

1 **Colonizations cause diversification of host preferences: a mechanism explaining increased**
2 **generalization at range boundaries expanding under climate change**

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13 **Abstract**

14 As species' poleward range limits expand under climate change, generalists are expected to be
15 better colonists than specialists, extending their ranges faster. This effect of specialization on
16 range shifts has been shown, but so has the reverse cause-effect: in a global meta-analysis of
17 butterfly diets it was range expansions themselves that caused increases of population-level diet
18 breadth. What could drive this unexpected process? We provide a novel behavioral mechanism
19 by showing that, in a butterfly with extensive ecotypic variation, Edith's checkerspot, diet
20 breadths increased after colonization events as diversification of individual host preferences
21 pulled novel hosts into population diets. Subsequently, populations that persisted reverted
22 towards monophagy.

23 We draw together three lines of evidence from long-term studies of 15 independently-
24 evolving populations. First, direct observations showed a significant increase of specialization
25 across decades: in recent censuses, eight populations used fewer host genera than in the 1980's
26 while none used more. Second, behavioral preference-testing experiments showed that
27 extinctions and recolonizations at two sites were followed, at first by diversification of heritable
28 preference ranks and increases of diet breadth, and subsequently by homogenization of
29 preferences and contractions of diet breadth. Third, we found a significant negative association
30 in the 1980's between population-level diet breadth and genetic diversity. Populations with
31 fewer mtDNA haplotypes had broader diets, extending to 3-4 host genera, while those with
32 higher haplotype diversity were more specialized. We infer that diet breadth had increased in
33 younger, recently-colonized populations.

34 Preference diversification after colonization events, whether caused by (cryptic) host
35 shifts or by release of cryptic genetic variation after population bottlenecks, provides a
36 mechanism for known effects of range shifts on diet specialization. Our results explain how
37 colonizations at expanding range margins have increased population-level diet breadths, and
38 predict that increasing specialization should accompany population persistence as current range
39 edges become range interiors.

40 **KEYWORDS**

41 butterfly, specialization, generalization, population bottleneck, additive genetic variance,
42 extinction-colonization dynamics, climate change, diet breadth, range expansion, host shift,
43 oviposition preference, Edith's checkerspot.

44 1 INTRODUCTION

45 Range shifts caused by human manipulation of wild species' habitats and by regional climate
46 warming are increasing in pace and prevalence (Parmesan 2006, Chen et al., 2011; Taboada et al.
47 2014; Platts et al., 2019; Lancaster, 2020). These shifts influence latitudinal patterns of
48 ecological specialization (Forister et al., 2015) by interacting in at least two ways with evolution
49 along a specialist-generalist axis. One of these ways is both expected and shown: generalists
50 should be better colonists than specialists, quicker to extend their ranges polewards as warming
51 creates opportunity (Platts et al., 2019, Monaco et al., 2020). However, a second, less expected
52 process, with the same result but the opposite direction of cause and effect, is also operating: the
53 process of range expansion itself can cause evolution of generalism at the population level
54 (Lancaster, 2020).

55 Using a global analysis of butterfly diets, distributions, and range dynamics, Lancaster
56 (2020) showed that, in this taxon, the trend for broader diets at higher latitudes has been caused
57 principally by range expansions themselves driving broadening of diets, rather than by
58 expansions being preferentially undertaken by generalists. This effect of range shifts on diet
59 breadth can complement the known tendency for large geographic range size to facilitate host
60 shifts (Jahner et al. 2011). However, unlike the effect of range size, the effect on diet breadths of
61 range expansion lacks an obvious mechanism. Here we reveal a candidate for the missing
62 mechanism: the fine-scale interactions between colonizations and host shifts in our study insect,
63 the butterfly, *Euphydryas editha*, generate an expectation of the cause-effect directionality shown
64 by Lancaster (2020).

65 In addressing host shifts and diets breadths of herbivorous insects, we are fortunate that
66 this topic has consistently fascinated evolutionary biologists for a century (Brues 1924, Ehrlich
67 & Raven 1964, Futuyma & Moreno 1988, Jaenike 1990, Thompson 1998, Agosta 2006, Tilmon
68 2008, Forister et al. 2012, Larose et al. 2019). The apparent conflict between the high degree of
69 host specialization of most species (Ehrlich & Raven, 1964) and the rapid accumulation of insect
70 communities on recently-introduced plants (Strong, 1974) has been particularly intriguing
71 (Agosta et al. 2010).

72 In recent decades, observed colonizations of exotic hosts have generated opportunities to
73 investigate ongoing episodes of diet evolution in real time. Forister et al (2013) found that
74 individual Melissa Blue butterflies sampled from populations that had colonized an exotic host,

75 alfalfa, were more generalized in their oviposition preferences than those sampled from
76 populations still using their traditional native host. Hardy (2017) asked whether the processes
77 that generate this type of pattern can be studied in captive insects: “does experimental adaptation
78 of a plant-eating insect population to a novel host result in host-use generalism, and improve the
79 odds of evolving additional new host associations?” Braga et al. (2018) used an experiment “in
80 silico” to answer this question in the affirmative.

81 Here we address these topics “in vivo” and in nature, applying a combination of long-
82 term observations and experiments to a single butterfly species and illustrating relationships in
83 real time between habitat colonizations, host shifts, population-level diet breadths and heritable
84 host preferences. Just as Braga *et al* (2018) discovered in their computers, we show in wild
85 populations that hosts whose use is opposed by natural selection have been briefly drawn into the
86 diets of *Euphydryas editha* butterflies after colonization events and then, after a few generations,
87 excluded again. We present evidence that this process is not confined to the populations in
88 which we observed it, and that colonizations have caused expansions of diet breadth across our
89 study species. This process can account for Lancaster's (2020) finding that range expansions,
90 each of which comprises multiple colonizations, have likewise caused broadening of population-
91 level diets.

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93 2 MATERIALS AND METHODS

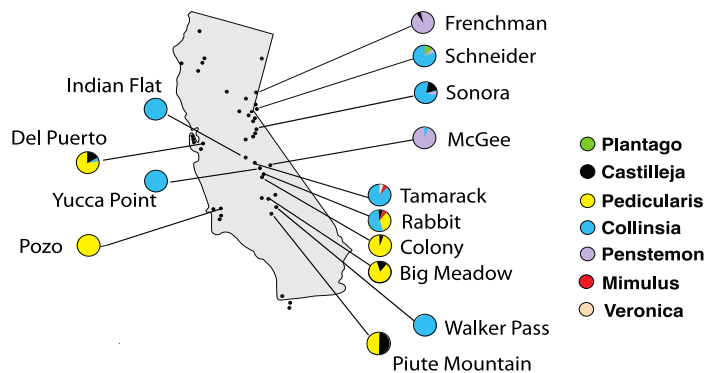
94

95 2.1 Study species

96 Edith’s checkerspot butterfly (*Euphydryas editha*; *Nymphalidae*, *Melitaeinae*), uses different
97 host genera in a geographic mosaic across its range (Singer, 1971; Singer & Wee, 2005; Singer
98 & McBride, 2012). The insect occurs as isolated populations and metapopulations, both of
99 which are typically localized, well-defined and subject to natural extinctions. This extinction-
100 recolonization dynamic revealed one of the earliest examples of a modern range shift linked to
101 anthropogenic climate warming (Parmesan 1996). Adult *E. editha* lay eggs in clutches on hosts
102 in the Orobanchaceae (*Pedicularis*, *Castilleja*) and Plantaginaceae (*Collinsia*, *Plantago*,
103 *Penstemon*, *Veronica*, *Mimulus*, *Antirrhinum*). When the proportion of *E. editha* eggs laid on
104 each host was ascertained by census at each of 57 sites, 43 populations were recorded as
105 monophagous, with the remainder using two to four host genera (Singer & Wee, 2005). These

106 populations showed strong isolation by distance but no isolation by host, so they did not
 107 comprise a set of host-associated cryptic species (Mikheyev et al., 2013). Figure 1 identifies to
 108 genus level hosts used in the 1980s at each of the sites used in the genetic study reported here.
 109 We treat insects at each of these sites as "populations," although some could equally be described
 110 as metapopulations. Dots without pie diagrams show sites where *E. editha* occurred that were not
 111 included in the current analyses.

112
 113 Figure 1. Snapshots of *E. editha* diet at the study sites in California and Nevada in the 1980s:
 114 results of censuses estimating proportions of eggs laid on each host genus at each site. The
 115 color-coding for host genera shown here is consistent through subsequent tables and figures.
 116 Identities to species level of hosts used at each site, and listing of potential hosts present but not
 117 used by the local butterflies, are given in supplemental Table 1; latitudes and longitudes are in
 118 supplemental Table 2.



119
 120 **2.2 Oviposition preference-testing technique and diet-breadth censuses**
 121 Population-level diet breadths at oviposition were recorded by physically searching known hosts
 122 and potential hosts for eggs and first instar or second-instar larvae, i.e., larvae young enough that
 123 they could be assumed to be feeding on the host species that had received eggs. We searched
 124 each individual of scarce plant species and censused quadrats placed in a stratified-random
 125 design to estimate the proportional use by the butterflies of more abundant plants.

126 Behavioral tests of oviposition preferences were performed by capturing butterflies in the
127 field and staging encounters with plants in their own habitats. Female *E. editha* that are
128 motivated to oviposit behave naturally in staged encounters with potential hosts, allowing an
129 experimenter to assess oviposition preferences by arranging a sequence of such encounters
130 (Singer et al., 1992). These preference tests used a standardized experimental technique in
131 which alternating encounters were staged between insect and plant. Plants were either left
132 undisturbed in their natural habitats or freshly transplanted into pots in their own soil.
133 Acceptance of plant taste was judged from full abdominal curling and extrusion of the ovipositor
134 for 3 sec. Acceptance and rejection were recorded at each encounter, but oviposition was not
135 allowed (videos showing acceptance in such staged encounters are linked in Singer & Parmesan
136 2019 and McBride & Singer 2010.)

137 During each test the range of plants that would be accepted, if encountered, expands over
138 time with increasing motivation to oviposit. Therefore, acceptance of plant A followed by
139 rejection of plant B is recorded as preference for A over B. The assumption that these insects'
140 preferences are not influenced by prior experience, either as larvae or as adults, is supported by
141 prior observation and experiment (McNeely & Singer 2001). Testing of other assumptions
142 underlying this technique is described in Singer et al. (1992). We used two experimental
143 designs, either testing each insect on the same individual plants, or on different plants sampled
144 independently from their populations; see footnote to Table 2.

145

146 2.3 Relationship between population-level diets and host preferences of individuals

147 Use of the behavioural preference assay has shown that, in populations of *E. editha* using more
148 than one host, this diversity of diet could be achieved either by weakness of oviposition
149 preference, allowing butterflies to accept hosts that they did not prefer, and/or by diversity of
150 preference rank within the population (Singer, 1983; Singer et al., 1989). Diversity of rank was
151 an important source of diet variation within two populations, Rabbit Meadow and Schneider,
152 where diet was known, from a combination of observation and experiment, to be rapidly-
153 evolving (Singer & Parmesan 2018, 2019). In contrast, weakness of preference was the principal
154 cause of diet variation in 6 populations that, judging from repeated censuses, were not indulging
155 in bouts of diet evolution (Singer et al., 1994).

156

157 2.4 mtDNA analyses

158 The original genetic study (Radtkey & Singer, 1995) included 24 populations of *E. editha* in
159 California, of which 14 had sample sizes of $n=4$ individuals or more and are included in the
160 current analysis. Butterflies used in these analyses were sampled independently of the censuses
161 used to assess diet breadths. We used 17 restriction endonucleases to identify 22 mtDNA
162 haplotypes of *E. editha*, the distributions of which were recorded within and among 24
163 populations of the butterfly. This molecular technique is long outdated, but the data that it
164 generated are not susceptible to bias for the questions we are asking, since we are not using them
165 to aim at a target, such as a phylogeny. Instead, we use them as an index of genetic diversity, to
166 ask whether an association existed between this index and diet breadth in the 1980s. For this
167 purpose our analysis is robust. It is particularly appropriate to use mtDNA, since it has half the
168 effective population size of nuclear DNA, which should augment the effects of bottlenecks
169 associated with population-founding events.

170 Haplotype diversity was itself diverse. Four populations were homogeneous, each
171 containing only a single haplotype despite sample sizes of 11, 13, 17 and 30 individual insects,
172 while at the opposite extreme one population in which 14 individuals were sampled produced 7
173 haplotypes and a second population with a sample size of only four contained no replicates.
174 Retention of this last informative sample was the reason for our choice of a sample size of four
175 as the cutoff for analysis. Exclusion of populations with sample sizes of less than four reduced
176 the number of populations from 24 to the 14 shown in Figure 1. We used a two-tailed Spearman
177 rank test to ask whether population diet breadths were associated with the numbers of haplotypes
178 found per individual sampled. Use of the per-individual statistic controls for variation of sample
179 size.

180

181 3 RESULTS

182

183 3.1 Changes of diet and diet breadth: long-term observations, 1980s-2010s

184 Detailed accounts of natural selection and behavioral mechanisms underlying evolution of diet
185 and preference have been published for two of our study sites, Schneider's Meadow and the
186 Rabbit metapopulation, each account filling an entire paper (Singer & Parmesan 2018, 2019).

187 This level of detail is beyond our current scope. Our dataset is heterogenous: censuses were
188 more frequent in some populations than in others, and we did not visit every population in every
189 year. Here we summarize decadal changes for the set of populations reported here in order to
190 focus on our primary question of the relationships between diet breadth and
191 extinction/colonization dynamics.

192 Table 1 shows time-trends of diet breadth across decades in 15 populations for which we
193 have long-term data on diet, 14 of which also provide data on mtDNA. The three left-hand
194 columns of Table 1 show population names, the numbers of host genera on which *E. editha* eggs
195 or larval webs were found during the 1980's, and the numbers of genera used in the most recent
196 decade of observation, which, with four exceptions, was the 2010s.

197 Figure 2 complements Table 1, adding information on diet-breadth oscillations and
198 extinction-colonization events. Populations where changes of diet breadth and/or extinctions
199 have occurred are identified in Figure 2 by two-letter or three-letter codes that link the
200 information in the Figure to that in Table 1. The timing of diet breadth observations in Figure 2
201 is positioned by the decade in which they were made. Although, for some populations, data
202 exist at greater accuracy than that, the overall trends are most easily seen at the decadal scale.
203 Each skull and crossbones icon indicates both an extinction and a subsequent recolonization, and
204 is not positioned by decade. It is placed between the last observation of diet breadth made before
205 the extinction and the first observation made after the recolonization. There were often multiple
206 censuses conducted after the initial extinction and prior to recolonization, that are not shown in
207 this figure.

208 Seven of the 15 study populations had the same diet breadth in the most recent census as
209 in the 1980's, while eight had narrower diets. None had broader diets. A two-tailed binomial
210 test rejects the hypothesis that diet breadth was equally likely to have expanded or contracted (P
211 = 0.008). Within our set of study populations, there has been a general trend for diet breadth to
212 be reduced over time (caveats in supplemental text 1).

213

214 Table 1. Diet diversity over time for 15 study populations and mtDNA diversity for 14.
 215 Columns 2 & 3: maximum numbers of host genera used simultaneously at each site in the 1980's
 216 and in the most recent decade when the site was censused, which is the 2010s unless otherwise
 217 indicated. Columns 4-6: numbers of mtDNA haplotypes sampled, sample sizes and numbers of
 218 haplotypes per individual sampled at each site in the 1980s. From Radtkey & Singer (1995).
 219 Hosts listed and identified to species in Supplemental Table 1.

population & lat-long	Number of host genera in 1980's	Number of host genera at last check	Number of mtDNA haplotypes	Sample size for mt DNA study (individuals)	mtDNA haplotypes / individual
Rabbit Meadow RM	4 ¹	2	1	30	0.03
Sonora Junction SON	3 ²	2	2	4	0.50
Tamarack Ridge TR6	3 ²	1	2	14	0.14
Schneider Meadow SCH	3 ⁴	2	2	7	0.29
Del Puerto Canyon* DP	3 ²	2 (1990s)	2	14	0.14
Frenchman Lake FR	3 ²	2	1	13	0.08
Tuolumne Meadow	3 ²	1 (2000s)	n/a	n/a	n/a
Piute Mountain	2 ²	2	3	10	0.33
Colony Meadow	2	2	1	17	0.06
McGee Creek MG	2	1 (2000s)	1	11	0.09
Big Meadow	2 ³	2	4	19	0.21
Yucca Point YP	1 ³	1	3	7	0.43
Walker Pass	1 ³	1	4	4	1.00
Indian Flat	1 ^{2,3}	1	7	14	0.50
Pozo	1 ^{2,3}	1 (2000s)	2	6	0.33

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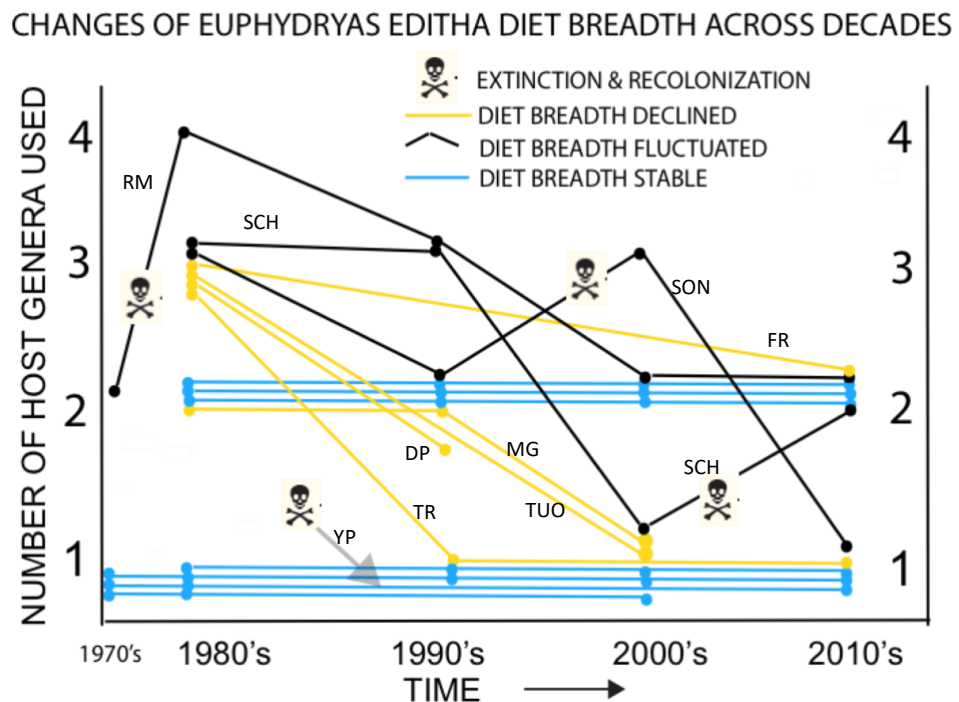
221 Notes for Table 1: references: ¹Singer 1983; ²Singer et al 1994 ³Radtkey & Singer 1995. Where
 222 no reference is given, as is the case for most of the “last check” column, data are previously
 223 unpublished. Location of named sites shown in Figure 1.

224 *Erratum: Singer et al. (1994) recorded only two hosts at Del Puerto, forgetting to include
 225 *Collinsia bartsiaefolia*, which had not been used since the high-rainfall spring of 1983.

226

227

228 Figure 2: Changes of *E. editha* diet breadth across decades. Population codes as in Table 1.
 229 Solid circles represent maximum diet breadths at each site for a given decade, usually
 230 representing results from multiple years. Censuses of eggs and young larvae were conducted as
 231 conditions permitted: all sites were censused at least once within each decade for which data are
 232 shown and some sites were censused multiple times/decade. Classification of a population as
 233 monophagous required a minimum sample size of 20 ovipositions (egg clusters or pre-diapause
 234 webs). There was greater variation in diet than evident on this figure: some sites had large shifts
 235 in proportions of eggs laid on the different host genera, but not in total number of genera used at
 236 the population level (this graphic). More detailed accounts of this type of change are beyond the
 237 scope of this paper. Sites not shown in the most recent survey were not censused in the 2010s.
 238 The skull and cross-bones icon indicates that the population went extinct for at least a year and
 239 was subsequently recolonized, with the post-recolonization diet breadth indicated by the solid
 240 circle in the subsequent decade. See sections 3.2.1, 3.2.2 and 4.8.2 for details of events at
 241 Sonora (SON), Rabbit (RM) and Schneider (SCH).



242

243

244

245 3.2 Diet breadths and preference diversities before and after colonizations

246 Here we provide details of changes at two sites where extinction/colonization events occurred
247 and from which we have not only diet data but also experimental data from behavioral tests of
248 individual female preferences, conducted both before the recorded extinction and after the
249 subsequent colonization event. At each site, diet breadth expanded and contracted, but the
250 mechanisms driving these dynamics were different in each case.

251

252 3.2.1 Site: Sonora (Fig. 1)

253 When we first worked at Sonora in the 1980's host preference ranks were almost invariant; we
254 found a single exception to the rule that butterflies either ranked *Castilleja pilosa*>*Collinsia*
255 *parviflora*>*Penstemon rydbergii* or they showed no preference (Table 2). Experimental
256 placement of eggs showed larval survival rates on the three hosts concordant with the rank order
257 of insect preference: survival was highest on *Castilleja*, lowest on *Penstemon* and intermediate
258 on *Collinsia*. However the top-ranked host, *Castilleja*, was estimated as receiving only 24% of
259 the eggs laid, with *Collinsia* receiving 75% and *Penstemon* 1% (Singer et al., 1989). *Castilleja*
260 was sufficiently rare that many searching insects failed to find it before reaching the level of
261 oviposition motivation at which they would accept either *Castilleja* or *Collinsia*, whichever they
262 encountered next. They were then more likely to encounter the more abundant host, *Collinsia*.
263 The population achieved diet diversity principally by interaction between weakness of preference
264 and rarity of the most-preferred host (Singer et al., 1989).

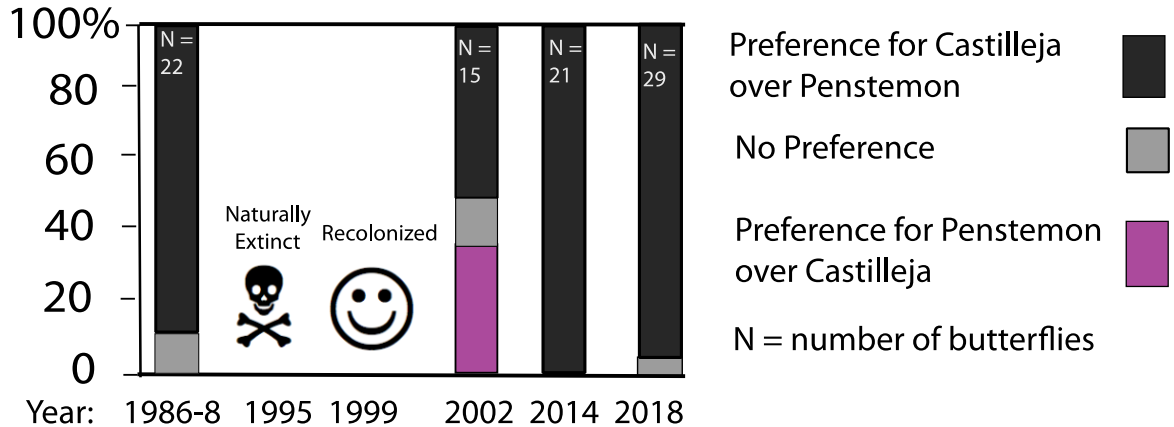
265 The Sonora population underwent a natural extinction in the 1990s, was absent for about
266 four years (confirmed with at least 2 intermediate censuses) and was recolonized by 1999. In
267 2002 we again conducted oviposition preference tests (Table 2, Figure 3). Preference ranks were
268 diverse: we found all possible rank orders for the three hosts, *Penstemon*, *Collinsia* and
269 *Castilleja*. Each of the three hosts was ranked by some individuals at the top of their preference
270 hierarchy and by others at the bottom. As expected from these preference tests, population-level
271 diet breadth at Sonora had increased in 2002: *Penstemon* had been added to the diet and all three
272 hosts were substantially used. We found 20 egg clutches on *Castilleja* in a total census of this
273 rare plant; 9 on *Collinsia* in a census covering approximately 40% of phenologically-suitable

274 plants and 14 on *Penstemon* in a census covering about 20% of these plants. We estimate that
 275 the most-used host was *Penstemon*, which had previously been the least-preferred of the three
 276 hosts and not used at all in some years.

277 Preference tests were once again performed at Sonora in 2014 and 2018 (Table 2, Figure
 278 3). With the exception of two butterflies that preferred *Penstemon* over *Collinsia*, preferences
 279 had reverted to their original homogeneous ranking of *Castilleja*>*Collinsia*>*Penstemon*. We
 280 found no females that preferred *Penstemon* over *Castilleja* and only one, out of 50 tested, that
 281 failed to discriminate between these hosts, so it is not surprising that *Penstemon* had once again
 282 disappeared from the diet. Despite intensive censuses, in neither 2014 nor 2018 did we find a
 283 single oviposition on *Penstemon*. Both the diversification of preferences and the inclusion of
 284 *Penstemon* into the diet as a major host had been ephemeral, appearing rapidly following the
 285 recolonization event, then disappearing just as quickly.

286

287 Figure 3. Proportions of butterflies preferring *Castilleja* or *Penstemon* at Sonora before and after
 288 natural extinction and recolonization. (additional data in Table 2).



289

290

291

Table 2: Preference ranks at Sonora before and after natural extinction and recolonization

		<<<<Prefer plant named at left	No preference	Prefer>>>> plant named at right	
1986-88	Castilleja	20	2	0	Penstemon
1986-88	Castilleja	13	9	0	Collinsia
1986-88	Collinsia	43	3	1	Penstemon
Extinction & Recolonization					
2002	Castilleja	7	1	6	Penstemon
2002	Castilleja	5	5	5	Collinsia
2002	Collinsia	12	2	10	Penstemon
2014	Castilleja	21	0	0	Penstemon
2014	Castilleja	21	0	0	Collinsia
2014	Collinsia	24	5	0	Penstemon
2018	Castilleja	28	1	0	Penstemon
2018	Castilleja	29	1	0	Collinsia
2018	Collinsia	18	5	2	Penstemon

293

294 *Footnote to Figure 3 & Table 2:* data from 1986-8 from Singer et al. (1989), Singer & Parmesan (1993).
 295 Data from 2002, 2014 & 2018 previously unpublished. We used two experimental designs: (1) tests in
 296 which all butterflies were offered the same individual plants, to reveal variation among individual
 297 butterflies and (2) tests in which each butterfly was offered a different set of individual hosts, to allow for
 298 effects of variable acceptability within host populations, which can generate apparent variability in the
 299 identity of the host species that is preferred (Singer & Lee 2000, Singer et al., 2002). Data shown from
 300 2002 are those obtained from the first design; the butterflies were truly variable. In 2014 and 2018 we
 301 used both designs but show results from the second design, which is conservative to the result,
 302 maximizing the likelihood of recording diverse preferences.

303

304

305 3.2.2 Site: Rabbit (represented as "Rabbit" in Figure 1 and as "RM" in Figure 2)
306 Prior to human intervention, *E. editha* in the Rabbit metapopulation used two perennial hosts and
307 occupied >20 habitat patches distributed across 8 x 10 km (Singer & Thomas 1996). The
308 principal diet was the perennial *Pedicularis semibarbata*, with minor use of the much rarer
309 *Castilleja disticha* (Singer & Parmesan 2019). Two potential hosts, both ephemeral annuals,
310 were present but not used: the super-abundant *Collinsia torreyi*, and the rare *Mimulus whitneyi*.
311 Natural selection opposed using *Collinsia* despite its abundance because its lifespan was so short
312 at this site that larvae hatching from eggs laid on it were almost certain to starve by failing to
313 reach diapause before host senescence (Moore 1989; Singer & McBride 2012).

314 Starting around 1967, humans made 18 clearings in which all trees were removed, fires
315 were set and ground was bulldozed, locally extirpating the butterflies from the cleared areas.
316 The effect of fertilization from the fires extended the size and lifespan of *Collinsia* to the point
317 where it could accommodate the life cycle of the butterflies. *Collinsia* in clearings suddenly
318 became a benign environment for the larvae, supporting higher fitness than the well-defended
319 *Pedicularis*, despite the butterflies being adapted to *Pedicularis* and demonstrably maladapted to
320 *Collinsia* in a suite of host-adaptive traits (Singer & Parmesan 2019).

321 The skull and crossbones along the RM line in Figure 2 represents the anthropogenic
322 local extinction caused by clear-cut and burn in the single "Rabbit Meadow" clearing, a large
323 (>2ha) clearing within the "Rabbit" metapopulation where we have studied diet intensively
324 across the decades. By 1979 the clearing had been colonized by butterflies immigrating from
325 adjacent unlogged patches, where the insects had persisted on their original diet of *Pedicularis*
326 and *Castilleja*. In 1981 a detailed census and map was made of the distribution of *E. editha*
327 ovipositions in the clearing. Eggs had been laid on four hosts: two novel hosts, *Collinsia* and
328 *Mimulus*, plus the two traditional hosts, *Pedicularis* and *Castilleja* (Singer 1983). *Pedicularis* is a
329 hemiparasite of gymnosperms, killed by logging, so it was restricted to the margins of the
330 clearing. *Collinsia* and *Mimulus* were used in the center of the clearing but remained unused in
331 the adjacent unlogged patch, where both occurred and *Collinsia* was abundant. This pattern of
332 host use sets the context for the two cases of preference diversification that occurred in the
333 Rabbit Meadow clearing during the 1980s and that are detailed in sections 3.2.2.1 and 3.2.2.2.

334 In the 1990s the direction of natural selection across the Rabbit metapopulation was
335 reversed, favoring preference for *Pedicularis* over *Collinsia*. The direction of evolution reversed

336 in response, and the use of *Collinsia* diminished until this host was permanently abandoned in
337 2001. Since then the metapopulation has reverted to its ancestral diet of *Pedicularis* and
338 *Castilleja*, with butterflies concentrated in the unlogged patches and rare in the clearings (Singer
339 & Parmesan, 2019).

340

341 3.2.2.1: Adaptive diversification of preference as part of host shift from *Pedicularis* to *Collinsia*.
342 Butterflies in Sequoia National Park (c.12 km from Rabbit) represent the putative pre-logging
343 state of the Rabbit metapopulation. We found no diversity of preference rank; most butterflies
344 from the Park showed varying strengths of preference for *Pedicularis* over *Collinsia* and a few
345 showed no preference, but none preferred *Collinsia* over *Pedicularis* (Singer & Thomas 1996).

346 In contrast, preference ranks for the same two hosts in the anthropogenically altered
347 Rabbit Meadow clearing were diverse and evolving through the 1980s. In the early 1980s, most
348 insects emerging in the centre of the clearing preferred to oviposit on *Pedicularis*, despite having
349 developed on *Collinsia* from eggs naturally laid on it. The proportion of these *Collinsia*-
350 emerging butterflies that preferred *Collinsia* increased significantly between 1984 and 1989
351 (Singer & Thomas 1996). This increase of preference for *Collinsia*, and the diversification of
352 preference from the starting condition lacking diversity of preference rank, are consistent with
353 adaptive evolutionary response to measured natural selection that favoured preference for
354 *Collinsia*, but that acted on an initially *Pedicularis*-preferring population (Singer & Parmesan
355 2019).

356

357 3.2.2.2: Non-adaptive preference diversification: incorporation of *Mimulus* into the diet as a side-
358 effect of host shift to *Collinsia*.

359 In the ancestral state *Mimulus* and *Collinsia* were present but neither was used for oviposition,
360 though *Collinsia* was fed upon by wandering late-instar larvae. In 1981-2, following logging and
361 burning, both hosts were used in the clearing and oviposition preferences for them were diverse
362 (Table 3B). Field experiments (Singer et al., 1994) estimated larval survival on *Collinsia* in the
363 clearing as three times higher than that on *Mimulus* (*Mimulus* is “host 4” in Figure 2 of Singer et
364 al., 1994). Natural selection favoured using *Collinsia* but not *Mimulus*. *Mimulus* had been
365 included in the diet despite natural selection against using it, but this situation proved short-lived:
366 by 1988 preferences for *Collinsia* over *Mimulus* had become homogeneous and *Mimulus* was no

367 longer used (Tables 3A, B). We found the same preference homogeneity again in 2019, but the
 368 experiment was not strictly comparable. We used butterflies from the unlogged patch adjacent to
 369 the Rabbit Meadow clearing rather than from the clearing itself, since *Collinsia* had not been
 370 used as an oviposition host since 2001, and butterflies in the clearing were scarce (Section 3.2.2).
 371
 372 Table 3A: Egg distributions on *Collinsia* and *Mimulus* in Rabbit Meadow clearing and adjacent
 373 unlogged patch. Each quadrat was 30cm x 30cm and could contain several hundred individual
 374 *Collinsia* plants and >1 egg clutch. * indicates that no census was done

year	Habitat: clearing patch		Habitat: unlogged patch	
	Clutches on <i>Collinsia</i> /quadrats searched	Clutches on <i>Mimulus</i> /plants searched	Clutches on <i>Collinsia</i> /quadrats searched	Clutches on <i>Mimulus</i> /plants searched
1979	16/41	*	0/22	*
1981	5/33	6/25	0/50	0/32
1982	37/118	13/36	0/56	0/46
1988	58/50	0/47	0/20	0/18
1989	9/69	0/37	0/25	0/35
1991	19/54	0/18	0/20	0/22
2019	0/40	0/13	0/25	0/61

375

376 Table 3B: Preferences for *Collinsia* vs *Mimulus* at Rabbit Meadow

377

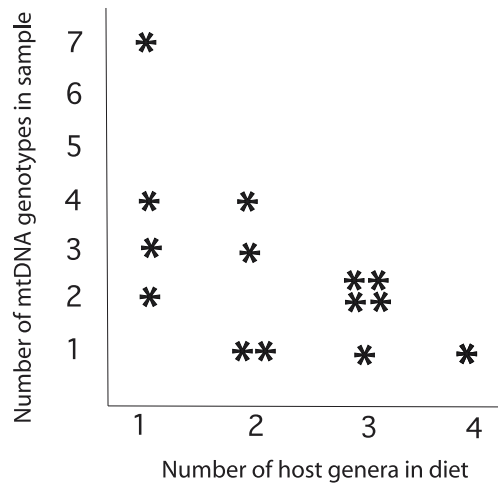
year	Butterflies preferring <i>Collinsia</i>	No preference	Butterflies preferring <i>Mimulus</i>
1981-2 (clearing)	11	12	8
1988-92 (clearing)	23	3	0
2019 (unlogged patch)	22	0	0

378

379 3.3 Genetic evidence: mtDNA and diet breadth

380 Re-examination of the mtDNA dataset first published long ago (Radtkey & Singer 1995)
381 reveals a relationship on which the original study did not comment: an inverse association
382 between population-level diet breadth in the 1980s and mtDNA diversity (Figure 4, Table 1);
383 samples from populations using fewer host genera contained more mtDNA haplotypes.
384

385 Figure 4. Numbers of mtDNA haplotypes found in the 14 study populations of *E. editha* plotted
386 against the 1980's diet breadths shown in Figure 1 and Table 1.



387
388

389 Because sample sizes were diverse, the association shown in Figure 4 might have
390 stemmed from sampling more individuals from populations that happened to be monophagous
391 than from those with broader diets. However, the opposite was the case: a regression of mtDNA
392 sample sizes on diet breadth, using the data in Table 1, gives a slope of +3.6 ($P = 0.06$, two-
393 tailed). The direction of this trend, with higher mtDNA sample sizes from populations with
394 broader diets, is opposite to that expected to produce the relationship in Figure 4. To control for
395 sample size in testing significance of the genetic diversity/diet breadth relationship, we
396 calculated an estimate of mtDNA diversity as the number of haplotypes per individual sampled
397 (Table 1, right hand column). The association between this statistic and the diet breadths listed

398 in column 2 of the Table is significant with $p = 0.024$, by Spearman rank test (two-tailed).

399 4 DISCUSSION

400 We exploit our study insect's extensive ecotypic variation and penchant for rapid evolution of
401 host preference to document a novel behavioral mechanism underlying Lancaster's (2020)
402 finding that butterfly range expansions have caused loss of population-level dietary
403 specialization. In species that resemble *E. editha* in the mechanics of their diet evolution,
404 colonizations at expanding range margins will increase both diversity of host preferences and
405 population-level diet breadths. At the same time, increasing specialization will accompany
406 population persistence in range interiors.

407 Following extinctions of two populations in the interior of *E. editha*'s range, diets
408 recorded after recolonization were broader than they had been at the same sites in pre-extinction
409 populations (section 3.1, Figure 2). We can generalize this effect of colonizations on diet breadth
410 at least to our study species as a whole, since we show a significant negative association among
411 populations between mtDNA diversity and diet breadth, suggesting that young populations had
412 broad diets and low genetic diversity, while older ones had acquired greater genetic diversity and
413 evolved local adaptation in the form of greater specialization (section 3.3, Table 1, Figure 4).

414 Behavioral preference tests administered in the field showed that the principal
415 mechanism of the post-colonization diet broadening events that we observed was not loss of
416 specialization by individuals, but diversification of specialists (section 3.2, Figure 3, Tables 2,
417 3B). Variability of oviposition preferences had increased in the newly-recolonized populations.
418 What might have caused this diversification? Two different, non-exclusive, processes are likely,
419 which we discuss in sections 4.5 and 4.6. First, the population bottleneck associated with the
420 colonization may reveal previously-cryptic additive genetic variance (van Heerwaarden et al.,
421 2008; Paaby & Rockman, 2014; Hoffmann et al., 2017). Second, colonizations may be
422 accompanied by host shifts and it may be the host shifts that cause preferences to diversify.

423

424 4.1 Oviposition preferences of *E. editha* are heritable

425 Past work, reviewed by Singer & Parmesan (2019) has shown that oviposition
426 preferences of our study insect are heritable and unaffected by experiences of larvae or adults.
427 Two sets of experiments were performed. First, preferences of freshly-caught females were

428 measured within a single season at a single site, Schneider, and offspring were raised in the
429 "laboratory" - a greenhouse - on a common host. Individual laboratory-raised daughters were
430 preference-tested "blind," in that the tester did not know the identities of their mothers or sibs.
431 The daughters resembled their mothers in preference, with $p < 0.005$ and $r = 0.45$ with 95% c.i.
432 0.16-0.74 (Singer et al., 1988). Given that male contribution in this experiment was random and
433 that male and female contributions to inheritance of oviposition preference were later shown to
434 be approximately equal (McBride & Singer 2010), we can estimate heritability of preference as
435 twice the mother-daughter correlation, with the lower bound of the 95% c.i. at 0.32.

436 The second set of experiments comprises measurements made at Schneider while rapid
437 changes of preference were occurring in nature. In two years, 1983 and 1990, we measured
438 preferences of freshly-captured butterflies for the exotic *Plantago* versus their traditional
439 *Collinsia* host. We also measured preferences of offspring of 1983 butterflies and 1990
440 butterflies that we had raised on *Collinsia* in a greenhouse. Changes of preference between the
441 two years were significant and similar in the field-caught and laboratory-raised butterflies,
442 indicating that the change in preference measured in nature was evolutionary (Singer et al.,
443 1993).

444

445 4.2 Long-term observations of increasing specialization: evidence for 446 independence of data

447 Seven of the 15 populations in our study used three or four host genera in the 1980s, but
448 by the 2010s none used more than two. In using a statistical test to reject the hypothesis that
449 specialization was equally likely to increase or decrease, we assume that changes of diet at
450 different sites were independent events. Our justification for this assumption is that *E. editha* is a
451 sedentary butterfly (Ehrlich, 1961) with a maximum recorded movement of 5.6km (Harrison,
452 1989). Observed evolution of host preference has differed between populations separated by
453 <10km and appeared independent between populations 12km apart (Singer & Thomas 1996).
454 No two populations in the current study underwent the same changes of diet. We therefore
455 assume that our study populations, scattered across California as they are (Figure 1), were
456 evolving independently of each other over the decadal timescales that we used and that the long-
457 term census data, showing an overall trend for increasing specialization across four decades, are
458 not effectively pseudoreplicated.

459

460 4.3 Two colonizations followed by diversification of preferences and expansions 461 of diet breadth.

462 Colonizations at the Rabbit Meadow clearing and at Sonora were followed by increases in
463 population-level diet breadth that were shown by experiment to be driven by within-population
464 diversification of oviposition preference. At Sonora, a natural extinction-colonization event was
465 followed in 2002 by an explosion of preference diversity (section 3.2.1, Table 2, Figure 3) and
466 expansion of diet breadth in which a formerly-avoided plant, *Penstemon*, became the principal
467 host. Experiment had previously shown natural selection against use of *Penstemon* at this site
468 (Singer et al., 1994). In the latest censuses, in 2014 and 2018, *Penstemon* was no longer used
469 and preferences for the most-preferred host, *Castilleja*, over *Penstemon* were homogeneous.

470 At Rabbit Meadow, colonization of a habitat patch in which humans had extirpated the
471 butterflies was accompanied by addition of a novel host, *Collinsia*, that supported high fitness
472 due to extended longevity after anthropogenic fire. This host shift was favored by natural
473 selection (Singer & Parmesan 2019), but the adoption of *Collinsia* was accompanied by addition
474 to the diet of a second novel host, *Mimulus* (Table 3A), that did not support high fitness but was
475 preferred for oviposition by some butterflies (Table 3B). Selection opposed the use of *Mimulus*
476 (Singer et al., 1994) and it was abandoned within a decade (section 3.2.2, Table 3A; shown in
477 Figure 2 as the decline from 4 to 3 genera at population RM), contributing to the overall trend for
478 increasing specialization as populations persisted.

479 We admit to being puzzled by the speed with which diet breadth expansions have been
480 followed by returns to specialization. Even though oviposition preferences of *E. editha* are
481 highly heritable and we have estimated strong natural selection on host use (Moore 1989, Singer
482 et al., 1994), returns to specialization have been unexpectedly fast: less than 12 generations at
483 Sonora (Table 2) and less than 6 for the loss of preference for *Mimulus* over *Collinsia* at Rabbit
484 Meadow (Table 3B).

485

486 4.4 Genetic evidence supports generality of diet breadth expansions following 487 colonizations

488 In the 1980s, populations with broader diets had significantly lower mtDNA diversity than

489 specialist populations using fewer host genera (section 3.3, Figure 4, Table 1). We expect these
490 mtDNA data to be subject to founder effects in the same manner as data gathered by more
491 modern techniques. Therefore, a negative association between variability of diet and of
492 genotype is expected if population-founding events frequently caused expansions of diet breadth
493 like those we observed in real time. Young populations would have broader diets and reduced
494 genetic diversity from founder effects; older populations would have evolved specialization as a
495 local adaptation while acquiring genetic diversity. Newly-founded populations are, indeed,
496 expected to have reduced genetic diversity and to acquire more genotypes as they age, from
497 some combination of immigration and mutation (Austerlitz et al., 1997, Excoffier et al., 2009).
498 However, it is not obvious that recently-colonized populations should have broader diets than the
499 sources from which they were derived. We address this question in section 4.6.

500 4.5 Two ways for host shifts to cause diversification of preferences.

501 Host shifts clearly diversify preferences when, during a shift, individuals retaining preference for
502 the traditional host occur in the same population and at the same time as those preferring the
503 novel host. This was the case both for the shift from *Pedicularis* to *Collinsia* at Rabbit Meadow
504 (section 3.2.2.1) and for the shift from *Collinsia parviflora* to *Plantago lanceolata* at Schneider
505 (Singer & Parmesan 2018).

506 A different possible role for host shifts stems from the evolutionary dimensionality of
507 preference. Evolutionary transitions from traditional to novel hosts may cause additional,
508 unexpected hosts to be drawn into to an insect's diet even if this addition is opposed by natural
509 selection. This hypothesis was put forward by Hardy (2017) and supported in a model by Braga
510 et al. (2018). We illustrate two examples: the apparently temporary use of *Penstemon rydbergii*
511 by the Schneider population during its host shift from *Collinsia* to *Plantago* (Singer & Parmesan
512 2018) and the clearly temporary use of *Mimulus* by the Rabbit Meadow population in the early
513 stages of its host shift from *Pedicularis* to *Collinsia* (section 3.2.2, Table 3; Singer & Parmesan,
514 2019). In both cases we estimated that use of the unexpected host was opposed by natural
515 selection and both the unexpected hosts were eliminated from the diets after only a few
516 generations.

517

518

519 4.6 Two ways for colonizations to cause diversification of preferences:

520 bottlenecks and host shifts.

521 4.6.1: Bottlenecks.

522 Cryptic genetic variation that exists in natural populations can be revealed by changing
523 conditions (Paaby & Rockman, 2014, Hoffmann et al., 2017). For example, experimental
524 application of population bottlenecks to *Drosophila bunnanda* revealed cryptic genetic variation
525 for desiccation resistance, causing an increase in additive genetic variance for this trait (van
526 Heerwaarden et al., 2008). This is not an isolated result; bottlenecks frequently augment additive
527 genetic variance, in apparent opposition to expected effects of genetic drift (Taft & Roff 2012).
528 We see a possible parallel between the result of the experiments with *Drosophila bunnanda* and
529 the increases of preference diversity that followed colonization events in our study of *E. editha*.

530

531 4.6.2. Association between colonizations and cryptic host shifts

532 If colonizations tended to cause host shifts, the host shifts could then diversify preferences as we
533 described in the previous section. Overall, it seems unlikely that colonizing female *E. editha*
534 would switch host genera with sufficient frequency to produce the association in Figure 4.

535 However, colonizations might routinely involve host shifts if those shifts were, from the
536 butterflies' perspective, more frequent than changes of host genus that are observable to humans.

537 We suspect this to be the case. Colonizations that don't shift host species will always involve
538 changes of host population, and variation among conspecific host populations can be extremely
539 important to butterflies (Harrison et al., 2011), including Melitaeines, the subfamily containing
540 *Euphydryas*. Prior studies of discriminations made by Melitaeine butterflies within and among
541 host species have shown that, from the perspectives of all three butterfly species investigated, (*E.*
542 *editha*, *Euphydryas aurinia* and *Melitaea cinxia*) variation of acceptability among host
543 individuals or conspecific populations was equivalent in magnitude to variation among host
544 genera (Singer & Parmesan, 1993; Singer & Lee, 2000; Singer et al., 2002) (See Glossary for
545 definitions of “preference” and “acceptability” and supplemental Text 2 for descriptions of
546 experiments and their results). Because variation among conspecific host populations is so
547 important to Melitaeines, it will often be the case that a colonizing female is effectively
548 undertaking a host shift even if the host she uses after migrating is the same species on which she
549 developed at her site of origin.

550 If each host population were effectively unique from the butterflies' perspective, then
551 adapting to a newly-colonized population of a traditional host species could cause additional host
552 species to be temporarily drawn into the diet, just as if the novel and traditional hosts were
553 different species. Although the underlying mechanism by which such "drawing in" might occur
554 has not been elucidated, our data suggest that it exists (section 4.5).

555

556 4.7 Rejection of gene flow and drift as causes of observed diet breadth variation.

557 A population may achieve a broad diet by containing a diversity of host-adapted genotypes. If
558 such a population sends out colonists that found new populations, those populations should,
559 through founder effects, have narrower diet breadths than their source. This process has been
560 called "specialization by drift" and there is phylogenetic evidence that it has been important in
561 scale insects (Hardy et al 2016). However, if it were important in *E. editha* we would expect
562 young populations to have narrower diets than established ones, the opposite of the inference that
563 we draw from our results.

564 As gene flow continues after population founding, established populations receiving
565 more diverse gene flow from multiple sources might become the ones with the broadest diets.
566 However, if this process were driving interpopulation variation we would expect a positive
567 association between genetic diversity and diet breadth, the opposite of our current findings
568 (section 3.3, Table 1, Figure 4). Gene flow and founder effects are not implicated as causes of
569 the variable diet breadths in our study system.

570

571 4.8. Specialization is not an evolutionary dead-end, either over millions of years or
572 decades; specialized populations contain cryptic variation of preference susceptible
573 to genetic assimilation.

574 4.8.1 Phylogenetic analyses .

575 These have tested the plausible hypothesis that specialists are derived from generalists more
576 frequently than evolution in the opposite direction, and that specialization can be an evolutionary
577 dead-end. This hypothesis was not supported (Janz et al., 2001; Nosil, 2002; Forister et al.,
578 2012); phylogenetic analyses indicate that diet breadth evolves readily in either direction. The

579 idea that this bidirectional evolvability causes oscillations between specialization and
580 generalization, and that these oscillations have acted as important drivers of insect speciation and
581 biodiversity, first emerged from analyses of the butterfly family Nymphalidae (Janz et al., 2001;
582 2006). This idea has stimulated lively and apparently unresolved debate (Janz et al., 2016;
583 Hamm & Fordyce 2016; Hardy, 2017; Nylin et al., 2018; Braga et al., 2018).

584 4.8.2. Three real-time observations of diet-breadth oscillation

585 We observed, in real time, three miniature versions of the diet-breadth oscillations deduced from
586 phylogenetic analyses. The oscillations at Sonora and Rabbit Meadow were described in
587 sections 3.2.2.1 and 3.2.2.2. The third example is the Schneider host shift, which we did not
588 include in Section 3 under "Results" since we present no new data. The most likely starting point
589 was monophagy on *Collinsia*, since that was the diet of the nearest-known population of the
590 same *E. editha* ecotype, at Curtz Lake, when the exotic *Plantago* had not yet arrived (Thomas et
591 al., 1987). In preference trials administered on site at Curtz Lake, no butterflies preferred
592 *Plantago* over their own *Collinsia* host, though around 10% had no preference (Thomas et al.,
593 1987). Assuming that this represents the initial condition at Schneider, we conclude that, during
594 its host shift from *Collinsia* to *Plantago*, the population passed from absence of preference for
595 *Plantago* over *Collinsia* prior to the start of our study, through the phase in 1982-1990 when
596 both preferences and diet were diverse, to eventually achieve monotonous preference for
597 *Plantago* over *Collinsia*, monophagy on *Plantago* and abandonment of the traditional host in
598 2002 & 2005 (Singer & Parmesan 2018).

599

600 4.8.3 Cryptic preference variation and genetic assimilation

601 Although we judged the initial diet of the Schneider population to be both monotonous and
602 monophagous (Section 4.8.2), the population contained variation of preference which was
603 cryptic, in the sense that it had no effect on diet until it was revealed by the arrival of the exotic
604 *Plantago*. From this point we can imagine increasing preference for *Plantago* evolving by a
605 classic genetic assimilation process (Paaby & Rockman, 2014).

606 As at Schneider, the starting condition for diet evolution in the Rabbit Meadow
607 population was absence of preference for the novel host *Collinsia*, with variable strengths of
608 preference for *Pedicularis* over *Collinsia* and a few individuals without preference (Section

609 3.2.2.1). Again, this variation was cryptic until humans, by logging, killed the *Pedicularis*
610 (which parasitizes trees) and created large patches of *Collinsia* from which individual butterflies
611 with weaker preferences were unable to escape before reaching the oviposition motivation at
612 which they would accept *Collinsia* (Singer & Parmesan 2019). The result was the creation of
613 booming populations in clearings that used *Collinsia* but in which most individuals retained
614 preference for *Pedicularis*. Natural selection for acceptance of *Collinsia* was strong in those
615 populations and evolution of preference was rapid (Singer & Thomas 1996).

616 Even in monophagous *E. editha* populations in which all individuals show the same
617 preference rank and none are without preference, preferences are not invariant, since the strength
618 of preference is still variable: individuals differ in the length of the fruitless search that they
619 would undertake before accepting a low-ranked host (Singer 1982). As in the Rabbit Meadow
620 example just described, it is possible for this variation to be revealed and exposed to selection
621 when, for whatever reason, butterflies fail to find their preferred host.

622 4.9 Contrary results to ours: colonizations cause specialization

623 In contrast to our results, two studies on other species have shown increased dietary
624 specialization after colonization. Hardy et al (2016) use phylogenetic analyses to argue that, in
625 scale insects, diet diversity is positively associated with genetic diversity, so founder effects
626 associated with colonizations and range expansions have caused population-level diet to become
627 more specialized, not less. Again conversely to our own results, the poleward range expansion
628 of the Brown Argus butterfly (*Aricia agestis*) in the UK has been associated with increasing host
629 specialization. In the expanding parts of the range of this insect oviposition preferences were
630 more specialized and homogeneous, both within and among populations, than in regions where
631 the insect was long-established (Bridle et al., 2014). In addition, larvae in the expanding regions
632 were physiologically more host-specialized and had lost evolvability, compared to their ancestral
633 populations (Buckley et al., 2014).

634 These contrary results give us pause in suggesting the level of generality of our result that
635 colonizations cause loss of specialization. However, the ability of our results to help explain both
636 the global latitudinal pattern of specialization documented by Forister et al. (2015) and the cause-
637 effect relation between range shifts and diet breadth documented by Lancaster (2020) suggests

638 that the mechanisms that we document here are not unique.

639

640 4.9 Implications of our study for ecological speciation

641 There are none. Much of the literature that ties insect diet evolution to generation of biodiversity
642 carries the assumption that host shifts facilitate speciation. In Melitaeine butterflies this does not
643 seem to be true. Host shifts are frequent, closely-related sympatric insect species typically have
644 overlapping diets (LaFranchis 2004), and *E. editha* itself shows strong isolation by distance but
645 no residual isolation by host (Mikheyev et al., 2013). The failure of Melitaeines to speciate with
646 host shift may reflect the fact that they don't mate on their hosts. Apart from this trait, we have
647 no reason to think that diet evolution in Melitaeines is unusual, so we expect its mechanisms, as
648 revealed in the current study, to be informative about processes that operate more widely than in
649 this butterfly subfamily. Whether the short-term changes we show are informative about long-
650 term diet breadth oscillations (Janz et al., 2001, 2006, 2016; Hamm 2016, Hardy 2017, Braga et
651 al., 2018a,b) is an open question, but the fact that insects tend to recolonize long-lost ancestral
652 diets suggests that processes measured on very different time scales are related.

653

654 4.10 Contribution to understanding relations between range shifts and diet evolution

655 The processes that we document here support the cause-effect directionality of range-shift effects
656 on diet breadth shown by Lancaster (2020) and help to account for the global latitudinal pattern
657 of specialization: the trend for temperate zone species to be less specialized than those in the
658 tropics (Forister et al., 2015; Settele et al., 2014; Monaco et al., 2020). As more and more
659 species track shifting climate spaces driven by current warming trends, the numbers experiencing
660 poleward range expansions will continue to rise. Yet we have little understanding of the
661 behavioural and evolutionary processes accompanying these ecological range expansions. The
662 mechanisms driving diet expansion and contraction that we document here are novel, especially
663 the finding that increases of generalization at the population-level can stem from diversification
664 of specialist individuals rather than from each individual becoming more generalist. These
665 results help us to better understand underlying dynamics operating at range boundaries and
666 during extinction/colonization episodes. Incorporating such behavioral evolution into our
667 understanding will better inform projection models and conservation planning under continued
668 anthropogenic climate change. Our analyses of the heritability, dimensionality and evolutionary

669 agility of host preferences should contribute to a mechanistic understanding of insect diets and
670 host shifts in general, in addition to their associations with range shifts.

671

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673

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904 GLOSSARY:

905 Population-level diet breadth: In the studies reported here, the number of host species on which
906 eggs of *E. editha* were laid in a particular population.

907 Host use. Again, in the work reported here, the proportion of eggs laid on each host species by
908 an insect or an insect population. In a practical sense, this must most often be measured from the
909 distributions of silken webs spun by young larvae, although groups that do not survive to this
910 stage are missed by this technique (see Methods).

911 Acceptance: a positive behavioural response by an insect to an encounter with a plant. It is a
912 description of an observable and measurable event. It is not a trait of either plant or insect, since
913 it depends on both insect preference and plant acceptability (see below). It is a trait of the plant-
914 insect interaction (Singer, 2000).

915 Insect preference: the set of likelihoods of accepting particular specified hosts that are
916 encountered. Defined in this way, it is a property of the insect that can vary among individuals
917 (Singer, 2000) and can be heritable. *E. editha* first encounters hosts visually, then chemically,
918 then physically, with separate preferences expressed at each stage (Singer & Parmesan, 2019).
919 Again, in *E. editha*, the strength of post-alighting preference for two hosts, say host A and host
920 B, is measured by the length of time that a female will search accepting only host B (if
921 encountered) until, after failing to find host B, she reaches the level of oviposition motivation at
922 which either A or B would be accepted, whichever is next encountered (details and justification
923 in Singer et al., 1992).

924 Plant acceptability. The set of likelihoods that a plant will be accepted by particular specified
925 insects that encounter it. Defined in this way, it is a property of the host that can vary among
926 individuals (Singer 2000) and can be heritable (Singer et al 1988).