by reading the literature. The adults learn to prefer what they ate as larvae. It's called Hopkins' host-selection principle.
>
> Mike: Oh, not in this case, I think. . . (but he was gone).

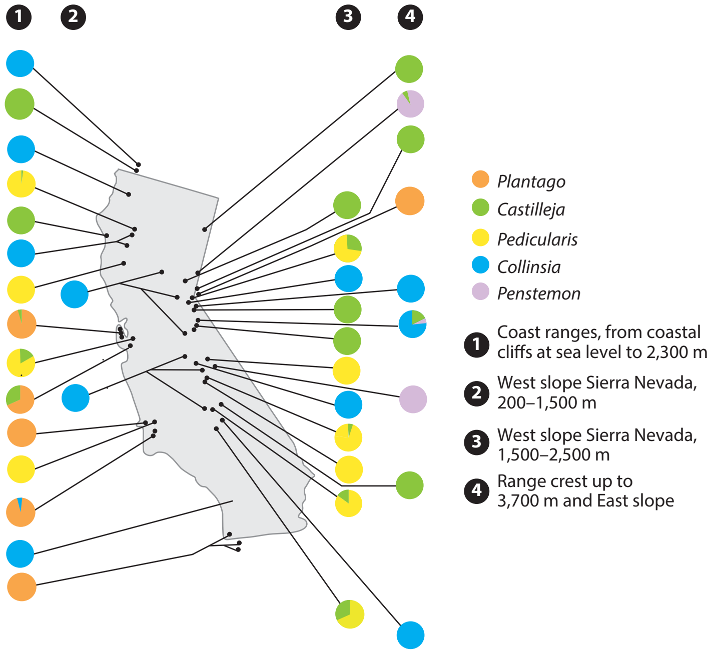

Figure 3

Proportions of eggs laid on each plant genus by Edith's checkerspot butterfly. Data gathered across decades from 1968–2018, during which diet changed over time at six of the sites; these changes are not shown (45, 55, 56, 59, 66, 67).

In retrospect, this dismissal was odd, because Dethier himself had noted exceptions from Hopkins' principle in his review of the evolution of preference (14).

Soon after my abject failure to impress an Influential Senior Scientist, I did learn more about oviposition mistakes. Paul took me and Larry to Trinidad. We stopped en route at Barro Colorado Island in the Panama Canal, where we had to climb 200 steps in high heat and humidity to reach the laboratory from the boat ramp. Halfway up the steps, a tiny white and russet satyrine, *Euptychia jesia*, bounced across the path in front of us and laid an egg on a clubmoss, *Selaginella horizontalis*. Larry said, "Ha! That one made a mistake!" and stomped on up the steps with Paul. I, on the other hand, wimp that I am, was already out of breath keeping up with my macho companions and desperate for an excuse to stop and rest, so I sat on my suitcase and watched to see whether the *Euptychia* repeated its choice. It did, over and over, leading me to start searching the *Selaginella*, where I found wonderfully camouflaged satyrine larvae feeding away and looking just like their host plant.

We had observed a host shift from angiosperms to a nonseed plant, an odd host for a butterfly. Later, on the same trip, I watched three other satyrine species in Trinidad also ovipositing on *Selaginella*. However, their larvae would not eat it. There may have been adaptive reasons for this, such as avoiding parasitoids or desiccation, but perhaps these choices of *Selaginella* by grass-feeding species were maladaptive "mistakes" like those Dethier had described. Whatever they were, they were interesting preludes to the host shift. I wrote a manuscript, with the three of us as authors, and suggested sending it to *The Canadian Entomologist*.

Paul boomed: Send it to *Science*!

Mike: I can't see much interest to non-entomologists.

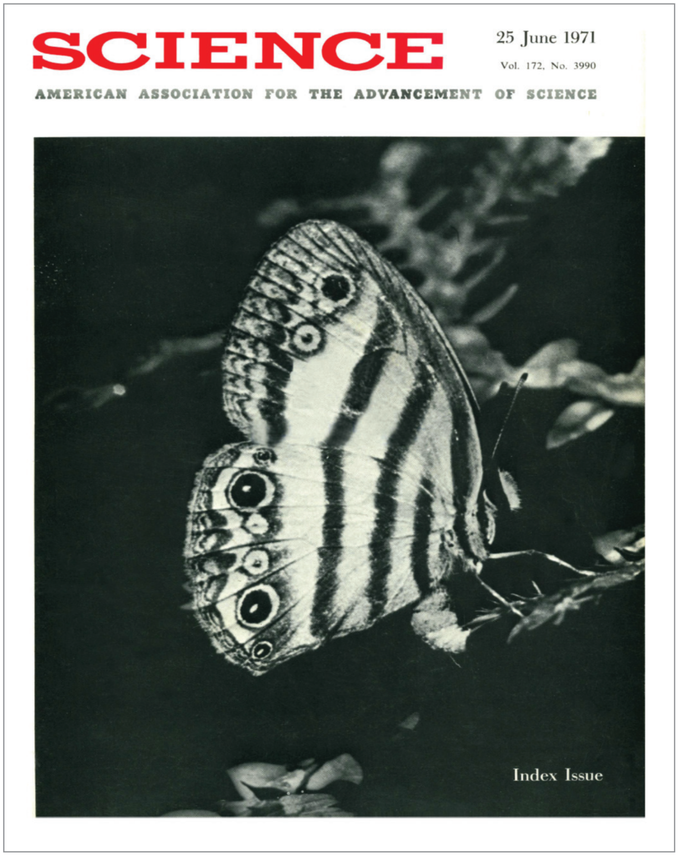

Figure 4

Cover of *Science Magazine*, June 25, 2971. Reprinted with permission from AAAS.

Paul: You should see the junk that comes out in *Science* these days.

Mike: Is that really an excuse?

Paul: Yes!

You can see from Larry's cover photo (**Figure 4**) what happened (51). Larry visited the molecular biologists and they said, "Oh, Gilbert, har, har, har! Next time we find *E. coli* in a funny place we'll put it in *Science*, har, har, har!!"

The molecular biologists had a point. There's nothing wrong with the paper, but it's pretty slight. Still, it was a happy start to my research career to have my first paper on the cover of *Science*, and it wouldn't have happened but for the fact that my supervisor had experience publishing at that level. From the moment I went to Oxford, I had an unfair advantage. Paul had evidently liked the content of my informal letter of application to Stanford, but I expect the Oxford address helped. And, had I chosen to stay in the United States after my PhD, the Oxford/Stanford "pedigree," with Paul's support, would have continued to help.

As a result of thinking about my own history, I downgrade the importance of pedigree when judging candidates for academic positions. Of course, if one person has clearly done the best, most imaginative work, then that person should be ranked first. But when I see research of similar

POSTCARD

Dear Mr Singer
In connection with your application for the lectureship please appear in room 24 on wednesday 15th May at 10:30am.. Your second-class rail fare will be refunded.
Yours faithfully
H.C. Bate
pp registrar

quality in competing candidates, I rank those with less prestigious pedigrees above those whose CVs look like mine.

My first wife, Pat, worked happily in Stanford during my PhD from 1967–1971, and then wanted to rejoin her family in the United Kingdom, so I looked for a faculty position there. I got a temporary one at Silwood Park, but then they needed a new van, so I had to go (I'm a decent mechanic, I could have fixed the van). My applications for UK lectureships failed, over and over and over. It became clear that I'd have done better if I'd stayed in Oxford. Take this example. After applying at Birmingham, I received a postcard with this message (see sidebar titled Postcard). Its signature was stamped on behalf of someone else.

This did not bode well, and so it proved. Here's the interview:

> Chair: Dr Singer, times are hard. No faculty member may spend more than £50 per year of Department money, including stationery. We do HOPE that your American experience has not spoiled you in matters financial?
>
> Mike: I hope so, too.
>
> Professor: Dr Singer, I notice from your CV that when you were in the United States you were involved in the teaching of UNDERGRADUATES! Tell me, IS THIS TRUE?
>
> Mike: Yes, I was a teaching assistant at Stanford.
>
> Professor: Don't you think it reprehensible that someone with so little qualification as you then had should be entrusted with this responsibility?
>
> Singer: No, I don't. I was teaching introductory biology to nonscience students.
>
> Professor: And WHAT does this tell us about the quality of American education?
>
> Mike: I think I got the best of both worlds by doing my undergraduate degree at Oxford and my doctorate at Stanford.

Of course, I didn't get that job, nor any that I interviewed for in the United Kingdom. I spent five years, 1972–1976, as an oddity, a lab technician with three papers in *Science*. I did my best to be a good technician, and forget that I had been trained to think about the data, but I still remember my boss's frustrated outburst: "I want the DATA from you, Mike, I'm not interested in your in-ter-pre-ta-tion!"

In spring 1976, with help from Paul and Larry (now back home in Texas), I looked for academic jobs in the United States. I was offered three in a week: in behavior at the University of Texas at Austin, in ecology at Purdue University, and in biological control of weeds at Oregon State University, Corvallis. For me, the United States really WAS the Land of Opportunity.

I started work in Texas in 1977. In 1980, Vince Dethier came to visit.

> Dethier: And what do you do, young man?

> Mike: I'm sure you don't remember this, but ten years ago I was beginning to suspect that the geographical mosaic of diet in Paul's *Euphydryas* butterflies was driven by genetic variation of oviposition preference. Well, now I can prove it!
>
> Dethier: Everybody knows that! Christer Wiklund's work shows that clearly.

And, with that, he walked off, in just the same way he'd done 10 years earlier. What had happened (2) to discredit Hopkins' principle between my two meetings with Dethier? First, Doug Futuyma (25) had noticed that most of the experiments confirming Hopkins went through more than one generation in the lab and did not exclude evolution. Second, John Jaenike (29) had found that Hopkins' effect in *Drosophila* disappeared if the pupae were washed, so although the adults did learn to prefer the larval environment, they did so when they emerged, as they encountered residues of larval food. And, yes, third, Christer Wiklund (80) had fed Swedish swallowtail larvae different hosts and found no effect on adult preferences. A similar experiment on North American swallowtails did seem to confirm Hopkins but was exposed as a fraud, forcing its author to resign his faculty position and join the CIA. Hopkins' principle isn't quite dead (12, 32), but it's no longer an obstacle to our assertions that oviposition preferences of our butterflies are not driven by larval experience (36, 40, 74).

I worked in Austin for 37 years, happily hauled into metapopulation ecology by Chris Thomas and Ilkka Hanski (10, 26, 33, 69); into hybrid fitness and speciation by Lindy McBride (35, 55, 56); and finally into climate change biology by my second and final spouse, Camille Parmesan (3, 41–43, 60). I was glad to have chosen Texas academia as a profession, since I enjoyed the challenge of teaching evolution to creationists and my health problems would have had me fired from almost any other profession. I tended to be ill for a couple of years at a time, but my CV was judged on a five-year moving time window, and on that basis, I was consistently able to look productive.

However, partly because of my original approach to preference testing (see below), I was not consistently funded. My proposals seemed to annoy reviewers quite personally. An inexpensive ($22,000) proposal to the National Science Foundation for summer field work with Ilkka Hanski in the 1,600 patch metapopulation of his butterfly, *Melitaea cinxia*, described experiments to ask whether spatial patterns of colonization of empty habitat patches containing different host genera were driven by variation of butterfly preference, host acceptability, or both. The proposal garnered miserable reviews and this panel summary: "nothing more than an excuse for a vacation in Finland... we wouldn't fund this, no matter what the program guidelines." I called the program director to say that for the panel to disclaim the program guidelines showed serious bias, and that I intended to lodge a formal complaint. He looked at what they had written and said: "Oh, no, Mike. . . er. . . please don't do that, we'll give you the money," thereby placing me in the rarefied category of researchers who have been funded because their reviews were so bad. If the panel had generated a rational critique, I'd have gotten nothing.

We went ahead with the Finnish project and it worked (26, 33, 78). Part of it required manipulating each individual *M. cinxia* to oviposit on an undisturbed host that we chose for it, growing in the same habitat patch where the butterfly had been captured. Oviposition requires sunlight, which was mostly lacking as the Åland Islands sat under the center of a large depression all summer. Still, I have happy memories of sitting in an old car in the rain with Saskya van Nouhuys (Thanks, Saskya!), watching the sky for a patch of blue and trying to see where it was heading. If it looked as though the sun might shine on patch 22 for a few minutes, we'd check our cages in the back seat to make sure that we had females from there. If we did, we'd take off at the maximum legal speed (50 kph/30 mph) to try to get to the patch in sunshine, release the butterflies onto their hosts and ask them to lay eggs.

Field work is inexpensive, so intermittent funding wasn't a problem for me—well, not until 2013, when grants became, seemingly overnight, the principal criterion used by the Department of Integrative Biology to assess our productivity. Accordingly, despite my CV from 2010–2013 showing four senior-author papers (35, 37, 41, 42) plus three first-author papers (55, 56, 59), all in good journals, I was publicly classified in a faculty meeting as "research-inactive." My office was cleared out without warning, leaving only the contents of file cabinets and a handwritten message from a student that had fluttered to the floor, thanking me for a "great class." Slides of study sites and letters from friends and luminaries (Ed Wilson, Bill Hamilton) were thrown out. I was lucky to have taken most of my field notebooks home; those that remained were destroyed.

Colleagues were wonderfully supportive; Mark Kirkpatrick offered me space in his lab, an astonishing kindness given my untidiness, and Dan Bolnick arranged an invitation for me to a prestigious symposium on maladaptation (11, 63). Nonetheless, I was miserable, and it was time to leave Austin for another reason: Old Mother Singer was increasingly in need of help in Plymouth, in response to which Camille had already established herself there in the Marine Institute. We moved our main base to Devon, and mother survived cheerfully past her 98th birthday. After she was gone, Camille intended to stay in the United Kingdom, but then came the Brexit vote, and she was an immigrant. So, she considered returning to the United States, but then Trump was elected, and Camille is a climate scientist. When the United States withdrew from the Paris climate accords, Macron brought out his MOPGA program ("Make Our Planet Great Again") to attract disaffected climate scientists to work in France. Camille was both disaffected and attracted, and as my address attests, I followed on her coat-tails and now write happily from the Pyrenees.

PART TWO: PREFERENCE

A Failed Experiment Generates an Idea and a Plan

My interest in preference began with a catastrophically failed experiment, teaching me a lesson that has stayed with me. In the United Kingdom, in the 1970s, a PhD had to be completed in three years or less; otherwise, you lost funding and were thrown out with nothing to show for your efforts. British that I was and am, I expected to easily do a three-year PhD at Stanford. Why not? However, to get this done in time, I had to complete one last experiment in 1969–1970. It was designed to ask whether within-population variation in host preference of *E. editha* larvae was heritable. Most known populations of the butterfly were monophagous, so I had been excited to find one in which larvae approaching pupation were distributed on two host genera, *Plantago* and *Castilleja*. The hosts were small and grew interdigitated, so larvae could easily sample both of them to express their preferences and choose which one to eat.

I recorded the identity of the host on which each larva was found, raised the larvae to adulthood, organized matings between individuals from the same or different hosts, and began an experiment to ask whether the presumed differences in larval host preference were heritable. I tried to raise 12,000 offspring. Too many! They caught disease and all died. This failure marked the end of my ambition to complete in three years, but in the end I didn't regret it because, in the following year, I watched the larvae more carefully in the field and observed that each one was actively selecting a diverse diet and spending about 40% of its time on *Plantago* and 60% on *Castilleja*. There were no permanent differences in preference. I had based my 12,000-larva experiment on a false premise and tested a non-question.

This failure forced me to realize that diversity of diet can occur with or without diversity of preference. However, in reading contemporary literature, I saw that some workers would assume, as I had done, that observed diet variation among individuals reflected variation of fixed preferences, while others assumed that preferences were labile or induced (Hopkins!), and still others assumed that preference variation within populations was unlikely, so individuals observed making different choices did so simply because they encountered different potential hosts.

I perceived that none of these assumptions was justified without experimental verification, and that it was important to understand the extent to which within-population variation of diet was caused by variation of preference. If individuals that made different choices did not have different preferences, then they were not following different behavioral rules, so there could be no heritable differences between those using different hosts, and no evolutionary response to natural selection on diet would be expected. However, if preference variation existed, and these preferences were expressed in nature, then they would need to be heritable if natural selection were to lead to diet evolution. As I finished my PhD in 1971, I imagined doing experiments in which I was able to test these questions and investigate the behavioral and evolutionary mechanisms underlying variation of butterfly diet.

I couldn't do these experiments in 1971–1977, since I was employed in the United Kingdom as a technician (see the section titled Part One: Personal History). My first opportunity came in 1978, when we discovered a metapopulation of *E. editha*, at Rabbit Meadow on the Generals' Highway in Sequoia National Forest (California), in which eggs were laid on four host genera. I applied oviposition preference tests to butterflies captured free-flying and to others captured in the act of ovipositing on different hosts. These experiments showed that there were three mechanistic causes of differences among individuals in the taxonomic identities of the hosts that they chose. First, preference ranks for responses to host chemistry were diverse with respect to two abundant hosts, *Pedicularis* and *Collinsia*; some adult butterflies preferred one and some the other. Because the plants were abundant, each insect could find the host that she preferred and express her preference. Second, there were insects with no preference between those same hosts, individuals equally likely to accept either plant after encounter. Finally, a third host, *Castilleja*, was so rare that most insects preferring it failed to find it and were deflected onto *Pedicularis*, on which I found them ovipositing (47). I was happy to get this result and felt proud that I was the first researcher to test what I perceived as an important question. But could I publish it? The kindly editor of *Evolution*, Doug Futuyma, told me that he would consider a submitted manuscript with these results provided that a description of my preference-testing technique were in press elsewhere. I was to be considered for tenure in 1982, so I needed to publish both the technique and results quickly if I were not to be shown the door at U. Texas.

I submitted the technique manuscript to *Ecological Entomology* in 1979. The eloquent but damning response is in the sidebar titled Editor's Letter. I eventually published the technique paper in *Oecologia* (46) and the results in *Evolution* (47). However, reviewers continued to be skeptical, even angry, for decades. Here is a memorable example: "The business of motivation involved in the so-called 'preference test' is incompetent, irrelevant, immaterial and without any foundation whatsoever in the established literature."

EDITOR'S LETTER

Dear Mike,
I sent your MS to three referees in the hopes of finding someone who might like it a little. Sadly, I failed. Clearly, you will have to think again. I would not be prepared to look at a revise [sic]. Yours, John
Review:

The interesting, if prolix, abstract led me into the introduction with great expectation. There, my interest was mired in the third sentence and never extracted. The overlapping and unclear denotations of preference, specificity and choice make the MS extremely difficult by the first page. These and ordinary problems of syntax make it impossible by the second, so that I was unable to figure out what the author is trying to say. At the risk of being wrong, it appears that the subject is interesting and the MS could be rewritten and rendered reviewable. As it stands, it is not.

PARALLEL LIVES

As a PhD student in her 20s, Liz fell in love with Reg Chapman, a professor in his 30s, and they collaborated happily for four decades. My life has been the mirror image. As a professor in my 30s, I fell in love with Camille Parmesan, a student in her 20s, and we have collaborated happily for four decades. Across the decades, Liz and Reg switched roles in their relative profiles, and we have done the same. Prior to 1996, Camille would be approached at meetings like this: "Hi Camille... er... er... er... er... Where's Mike?" Post-1996, it's been I who am approached by folk saying, "Hi Mike, how ya doin'? Still in Texas, eh?... er... er... Is Camille here?"

It wasn't just reviewers who didn't believe me; my friends didn't, either. I took Jim Mallet to an *E. editha* population and asked him to help with a preference test. Although he must have heard me describe my testing technique dozens of times, he can't have believed it because he was so obviously surprised: "WOW! This really works!" After I gave a seminar to Liz Bernays and Reg Chapman's group in Berkeley, Liz said, in her driest tones: "I wouldn't believe you could do what you SAY you can do... except that it produces such sensible results." Incidentally, I'm following Liz here in at least three ways, since we have an overlapping interest in insect diet breadth (4–9, 17, 71), she and Reg have a parallel life story to me and Camille (see sidebar titled Parallel Lives), and she wrote the 2019 autobiographical paper in this journal (5).

I didn't set out to annoy people or to generate mistrust, so how did I get into this damaging professional situation? To explain, I need to describe why I needed to develop my own preference-testing technique, what it comprised, and how I think my writing about it was misinterpreted.

Why Did I Need My Own Preference-Testing Technique?

Study of butterfly oviposition preferences became topical in the early part of my career (13, 30, 75, 76), so surely there must have been well-established techniques for testing preference that would not have been controversial? There were—the most common method of testing oviposition preference was to put the insect in a cage with alternate hosts and record the eventual distribution of eggs. This design is satisfyingly simple, though analysis of its results can be complex. Butterflies may respond to previously laid eggs (27, 52), and eggs themselves may induce responses in the hosts that alter plant acceptability and influence subsequent choices by the insects. Therefore, unless there is evidence that eggs laid at different times are independent events (e.g., 54), analyses should not assume independence. Modern analytical methods can tackle these difficulties (23, 72).

My problem with the accepted technique was not with analysis, but with the fact that the butterflies declined to behave as the experimental setup required. When caged, they didn't reproduce natural search behavior. Instead, they sat on the walls of their cages and often failed to find any of the test plants, let alone compare them. Fine discriminations were lost because, if a female did find a plant, she was likely to be so highly motivated by that time that she would accept almost any host. She could also get so excited by finding a highly acceptable host that she ran around and laid eggs on a different plant. I needed to think of something else.

Development and Validation of a Staged-Encounter Preference-Testing Technique

To test preference, it's useful, perhaps necessary, to understand details of habitat choice and oviposition behavior (24). As an example, when a *Euphydryas* forages for an oviposition site, she shows

CHRIS THOMAS

Chris Thomas began his PhD defense in 1988 like this:

"When Mike Singer was twelve, he discovered how much NICER it is than sticking pins in butterflies to HELP them to find places to lay their eggs."

We had begun our collaboration in 1984 after he approached me with this comment on my 1983 Rabbit Meadow paper:

"Dr Singer, I read your paper in *Evolution* and I thought it would be very interesting IF TRUE, but I did my MSc on butterflies and I have difficulty believing that you could do things like that with them. However, if you CAN do that, I would be rather interested in pursuing it for my PhD. So, what I propose to you is this: I'll join your field trip to California this summer, provided that I can spend the first field season double-checking your published work."

Which he did. Our collaboration continued for more than a decade; our friendship continues still.

a fixed sequence of behaviors, at each of which a preference can be expressed. She begins by alighting in response to visual stimuli; that's her first preference (39, 40). Next, she tastes the plant on which she has alighted. If the chemical stimuli (55) are accepted (second preference), then she expresses her third preference, for egg height (see below), and she curls her ovipositor, presses it upwards, and responds to acceptable tactile stimuli (fourth preference) by laying eggs (unlike in some butterflies, the ovipositor lacks chemical sensors and responds only to tactile stimuli).

In 1956 (not a typo, see sidebar titled Chris Thomas), I discovered that Melitaeine butterflies are surprisingly manipulable. If I simulated alighting by placing an oviposition-motivated female on a potential host, then I could watch her tasting it with her atrophied foretarsi. If the taste were accepted, then she would produce abdominal curl and ovipositor extrusion, then lay eggs, just as if she had not been handled. In my first attempt to utilize this manipulability of the butterflies, in 1971 (45), I reported the use of 4 different host genera (**Figure 2**) by 17 *E. editha* populations in a mosaic distribution across California (**Figure 3**). To ask whether this pattern was caused, at least in part, by interpopulation variation of preference, I performed preference tests by keeping butterflies for 24 hours with no opportunity to lay eggs and then placing them gently on each host until one was chosen and oviposition occurred. Crude though they were, these tests generated the information that I failed to communicate to Vince Dethier in 1970 (see section titled Part One: Personal History). Butterflies from *Collinsia*-feeding populations accepted only their own hosts and were more host specific than those from populations using *Plantago* or *Pedicularis* (45).

A second and more thought-provoking result from this same experiment was that butterflies from the only site where *Penstemon heterodoxus* occurred, Gardisky Lake (Mono County, California), were the only ones to accept that plant but did not seem to use it in nature, laying eggs only on their preferred host, *Castilleja nana*. However, Gardisky larvae could eat *Penstemon* and occasionally migrated to it. This result led me to suggest that there was an adaptive "preference hierarchy" at Gardisky, enabling the butterflies to add the possibility of oviposition on *Penstemon* to their repertoire, without changing their preference, if *Castilleja* became rare (45; for hierarchical preferences in swallowtails, see 81). The idea could be formalized like this: Each oviposition search by *Euphydryas* begins with a motivation level at which only the most preferred hosts would be accepted if encountered. During the search, oviposition motivation increases, and the range of plants that would be accepted (if encountered) expands until, eventually, acceptance and oviposition occur.

After migrating to the United Kingdom in 1971, I recognized that testing insects after holding them for 24 hours, as I had done (45), merely divided hosts into two categories: those accepted and those rejected by highly motivated insects. What I should have done was to begin testing insects before they were ready to oviposit, staging repeated encounters with each test plant in alternation, recording full abdominal curling and ovipositor probing as acceptance of plant chemistry, and removing the insects from test plants before oviposition began. It was clear that a female subjected to repeated staged encounters like this, without being allowed to oviposit, would accept a wider range of hosts as time passes. Thus, if she preferred plant Z over plant X, then it should be possible for me to estimate the length of time during which Z was consistently accepted and X consistently rejected before she reached the motivation at which either would be accepted. I decided to call this length of time a "discrimination phase." Given the behavior of the butterflies, this seemed a potentially useful, practical measure of their post-alighting preferences for host chemistry.

I was unable to follow up on this idea until I arrived back in the United States and restarted field work in California in 1978. Then, I was able to test the hypothesis that discrimination phases existed in *E. editha*. Indeed they did! Not only that, but the length and direction of these phases, i.e., the strength of preference and the identity of the preferred hosts, were (*a*) diverse both within populations and among them (46, 47, 59, 65); (*b*) repeatable when individuals were tested more than once (53); and (*c*) heritable, judging both by mother–daughter regression (57) and by crosses between populations (35). Estimating preferences in this way was facilitated by the fact that, although the butterflies learned which flowers to visit for nectar and how to find nectar in different flowers (36), behaviors involved in oviposition were unaffected by learning (36, 40, 74). Staged encounters with hosts administered in the course of preference tests had no detectable effects on subsequent responses to the same or different hosts (74).

Experimental Validation of Preference Test for Discrimination Among Individual Hosts

With my graduate student, Duncan Mackay, Mark Rausher (see sidebar titled The Importance of Money) and I followed natural oviposition searches by *E. editha* in Rabbit Meadow, Tulare County, California, where the principal host was *Pedicularis*. Mark noted that each butterfly usually alighted on many *Pedicularis* individuals before choosing one. Bearing in mind my assertion that the insects became less choosy as they searched, he devised an experiment to test whether a butterfly searched until she would accept the next *Pedicularis* that she encountered, or whether she was discriminating among conspecific hosts. We numbered each plant in an area where natural search was occurring. Then, when a butterfly was just beginning to settle down to lay eggs at the end of a natural search,

THE IMPORTANCE OF MONEY

My 1971 paper on preference of *E. editha* (45) was read in 1977 by a graduate student finishing up his PhD at Cornell University, Mark Rausher. He noticed that I had published nothing further on this system and wrote to me, interested in following it up if I had abandoned it. Since I was just restarting my academic career after five years as a techie, I explained that I was picking up *E. editha* where I had left it in 1971. Mark swallowed his disappointment and asked if he could work on the system as my postdoc if he wrote the grant proposal. "Oh, sure," I said absently, totally blind to the importance of grants. He wrote it, I was the principal investigator, and we got the money. Thanks, Mark! This proved essential to my tenure, since the proposals that I wrote in 1978–1982 all succumbed to skepticism of my preference-testing technique.

we captured her before the first egg was laid, gave her a five-minute rest, then flipped a coin to decide whether to replace her on the plant she had accepted or on the penultimate *Pedicularis*, the last one that she had rejected after alighting.

The manipulated butterflies strongly tended to duplicate the responses to individual plants that they had shown prior to capture. This result both validated the manipulated-encounter technique and showed that butterflies were truly discriminating among individual *Pedicularis* plants after alighting (44).

Video Generates Credibility!

Nowadays, the credibility of my staged-encounter preference tests has recovered from its decades of suspicion, mainly thanks to videos such as the one published by *PLOS Biology* (**https://doi.org/10.1371/journal.pbio.1000529.s015**).

The video shows a female *E. editha* at Rabbit Meadow being placed by hand on a *Pedicularis* growing naturally in the field. She is free to fly away, but instead she basks until the experimenter, Lindy McBride, gives her a nudge to remind her that she has a task, which is to consider the plant for oviposition. She obliges, tasting the plant and responding to acceptance of the taste by expressing her positive geotaxis, dropping to the ground, and searching for the base of the plant, which she fails to find. She takes off and starts again, alighting at the spot where she had been initially placed, then tasting and dropping again. Eventually she does find the base and lays eggs.

Supplemental Material >

The geotaxis seen in the video influences the height of eggs above the ground, a trait that forms part of a complex suite of host-related adaptations that varies among *E. editha* populations (3, 35, 55). Positive geotaxis at Rabbit Meadow protects eggs from grazing by vertebrates (3, 63) but increases their exposure to thermal stress (3). At most sites where *Collinsia* is the host, geotaxis is omitted from the oviposition sequence, as shown in the **Supplemental Video**.

You can see from the videos that *E. editha* individuals behave naturally after staged host encounters. I suspect that the strong negative emotions aroused in reviewers have stemmed from suspicion that I was advising them to duplicate my methods. Absolutely not! I make no assumption that my practical techniques for preference-testing in Melitaeine butterflies should be useful to others; indeed, I have advocated that testing be designed around the specific behavioral sequences that precede oviposition of each study species (48).

Concepts of Insect Preference and Host Acceptability

Practical methods of preference-testing may be specific to study organisms, but the concept of preference should apply widely. This does not seem to be the case. For example, students of behavior define preference as a set of responses to stimuli, while ecologists view it as the proportion of particular items in the diet relative to their abundance in the habitat. To see that this makes an important difference, consider this thought example (49). Two host plants have identical overall densities, but plant C occurs in a clumped distribution, while plant W is more widely dispersed. A butterfly that has equal likelihoods of accepting both plants after encounter begins each oviposition search at a random point in space. She will encounter W more often than C, place more eggs on it, and prefer it by the ecological definition (34, 49). In contrast, by the behavioral definition, she has no preference. Therefore, preference by the ecological definition is at least partly a trait of the plants, depending on their dispersion, while preference by the behavioral definition is a potentially heritable property of the insect (49). I consider it useful to render preference a potentially heritable trait of the insects, rather than a more complex trait of the plant–insect interaction.

For this reason, I have advocated a behavioral definition of preference: "the set of likelihoods of accepting particular resources that are encountered" (48, 49).

The trait of a plant that interacts with insect preference—the mirror image of preference—is the set of likelihoods of the plant being accepted by particular insects that encounter it. For this trait, I have used the term "acceptability" (48, 49). This conceptual approach separates traits of plants ("acceptabilities") and traits of insects ("preferences") from traits of the plant–insect interaction, which could be "insect diet" if viewed entomocentrically or "guild structure" of insects on a plant if viewed phytocentrically (48). It allowed us to show that the mechanism causing two *E. editha* populations to select different host genera from apparently identical plant communities was twofold. The sites differed both in butterfly oviposition preference and in host acceptability, both traits being heritable and neither alone being sufficient to account for the geographic mosaic of insect–plant association (59; for a definition of this form of ecological geographic mosaic, see 56).

Preference Tests Reveal a Cryptic Preference–Performance Association

The manipulability of *Euphydryas*, as seen in videos, has facilitated studies of correlations between preference and other traits—for example, fecundity (1) and dispersal (2, 74), as well as offspring performance (38, 55, 57). It has also revealed underlying behavioral complexity in host choice, especially in relationships between preferences expressed within and between host species.

Our first finding of complexity came from an experiment designed to ask whether, in their discriminations among individual *Pedicularis* plants at Rabbit Meadow (see above), female *E. editha* were choosing plants on which survival of offspring would be high. However, only about half of the adults had discriminated among individual plants; the others showed no detectable preference. The preferences that existed were all in the same direction; if one butterfly preferred *Pedicularis* plant 52 over plant 23, then none would prefer plant 23 over plant 52. So, we could place the plants into two classes of acceptability: those accepted and those rejected by discriminating butterflies. David Ng asked whether mean larval survival differed between the two classes of *Pedicularis*. It did not. However, when we looked at offspring survival as a function of maternal preference, we found a significant difference between offspring of discriminating and nondiscriminating mothers in relative survival on the two plant classes. Offspring of discriminators survived better on plants accepted than on those rejected by discriminating parents, while offspring of nondiscriminators survived equally well on the two classes of plant. We had used information provided by the discriminating butterflies to divide the plant population into two classes of acceptability. Having done that, we could then determine that the butterfly population contained specialist and generalist phenotypes, with respect to those two acceptability classes. Further, the specialist–generalist axis of variation was expressed in correlated preferences and performances (38, 58).

Independent Evolution of Discrimination Within and Among Hosts

Building on the finding of variation in discrimination among *Pedicularis* plants, we asked whether this axis of variation was related to preferences among host species. We worked in a large (>2 ha) clearing adjacent to the *Pedicularis*-feeding site where we had studied within-host discrimination. Within the clearing, loggers had removed all of the trees, killing *Pedicularis*, which parasitizes gymnosperms. The loggers burned the wood that they did not remove, fertilizing the soil, extending the lifespan of *Collinsia*, and thereby allowing it to support a *Euphydryas* population (63). Butterflies emerging in the clearing that had all developed on *Collinsia* showed strong variation of preference:

A few preferred *Collinsia* over *Pedicularis*, some had no preference, and some completely rejected the host that their mother had chosen and on which they themselves had developed (47, 65). We found a significant association between preferences expressed within and among host species. Acceptance of *Collinsia* was negatively associated with discrimination among *Pedicularis* plants. This strongly suggested that colonization of a novel host had caused loss of adaptive discrimination among individuals of the traditional host. Thanks principally to work by Bernays (4) and Janz & Nylin (31), this result would not now be newsworthy, but in 1988 it would have been. We had our title ready: "A novel source of natural selection for evolution of host specialization."

Alas, instead of publishing this nice result, we tested it by comparing butterflies in the rapidly evolving Rabbit Meadow metapopulation with those from Sequoia National Park 12 km away, where no logging had occurred and *Collinsia* had not been colonized. Although acceptance of *Collinsia* had increased dramatically at the evolving site, discrimination within *Pedicularis* had not diminished. In fact, the correlation between preferences expressed within and among hosts had itself evolved significantly. So, our new title (58) was less catchy: "Rapidly evolving associations among preferences fail to constrain evolution of insect diet breadth." Is this widely cited? Guess! But it did illuminate the evolutionary dimensionality of preference.

Preferences Within and Among Host Species: Bad News for Experimental Design

Ilkka Hanski's wife, Eeva Furman, told me that she gave him a book of Finnish butterflies for his 40th birthday. He sat down with it after dinner, turned the pages slowly, stopped at *Melitaea cinxia* (pronunced "keenksia" in Finnish), and said: "Keenksia. . .hmmm. . .keenksia. Yes. . .keenksia. I'll work on that one" and snapped the book shut. Six years later, I met Ilkka for the first time at a workshop he organized. His talk began thus: "We have sequenced an entire landscape comprising 1.6 kilopatches," which seemed to me like extraordinary hubris until he explained that he and his entourage had counted the larval groups of *M. cinxia* on two host genera, *Veronica* and *Plantago*, in 1,600 habitat patches each year for five years. Not hubris, an amazing data set just waiting to be complemented with preference tests!

Ilkka and I had a long and happy collaboration; he is much missed. In Texas, I was able to keep separate cultures of *M. cinxia* originating from *Plantago*-feeding and *Veronica*-feeding sites in Finland, along with laboratory populations of the Finnish hosts. One of the results from these cultures has helped to further illustrate the dimensionality and complexity of oviposition preference. Butterflies from the two laboratory cultures were asked to rank the same six individual plants, three *Veronicas* and three *Plantagos.* About half of the insects from the *Veronica* culture ranked all three *Veronicas* over all three *Plantagos*. None showed the reverse order. About half of the butterflies from the *Plantago* culture ranked all three *Plantagos* over all three *Veronicas*. None showed the reverse order. The other butterflies in each culture told us that THIS *Plantago* is better than THAT *Veronica*, but THAT *Veronica* is better than THIS *Plantago*. So, in each culture, some butterflies had responded more to variation within host species than between them, while others had done the reverse. In our paper (53), we explain how this experiment shows that, when experiments are designed to ask if insects vary in the identities of the species they prefer, variation of within-species discrimination can masquerade as variation of between-species discrimination.

My group then added work on *Euphydryas aurinia*, which surprised us with yet another aspect of preference variation. Many European populations of this species are monophagous on *Succisa*. However, in southern France and Catalonia, there are populations that are not exposed to *Succisa* and that use *Gentiana*, *Lonicera*, or *Cephalaria*. A French researcher, Robert Mazel, had maintained that all *E. aurinia* preferred *Succisa*, whether or not it was their own host. This suggested a more constrained evolutionary dimensionality of preference than in *E. editha*, which can prefer any one of at least five different host genera. When we sampled hosts at random from sites where *E. aurinia* used *Lonicera* or *Cephalaria*, we found that Mazel was correct; the insects preferred *Succisa*, which did not grow in their habitats, over their own hosts. They appeared to waste time searching for a nonexistent plant before they could oviposit. However, when we tested *Succisa* against individual *Lonicera* or *Cephalaria* that had naturally received eggs, it was no longer preferred. When the hosts were sampled at random from their populations, as researchers frequently do, variation of acceptability within those host populations was so great as to generate a misleading preference for a nonexistent plant, *Succisa* (64).

What to Do?

Stepping back and considering all of these studies on different species of Melitaeine, some lessons can be learned. One lesson stemming from the existence of unexpected axes of variation is that choosing individual plants or insects to represent their populations or species is risky because the chosen ones may not be at all representative. But if you don't make a choice, for example, resampling a host population at random for each data point, then you can run into a different kind of difficulty because the insects are not using their hosts at random. Some limitations of design are illustrated by our experiment (59, section titled Concepts) that looked for the mechanistic cause of diet difference between two *E. editha* populations in eastern California, one at Frenchman Lake and the other at Sonora Junction. We began by sampling one *Collinsia* and one *Penstemon* plant from each site. We asked one Frenchman butterfly and one Sonora butterfly to rank *Penstemon* versus *Collinsia* twice, once for each plant pair. We then resampled the butterfly and plant populations and asked the same question again and again, so each data point represented independent samples of both butterflies and plants from the respective populations. This experiment told us that Frenchman butterflies always preferred *Penstemon*, no matter the origin of the plant pair. With one exception, Sonora butterflies preferred *Collinsia* when offered plant pairs from their own site, but were more likely to prefer *Penstemon* if the plant pair came from Frenchman, where *Penstemon* was the host. It took a separate set of experiments, with plant pairs each comprising two *Penstemons*, one from each site, to show that *Penstemons* from Frenchman were more acceptable than those from Sonora to butterflies from either site. This gave us a mechanism to explain the diet difference between the sites as a combination of variable insect preference and variable plant acceptability, but it still left unanswered questions, such as whether the one Sonora butterfly that preferred *Penstemon* was an outlier as a butterfly in its population, or whether it encountered an unusually acceptable *Penstemon* or an unusually unacceptable *Collinsia*.

CONCLUSION

I hope that readers will find my examples of complexity helpful to experimental designs rather than daunting. To finish on a more optimistic note, the "unreasonable fixation on oviposition preferences of Melitaeine butterflies" of which I accused myself in the Introduction has led to documentation of six independent host shifts observed in real time. Remarkably, they are all different; a host shift is a living entity, and if you watch carefully, it will shimmer and dance under your gaze. I'm hoping to publish all six eventually. Only two have been published so far (61, 63, 65, 68, 73), but one was highlighted by the journal on the cover (**Figure 5**), in an editorial and in an extraordinary puppet show that won an award for scientific journalism. The link follows, with my congratulations and thanks to the puppeteers: **https://www.nature.com/articles/d41586-018-05132-x**.

Figure 5

Edith's checkerspot on the cover of *Nature*, May 10, 2018, referencing a paper about diet evolution of the butterfly at Schneider's Meadow (61). Reprinted with permission.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

I wouldn't have been able to maintain my "unreasonable fixation" on oviposition preferences of not-very-intelligent butterflies without my long list of collaborators, especially Davy Boughton, Marie Butcher, Alexa Carleton, Ellen Crocker, Paul Ehrlich, Larry Gilbert, Ilkka Hanski, Ahmar Hashmi, Duncan Mackay, Jim Mallet, Lindy McBride, Camille McNeely, Sasha Mikheyev, Rick Moore, Sandy Moore, David Ng, Camille Parmesan, Mark Rausher, Paul Severns, Chris Thomas, Saskya Van Nouhuys, Dan Vasco, Brian Wee, and Ray White. Thanks also to a long list of lepidopterists who led me to their sites, especially Sterling Mattoon. Folk who have encouraged me in the face of professional criticism are Carol Boggs, Dan Bolnick, Francie Chew, Matt Forister, Tad Kawecki, Bill Hamilton, Susan Harrison, Niklas Janz, Mark Kirkpatrick, Lesley Lancaster, Dennis Murphy, Isabelle Olivieri, Ophélie Ronce, Miriam Rothschild, Dick Southwood, Sharon Strauss, Chris Wheat, and Christer Wiklund. Intermittent funding from the National Science Foundation, most recently in 2002–2004, has been a great help.

LITERATURE CITED

1. Agnew K, Singer MC. 2000. Does fecundity drive the evolution of insect diet? *Oikos* 88:533–38
2. Barron AB. 2001. The life and death of Hopkins' host-selection principle. *J. Insect Behav.* 14:725–37
3. Bennett NL, Severns PM, Parmesan C, Singer MC. 2015. Geographic mosaics of host preference, phenology, adult size and microhabitat choice predict butterfly resilience to climate warming. *Oikos* 124:41–53
4. Bernays EA. 1998. The value of being a resource specialist: behavioral support for a neural hypothesis. *Am. Nat.* 151:451–64
5. Bernays EA. 2019. An unlikely beginning: a fortunate life. *Annu. Rev. Entomol.* 64:1–13
6. Bernays EA, Bright KL, Howard JJ, Raubenheimer D, Champagne D. 1992. Variety is the spice of life: frequent switching between foods in the polyphagous grasshopper, *Taeniopoda eques*. *Anim. Behav.* 44:721–31
7. Bernays EA, Funk D. 1999. Specialists make faster decisions than generalists: experiments with aphids. *Proc. R. Soc. B* 266:151–56
8. Bernays EA, Graham M. 1988. On the evolution of host specificity in phytophagous arthropods. *Ecology* 69:886–92
9. Bernays EA, Wcislo W. 1994. Sensory capabilities, information processing and resource specialization. *Q. Rev. Biol.* 69:187–204
10. Boughton DA. 1999. Empirical evidence for complex source-sink dynamics with alternative states in a butterfly metapopulation. *Ecology* 80:2727–39
11. Brady SP, Bolnick DI, Angert AL, Gonzalez A, Barrett RDH, et al. 2019. Causes of maladaptation. *Evol. Appl.* 12(S1):1229–42
12. Cahenzli F, Wenk BA, Erhardt A. 2015. Female butterflies adapt and allocate their progeny to the host-plant quality of their own larval experience. *Ecology* 96:1966–73
13. Chew FS, Robbins RK. 1984. Egg laying in butterflies. In *The Biology of Butterflies*, ed. RI Vane-Wright, P Ackery, pp. 65–79. Cambridge, MA: Academic
14. Dethier VG. 1954. Evolution of feeding preferences in phytophagous insects. *Evolution* 8:33–54
15. Dethier VG. 1959. Egg-laying habits of Lepidoptera in relation to available food. *Can. Entomol.* 91:554–61
16. Dethier VG, MacArthur RH. 1964. A field's capacity to support a butterfly population. *Nature* 201:728–29
17. Dukas R, Bernays EA. 2000. Learning improves growth in the grasshopper, *Schistocerca americana*. *PNAS* 97:2637–40
18. Dyer LA, Forister ML, eds. 2015. *The Lives of Lepidopterists*. Berlin: Springer
19. Ehrlich PR. 1961. Intrinsic barriers to dispersal in checkerspot butterfly. *Science* 134:108–9
20. Ehrlich PR. 1965. The population biology of the butterfly *Euphydryas editha*. II. The structure of the Jasper Ridge colony. *Evolution* 19:327–36
21. Ehrlich PR. 1968. *The Population Bomb*. San Francisco/New York: Sierra Club Books/Ballantine Books
22. Ford HD, Ford EB. 1930. Fluctuation in numbers and its influence on variation in *Melitaea aurinia* Rott. (Lepidoptera). *Trans. Entomol. Soc. Lond.* 78:345–51
23. Fordyce JA, Gompert Z, Forister ML, Nice C. 2011. A hierarchical Bayesian approach to ecological count data: a flexible tool for ecologists. *PLOS ONE* 6(11):e26785
24. Friberg M, Olofsson M, Berger D, Karlsson B, Wiklund C. 2008. Habitat choice precedes host plant choice—niche separation in a pair of a generalist and a specialist butterfly. *Oikos* 117:1337–44
25. Futuyma DJ. 1983. Evolutionary interactions among herbivorous insects and plants. In *Coevolution*, ed. DJ Futuyma, M Slatkin, pp. 207–31. Sunderland, MA: Sinauer
26. Hanski I, Singer MC. 2001. Extinction-colonization dynamics and host plant choice in butterfly metapopulations. *Am. Nat.* 158:341–53

27. Hilker M, Fatouros NE. 2015. Plant responses to insect egg deposition. *Annu. Rev. Entomol.* 60:493–515
28. Hopkins AD. 1917. A discussion of C.G. Hewitt's paper on insect behaviour. *J. Econ. Entomol.* 10:92–93
29. Jaenike J. 1983. Induction of host preference in *Drosophila melanogaster*. *Oecologia* 58:320–25
30. Jaenike J. 1990. Host specialization in phytophagous insects. *Annu. Rev. Ecol. Syst.* 21:243–73
31. Janz N, Nylin S. 1997. The role of female search behaviour in determining host plant range in plant feeding insects: a test of the information processing hypothesis. *Proc. R. Soc. Biol. Sci.* 264:7017
32. Janz N, Soderlind L, Nylin S. 2009. No effect of larval experience on adult host preferences in *Polygonia c-album* (Lepidoptera: Nymphalidae): on the persistence of Hopkins' host selection principle. *Ecol. Entomol.* 34:50–57
33. Kuussaari M, Singer MC, Hanski I. 2000. Local specialization and landscape-level influence on host use in a herbivorous insect. *Ecology* 81:2177–87
34. Mackay DA, Singer MC. 1982. The basis of an apparent preference for isolated plants by ovipositing *Euptychia libye* butterflies. *Ecol. Entomol.* 7:299–303
35. McBride CS, Singer MC. 2010. Field studies reveal strong postmating isolation between ecologically divergent butterfly populations. *PLOS Biol.* 8(10):e1000529
36. McNeely C, Singer MC. 2001. Contrasting the roles of learning in butterflies foraging for nectar and oviposition sites. *Anim. Behav.* 61:847–52
37. Mikheyev AS, McBride CS, Mueller UG, Parmesan C, Smee MR, et al. 2013. Host-associated genomic differentiation in congeneric butterflies: Now you see it, now you don't. *Mol. Ecol.* 22:4753–66
38. Ng D. 1988. A novel level of interaction in plant-insect systems. *Nature* 334:611–12
39. Parmesan C. 1991. Evidence against plant "apparency" as a constraint on evolution of insect search efficiency (Lepidoptera: Nymphalidae). *J. Insect Behav.* 4:417–30
40. Parmesan C, Singer MC, Harris I. 1995. Absence of adaptive learning from the oviposition behavior of a checkerspot butterfly. *Anim. Behav.* 50:161–75
41. Parmesan C, Duarte C, Poloczanska E, Richardson AJ, Singer MC. 2011. Overstretching attribution. *Nat. Clim. Chang.* 1:2–4
42. Parmesan C, Burrows MT, Duarte C, Poloczanska E, Richardson AJ, Singer MC. 2013. Beyond climate change attribution in conservation and ecological research. *Ecol. Lett.* 16:58–71
43. Parmesan C, Williams-Anderson A, Moskwik M, Mikheyev AS, Singer MC. 2015. Endangered Quino checkerspot butterfly and climate change: short-term success but long-term vulnerability? *J. Insect Conserv.* 9:185–204
44. Rausher MD, Mackay DA, Singer MC. 1981. Pre- and post-alighting host discrimination by *Euphydryas editha* butterflies: the behavioral mechanisms causing clumped distributions of egg clusters. *Anim. Behav.* 29:1220–28
45. Singer MC. 1971. Evolution of food-plant preferences in the butterfly *Euphydryas editha*. *Evolution* 25:383–89
46. Singer MC. 1982. Quantification of host preference by manipulation of oviposition behavior in the butterfly *Euphydryas editha*. *Oecologia* 52:224–29
47. Singer MC. 1983. Determinants of multiple host use by a phytophagous insect population. *Evolution* 37:389–403
48. Singer MC. 1986. The definition and measurement of oviposition preference. In *Plant-Insect Interactions*, ed. J Miller, TA Miller, pp. 65–94. Berlin: Springer
49. Singer MC. 2000. Reducing ambiguity in describing plant-insect interaction: "preference," "acceptability" and "electivity." *Ecol. Lett.* 3:159–62
50. Singer MC. 2015. Adaptive and maladaptive consequences of "matching habitat choice": lessons from a rapidly-evolving insect metapopulation. *Evol. Ecol.* 29:905–25
51. Singer MC, Ehrlich PR, Gilbert LE. 1971. Butterfly feeding on Lycopsid. *Science* 172:1341–42
52. Singer MC, Kuussaari M, van Nouhuys S. 2017. Attraction of *Melitaea cinxia* butterflies to previously-attacked hosts: a likely complement to known Allee effects? *Ann. Zool. Fenn.* 54:205–11
53. Singer MC, Lee JR. 2000. Discrimination within and between host species by a butterfly: implications for design of preference experiments. *Ecol. Lett.* 3:101–5

54. Singer MC, Mandracchia J. 1982. On the failure of two butterfly species to respond to the presence of conspecific eggs prior to oviposition. *Ecol. Entomol.* 7:327–30
55. Singer MC, McBride CS. 2010. Multitrait, host-associated divergence among sets of butterfly populations: implications for reproductive isolation and ecological speciation. *Evolution* 64:921–33
56. Singer MC, McBride CS. 2012. Geographic mosaics of species' association: a definition and an example driven by plant-insect phenological synchrony. *Ecology* 93:2658–73
57. Singer MC, Ng D, Thomas CD. 1988. Heritability of oviposition preference and its relationship to offspring performance within a single insect population. *Evolution* 42:977–85
58. Singer MC, Ng D, Vasco DA, Thomas CD. 1992. Rapidly evolving associations among oviposition preferences fail to constrain evolution of insect diet breadth. *Am. Nat.* 139:9–20
59. Singer MC, Parmesan C. 1993. Sources of variation in patterns of plant-insect association. *Nature* 361:251–53
60. Singer MC, Parmesan C. 2010. Phenological asynchrony between herbivorous insects and their hosts: signal of climate change or pre-existing adaptive strategy? *Phil. Trans. R. Soc. Lond. B* 365:3161–76
61. Singer MC, Parmesan C. 2018. Lethal trap created by adaptive evolutionary response to an exotic resource. *Nature* 557:238–41
62. Singer MC, Parmesan C. 2018. A poison'd chalice tale: butterfly extinction in eco-evolutionary trap. *Nature Research Ecology & Evolution Community*, May 10. **https://natureecoevocommunity.nature.com/channels/521-behind-the-paper/posts/32973-a-poison-d-chalice-tale-butterfly-extinction-in-eco-evolutionary-trap**
63. Singer MC, Parmesan C. 2019. Butterflies embrace maladaptation and raise fitness in colonizing novel host. *Evol. Appl.* 12:1417–33
64. Singer MC, Stefanescu C, Pen I. 2002. When random sampling doesn't work: Standard design falsely indicates maladaptive host preferences in a butterfly. *Ecol. Lett.* 5:1–6
65. Singer MC, Thomas CD. 1996. Evolutionary responses of a butterfly metapopulation to human and climate-caused environmental variation. *Am. Nat.* 148:S9–39
66. Singer MC, Thomas CD, Billington HL, Parmesan C. 1989. Variation among conspecific insect populations in the mechanistic basis of diet breadth. *Anim. Behav.* 37:751–59
67. Singer MC, Thomas CD, Billington HL, Parmesan C. 1994. Correlates of speed of evolution of host preference in a set of twelve populations of the butterfly *Euphydryas editha*. *Ecoscience* 1:107–14
68. Singer MC, Thomas CD, Parmesan C. 1993. Rapid human-induced evolution of insect diet. *Nature* 366:681–83
69. Singer MC, Vasco D, Parmesan C, Thomas CD, Ng D. 1992. Distinguishing between preference and motivation in food choice—an example from insect oviposition. *Anim. Behav.* 44:463–71
70. Singer MC, Wee B, Hawkins S, Butcher M. 2008. Rapid natural and anthropogenic diet evolution: three examples from checkerspot butterflies. In *The Evolutionary Ecology of Herbivorous Insects: Speciation, Specialization and Radiation*, ed. KJ Tilmon, pp. 311–24. Berkeley, CA: Univ. Calif. Press
71. Singer MS, Mace KC, Bernays EA. 2009. Self-medication as adaptive plasticity: increased ingestion of plant toxins by parasitized caterpillars. *PLOS ONE* 4:389–97
72. Steward RA, Boggs CL. 2020. Experience may outweigh cue similarity in maintaining a persistent host plant-based evolutionary trap. *Ecol. Monogr.* 90:e01412
73. Thomas CD, Ng D, Singer MC, Mallet JLB, Parmesan C, Billington HL. 1987. Incorporation of a European weed into the diet of a North American herbivore. *Evolution* 41:892–901
74. Thomas CD, Singer MC. 1987. Variation in host preference affects movement patterns in a butterfly population. *Ecology* 68:1262–67
75. Thompson JN. 1988. Evolutionary genetics of oviposition preference in swallowtail butterflies. *Evolution* 42:1223–34
76. Thompson JN. 1988. Variation in preference and specificity in monophagous and oligophagous swallowtail butterflies. *Evolution* 42:118–28
77. Thorpe WH. 1930. Biological races in insects and allied groups. *Biol. Rev.* 5:177–212
78. Van Nouhuys S, Singer MC, Nieminen M. 2003. Spatial and temporal patterns of caterpillar performance and the suitability of two host plant species. *Ecol. Entomol.* 28:193–202

79. Wee B, Singer MC. 2007. Variation among individual butterflies along a generalist-specialist axis: no support for the “neural constraint” hypothesis. *Ecol. Entomol.* 32:257–61

80. Wiklund C. 1975. The evolutionary relationship between adult oviposition preferences and larval host plant range in *Papilio machaon*. *Oecologia* 18:185–97

81. Wiklund C. 1981. Generalist versus specialist oviposition behaviour in *Papilio machaon* (Lepidoptera) and functional aspects on the hierarchy of oviposition preferences. *Oikos* 36:163–70

Annual Review of Entomology

Volume 66, 2021

Contents