Evolution of trade-offs across environments following experimental evolution of the generalist *Drosophila suzukii* to different fruit media

Short title: Trade-offs evolution in a generalist species

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Data accessibility

Our data will be archived on dryad upon acceptance.

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Abstract

Adaptation to divergent environments can result in ecological specialization. The detection of trade-offs across environments (i.e., negative correlations in performance between different environments) is the hallmark of specialization. Although such trade-offs are predicted by theory, experimental evidence that trade-offs can readily evolve in the laboratory remains scarce. Here, we investigated the evolution of adaptation to distinct environments, including potential fitness trade-offs by maintaining populations of the generalist fruit pest, *Drosophila suzukii*, for 26 generations on media made with different fruits. We measured the performance and preference of each evolved population on the different fruits using reciprocal transplant experiments after five generations and at the end of our experiment. After five generations, experimental populations on most fruits had gone extinct, but they had adapted to three test fruit media, without exhibiting trade-offs. By generation 26 on these three fruits, specific adaptation to each fruit media had evolved, with trade-offs across media for some populations. The evolution of fruit-specific performance did not drive the evolution of corresponding preferences (i.e., preferences for the evolution fruit). This study suggests that ecological specialization can evolve in generalist species, even if only transiently, when hosts or habitats are heterogeneous over time and space.

Key words

adaptation, experimental evolution, generalist/specialist, specialization, trade-offs

Introduction

Ecological specialization is the process of adaptation to a restricted spectrum of environments originally used by a given species (reviewed in Bolnick and SvanbÃ 2003; Holt 2009; Ravigné et al. 2009; Devictor et al. 2010; Poisot et al. 2011). This process is widely recognized as a major determinant of the emergence and maintenance of biodiversity (Futuyma and Moreno 1988). It can be the first step in the evolution of host races and even speciation (e.g., Filchak et al. 2000). The process of ecological specialization leads to a pattern of increased fitness in a local environment and decreased fitness in other environments (Futuyma and Moreno 1988). When distinct trait values are optimal in different environments (for example, the ability of plants to tolerate salts or metals in the soil), fitness trade-offs across environments can emerge. Trade-offs can be measured phenotypically as a decrease in fitness in an environment associated with an increase in fitness in another environment (Angilletta et al. 2003). Genetically, adaptation to a subset of environments (i.e., specialization) is due to an increase in the presence or frequency of alleles favorable in those environments. Phenoptypic trade-offs appear when such alleles are either neutral or deleterious with respect to fitness in other environments (antagonistic pleiotropy ; Fry 1996). Phenothypic trade-offs also arise when alleles that are neutral in the focal environment (and thus not selected against and can accumulate over time) are deleterious in other environments (Kawecki et al. 1997).

Darwin's finches provide one of the most famous illustrations of trade-offs leading to ecological specialization (Grant and Grant 1995). Species or populations with small beaks can open small seeds but not large seeds and reciprocally for species or populations with large beaks. Environmental changes in the size and toughness of the seeds available results in the evolution of both beak shape and beak size in these finches. While the inability of a bird to have two different beaks is self-evident, trade-offs are generally more challenging to detect

53 in general (Fry 1996). This challenge has been addressed by performing reciprocal transplant
54 experiments of natural or experimentally evolved populations. In natural populations,
55 reciprocal transplant experiments offer a direct way of revealing the presence of fitness trade-
56 offs and patterns of local adaptation (Bradshaw 1984; Kawecki and Ebert 2004). In such
57 experiments, the fitness of organisms is measured in their evolved environment (also called
58 natal, local, sympatric, source or home environment) and in one or more alternative
59 environments (also called novel, away or allopatric environments; Bradshaw 1984).
60 Reciprocal home site advantage indicates that adaptation to one environment involves
61 performance trade-offs with other environments (Kawecki and Ebert 2004), or that alleles
62 favorable in one environment are simply neutral in other environments (Fry 1996). An
63 important question in evolutionary biology is to know whether trade-offs lead to
64 specialization or whether the evolution of specialization leads to the evolution of trade-offs.
65 The order of such processes is difficult if not impossible to discern through the direct study of
66 natural populations (Kawecki et al. 2012; Magalhães and Matos 2012). Experimental
67 evolution aids in establishing the order and causality of evolutionary processes such as the
68 evolution of organisms in response to selection pressures induced by an environment over
69 time (Magalhães and Matos 2012).
70 Although fitness trade-offs across environments are expected predicted by theory
71 (Futuyma and Moreno 1988; though see Fry 1996), experimental evidence for their evolution
72 in experimental populations is mixed (Hereford 2009). Experimental evolution of organisms
73 ranging from bacteria, viruses, insects and plants in different abiotic and biotic environments
74 have found trade-offs in some cases (Fry 1990; Mackenzie 1996; Bedhomme et al. 2012;
75 Messina and Durham 2015; Gompert and Messina 2016), but not others (Magalhães et al.
76 2009; Messina et al. 2009; Laukkanen et al. 2012; Messina and Durham 2013). The lack of
77 trade-offs observed in many experimental evolution studies can have different causes (Fig. 1).

Trade-offs are likely to evolve and be detected when populations adapt to environments with distinct phenotypic optima (Travisano and Lenski 1996; Cooper and Lenski 2000; Fig. 1A). Using this reasoning, trade-offs are unlikely to evolve when the environments are similar (Fig. 1B). For example, in the context of plant-insect interactions, the host plant determine the environment to which the herbivorous insects must adapt. If plant species share secondary (defensive) compounds, they may not represent substantially different selective environments (Agrawal 2000; Fukano and Nakayama 2018). Even when the environments to which populations are adapting differ, they might differ in similar ways from the ancestral environment, so that organisms adapting to one of the environments will also adapt to the other environment, increasing their fitness in both environments (Fig. 1C; Reboud and Bell 1997; Kassen and Bell 1998). For both these scenarios (Figs. 1B & 1C), trade-offs can ultimately evolve over multiple generations as mutations accumulate that are neutral in the home environment but deleterious elsewhere. Other more technical reasons may also explain the failure to detect trade-offs, including low statistical power and measurement of traits not involved in trade-offs (Phillips and Shine 2007). Finally, the multidimensionality of trade-offs makes them inherently difficult to measure (Poisot et al. 2011).

Herbivorous arthropods represent one of the most diverse taxa on Earth and many are important pests (Messina 1985). They are therefore often studied in the context of the evolution of specialization (Via 1990). Theory predicts that polyphagy and hence generalism in herbivorous arthropods will be restricted by fitness trade-offs when attacking distinct host plants (Futuyma and Moreno 1988; Jaenike 1990; Joshi and Thompson 1995) that differ in their physical or chemical defenses, nutrient profiles, phenologies and other traits (Behmer 2009; Simpson et al. 2015). However, even when host plants differ strongly, experimental evolution does not consistently find evidence of theoretically predicted trade-offs. For

instance, Magalhães et al. (2009) did not find any trade-offs across host plants in spider mites that had evolved on distinct hosts for 25 generations.

Studying the evolution of specialization in herbivorous arthropods can strengthen our current understanding of when and why trade-offs evolve. In herbivorous insects, preference of adult females for different hosts and performance of juveniles on those hosts are two important fitness components. Theory shows that both preference (also called habitat choice) and performance are important drivers of the evolution of ecological specialization (Ravigné et al. 2009). Because the evolution of preference can reinforce specialization through habitat sorting, by decreasing migration between environments, preference and performance are expected to be positively correlated (Jaenike 1990). Such a correlation means that females should choose to lay eggs onto hosts that are suitable for their offspring, an idea called the "Mother Knows Best" hypothesis (Thompson 1988; Jaenike 1990). Several experimental studies have found support for this hypothesis in a variety of taxa (Gripenberg et al. 2010). During experimental evolution of performance, preference might hence co-evolve, for example towards higher preference for the fruit a population was reared on, especially when preference and performance are genetically linked (Via and Hawthorne 2002).

Drosophila suzukii Matsumura (Diptera: Drosophilidae), the spotted wing drosophila, represents an excellent biological model to study the potential for specialization and the evolution of trade-offs in a generalist species. It is a generalist vinegar fly that has invaded several regions of the world (Lee et al. 2011). *Drosophila suzukii* uses more than 80 host plant species, including wild (e.g., rose hips and blackberries), ornamental (e.g., *Phytolacca americana*) and crop host plants (e.g., cherries and strawberries, Kenis et al. 2016). Moreover, spotted wing drosophila is a major pest of berries and stone fruits (Asplen et al. 2015). A better understanding the potential adaptations of this species to different hosts is hence of agronomic interest.

127 Here, we evaluated whether experimental evolution on different environments (i.e.,
128 media based on different fruits) could lead to the evolution of trade-offs in a generalist
129 species. We more specifically addressed the following four questions: 1) Does adaptation to
130 contrasting fruits occur ? ; 2) How does adaptation to one fruit influence performance across
131 fruits? ; 3) If fitness trade-offs evolve, how are they influenced by the duration of the
132 experiment or features of the fruit?; and 4) How does selection on performance on a given
133 fruit influence preference for different fruits? We addressed these questions by
134 experimentally evolving populations of *D. suzukii* from a wild population on media made
135 with different fruits. We assessed the evolutionary response of populations from our
136 experimental populations after 5 generations and after 26 generations by measuring their
137 performance in the fruit they were maintained on (hereafter "sympatric fruit") and alternative
138 fruits (herafter "allopatric fruits"), as well as their oviposition preference for those fruits.

139 Methods

140 **Field sampling and laboratory maintenance**

141 *Drosophila suzukii* were collected in the vicinity of Montpellier, France (six sampling sites
142 within 10 km of Montpellier) in October 2016 using baited cup traps (Lee et al. 2013). About
143 1,000 flies were used to initiate a laboratory population. Prior to the start of the experiments,
144 flies were maintained for nine generations on standard laboratory fly food ("German food"
145 formulation consisting of sugar, dry yeast, minerals and antifungal
146 solution;http://flystocks.bio.indiana.edu/Fly_Work/media-recipes/germanfood.htm) at 21°C ±
147 2°C, 65% relative humidity, and 16:8 day/night light cycle in a controlled climate chamber.
148 Experimental evolution and phenotyping were performed under the same temperature,
149 humidity and light conditions. We initially used the neutral (German food) diet to avoid
150 selecting for higher performance on or preference for any given fruit prior to the start of the

151 experiment. The base population was maintained at a population size of approximately 2,000
152 individuals distributed in 100 tubes. Adult flies were allowed to oviposit for approximately
153 24 hours, and 15 days later, the emerging adults were randomly mixed to maintain a
154 panmictic population. Those adults were allowed to mature for six days. To form the
155 subsequent generation, approximately 2,000 mature individuals (approximately 20 per tube)
156 were placed on fresh media. Generations were non-overlapping.

157 **Fruit media**

158 During experimental evolution, flies were reared on artificial fruit media made using eight
159 different fruit purees. We selected a range of fruits that included crops of agronomic interest
160 that are all attacked to varying degrees (blackcurrant, cherry, cranberry, fig, grape,
161 strawberry), crops that are not currently attacked (tomato), and one non-crop species (rose
162 hips). To choose the fruits, we considered their phylogenetic relationships and composition
163 (Fig. S1). We also informed our choice with feedback from local pest management partners
164 (FREDON Languedoc Roussillon, France and CTIFL, France) regarding the importance of
165 *D. suzukii* as an agricultural pest. A preliminary experiment allowed the selection of fruits
166 with similar distributions of fly development times (see Fig. 4 in Olazcuaga et al. 2019). This
167 was necessary because, during experimental evolution, the same generation time was used for
168 all fruits (see below).

169 We used media made with fruit purees rather than whole fruit for two main reasons:
170 purees allowed us to maintain consistent quality of fruits throughout the experiment and to
171 compare fruits that ripen at different times of year directly. All purees were produced by
172 private companies with agro-industrial techniques that preserve the properties of fresh fruits,
173 without added sugar, coloring or preservatives. Purees were held frozen prior to use to
174 maintain their original flavors and colors. Agar, inactive yeast and antifungal were added to

175 produce each fruit medium. See Olazcuaga et al. (2019) for details of the recipe composition
176 and references for each product used to make the experimental fruit media.

177 **Experimental evolution**

178 Experimental populations of flies were established in March 2017 by placing adults from the
179 9th generation after collection from the field, on eight different fruit media (400 adults per
180 fruit population; Fig. 2). No populations were maintained on the neutral media, and a fruit
181 media control was not feasible, because the host plants used by the population sampled in the
182 wild was unknown, as wild adult individuals were collected using traps late in the season
183 when crops were no longer grown in the field. Five replicate populations were established per
184 fruit (40 populations in total). Each population consisted of 20 tubes of 20 flies. For each
185 tube, we put 20 flies that had emerged as adults six days earlier into a tube with a single fruit
186 medium (approximately 10 ml) to mate and oviposit. At this stage of adult development, all
187 adults would be ready to oviposit (Emiljanowicz et al. 2014). The sex ratio of the adults was
188 neither controlled nor measured. After 18 hours, adults were removed. After 15 days, adults
189 of the new generation were counted in every single tube. With this measure, we could keep
190 track of the population size of each population, over the course of the experiment. 400
191 individuals per population were kept to produce the next generation, on a 21 day cycle. Each
192 generation, adults were anesthetized using CO_2 and mixed to produce 20 tubes of 20 flies and
193 were placed on fresh medium. If fewer than 400 individuals emerged, less than 20 tubes were
194 made but always with 20 adults each. Populations were randomly distributed among eight
195 racks (100 tubes per rack, composed of five populations of 20 tubes each) and randomly
196 arranged spatially in a climate chamber. The 40 populations were reared in two temporal
197 blocks separated by two days.

198 After five generations, individuals were phenotyped (see below). Only three fruits -
199 cherry, cranberry, and strawberry - were further maintained, as populations from other fruits

200 went extinct (see Results). The remaining populations on the three fruits were of relatively
201 small size. Therefore, to reduce the potential for inbreeding depression, the separate lines for
202 each of the three fruits were mixed and maintained as three populations of large size (not
203 limited to 400 individuals) for five generations, following the same experimental protocol.
204 These three pooled populations (one for each fruit) were then divided again into separate
205 replicates: three for cherry, five for cranberry and three for strawberry (Fig. 2). The number
206 of replicate populations per fruit depended on the number of individuals available. During
207 this third phase of experimental evolution, each population was maintained at a size of 500
208 individuals (25 tubes of 20 individuals) using the same protocol. Populations were randomly
209 distributed among four racks (75 tubes per rack i.e., three populations of 25 tubes) arranged
210 randomly in the climate chamber. The 11 populations were reared in a single temporal block.
211 16 generations after mixing (thus 26 generations total on each fruit) populations were again
212 phenotyped (see below).

213 **Phenotyping**

214 During our experiment, we phenotyped populations three times - in the 1st, 7th, and 29th
215 generation (Fig. 2; G1, G7, and G29), by measuring the number of eggs laid and the number
216 of adults that emerged in standardized conditions. G7 and G29 respectively experienced five
217 generations and 26 generations on fruit media.

218 Prior to phenotyping, we reared all populations for one generation on neutral German
219 food medium to standardize maternal environmental effects. After this step, we measured the
220 performance and preference of each population on cherry, cranberry and strawberry media.
221 Performance was measured in two ways: emergence rate (the proportion of eggs laid that
222 emerged as adults) and the total number of adults (which integrates larval and adult survival
223 and female fecundity). To do this, we put groups of 20 six-day old adults into tubes
224 containing one of the three fruit media to mate and oviposit for 18 hours. For the initial and

225 intermediate phenotyping steps, adults matured for 6 days on neutral medium and for the
226 final phenotyping step adults matured on the test fruit medium. As during the experimental
227 evolution, sex ratios of the groups of 20 adults were neither controlled nor measured. After
228 adults were removed, all eggs were counted, and fifteen days later, the new adults that
229 emerged were counted. During the initial phenotyping of the base population prior to
230 experimental evolution, we replicated this measure with 100 tubes per treatment over two
231 temporal blocks. During the intermediate phenotyping, we used an average of nine tubes per
232 treatment and per population over six temporal blocks. For the final phenotyping, we used 30
233 tubes per treatment and per population over three temporal blocks.

234 To test for the possibility of that preference evolved in response to experimental
235 evolution of performance, preference was measured during the initial, intermediate and final
236 phenotyping steps in two ways: no-choice and choice conditions. Without a choice, the
237 number of eggs represents how much a given media simulates oviposition. With a choice of
238 different fruits to oviposit onto, the number of eggs on different fruits reflects oviposition
239 preference. No-choice measurements of stimulation of oviposition were done in single tubes,
240 with 20 flies allowed to oviposit for 18 hours, and all eggs were then counted. To measure
241 preference, we created rectangular experimental arenas containing 12 different fruit media
242 (apricot, blackberry, blackcurrant, cherry, cranberry, fig, grape, kiwi, raspberry, rose hips,
243 strawberry, and tomato) distributed randomly into 12 compartments. We allowed groups of
244 20 six-day-old flies (held six-days after emergence as adults on neutral medium) to oviposit
245 for 18 hours in such arenas (Olazcuaga et al. 2019). All eggs laid in each compartments were
246 then counted. The sex ratio of the adults was neither controlled nor measured. The data from
247 this initial phenotyping step, are published in Olazcuaga et al. (2019) and not analyzed here.
248 During the intermediate phenotyping step, we replicated this experiment with an average of 8
249 arenas per population over six temporal blocks. During the final phenotyping step, we

250 replicated this experiment with an average of 15 arenas per population over three temporal
251 blocks.

252 **Statistical analyses**

253 All analyses were performed using the R statistical software (R Core Team 2014).

254 *Adaptation during experimental evolution*

255 To test how adaptation to different fruits evolved over time during experimental evolution,
256 we performed two different analyses on the number of adults. Count (number of adults
257 produced) data were analysed using generalized linear mixed models (GLMM) using the
258 "lme4" package (Bates et al. 2015). The significance of each effect was tested using a model
259 selection approach based on likelihood ratio tests (LRT) with a 5% threshold.

260 First, we analyzed the performance of populations measured on their evolution fruit
261 (the fruit they were maintained on). We did this for all generations (except when populations
262 were pooled) to evaluate trends in performance over time. We analyzed the number of adults
263 y_{ijk} produced, from populations evolved on fruit *i* (i=1,..,3) at generation *j* (j=1,...,29) in each
264 tube *k*. y_{ijk} was assumed to follow a Poisson distribution with parameter λ_{ijk} which was
265 modelled as follows:

266 $$\log(\lambda_{ijk}) = \text{evolution fruit}_i + \text{generation}_j + \text{evolution fruit:generation}_{ij} + \text{obs}_{ijk} \quad (1),$$

267 where fixed effects included the effect of the fruit on which the populations evolved
268 (evolution fruit_i), the effect of generation *j* (generation_j), and the interaction between these
269 terms (evolution $\text{fruit:generation}_{ij}$). To account for overdispersion, we added an observation-
270 specific random effect obs_{ijk}, assumed to be normally distributed with mean zero and a
271 variance σ^2_{obs}.

272 Second, we compared the performance between the initial and the intermediate
273 phenotyping steps and between the initial and the final phenotyping steps. For each pair of

274 comparison, we analyzed the number of adults y_{ijkl} measured on the three test fruits *i*

275 (i=1,..,3), for a population evolved on the fruit *j* (j=1,..,3) at generation *k* (k=1 and 7 or 29 for

276 the first and second comparison, respectively), and in each tube *l*. y_{ijkl} was assumed to follow

277 a Poisson distribution with parameter λ_{ijkl} which was modelled as follows:

278 $\log(\lambda_{ijkl}) =$ test fruit_i + evolution fruit_j + generation_k +

279 test fruit:evolution fruit_{ij} + test fruit:generation_{ik} +

280 generation:evolution fruit_{jk} +

281 test fruit:evolution fruit:generation_{ijk} + obs_{ijkl} (2),

282 where fixed effects included the effect of the test fruit (test fruit_i) the fruit on which the

283 populations evolved (evolution fruit_j), the effect of generation (generation_k) and all the

284 interactions between those terms. To account for overdispersion, we added an observation-

285 specific random effect obs_{ijkl}, assumed to be normally distributed with mean zero and

286 variance σ^2_{obs}.

287 *Detection of trade-offs across fruits*

288 We tested for 'sympatric–allopatric' (SA) contrasts following Blanquart et al. (2013). Briefly,

289 this method compares the performance of populations measured on their host of origin (their

290 sympatric fruit) to that of populations measured on alternative hosts (all allopatric fruits)

291 while controlling for variation in intrinsic quality among environments and for variation in

292 genetic background among populations (e.g., due to differences in inbreeding depression).

293 Detecting local adaptation with this method is equivalent to detecting trade-offs in

294 performance across fruits.

295 For both the intermediate and final phenotyping steps, we analyzed performance y_{ijkl}

296 (log-transformed number of adults produced and emergence rate), measured on the test fruit *i*

297 (i=1,..,3), for a population evolved on fruit *j* (j=1,...,3), in the replicate population *k* (with

k=1,..,14 or k=1,..,11 for the intermediate and final phenotyping steps, respectively), and in each tube *l*. y_{ijkl} was fitted with a linear model as follows:

$$y_{ijkl} = \text{test fruit}_i + \text{population}_k + \text{test fruit:evolution fruit}_{ij} + SA_{ij} + \varepsilon_{ijkl} \quad (3),$$

where fixed effects included the effect of the test fruit (test fruit_i), the effect of the population (population_k) and the fruit on which the populations evolved (evolution fruit_j). SA_{ij} is the sympatric vs. allopatric effect that measures local adaptation. ε_{ijkl} is a random error term assumed to be normally distributed with a mean of zero and variance σ^2_{res}. To test for local adaptation (and hence trade-offs), we used a two-way ANOVA to compute a *F*-test statistics (eq. D1 in Blanquart et al. 2013). Statistical significance was assessed against the expected Fisher-Snedecor distribution with degrees of freedom df1 and df2. The proportion of variance explained by each effect was calculated with the coefficient of determination (R^2) using the "rr2" package (Ives 2019).

The previous test detects local adaptation providing that replicate populations adapt at similar rates. Since each population can follow a distinct evolutionary path, we also tested for local adaptation at the population level. We performed a similar statistical analysis considering the interaction between the replicate population *k* and the test fruit *i* (instead of the interaction between the fruit on which the population evolved *j* and the test fruit *i* as above) using the following model:

$$y_{ijkl} = \text{test fruit}_i + \text{population}_k + \text{test fruit:population}_{ik} + SA_{ij} + \varepsilon_{ijkl} \quad (4)$$

Because the experimental performance data were discrete counts (number of adults emerged), we log-transformed them prior to analysis (Miller 1997). We performed a set of computer simulations mimicking our experimental setup to evaluate the performance of the *F*-test when applied to log-transformed count data. Such simulations are detailed in the Supplementary Appendix S1. They confirm that, at least in our experimental setup (high

322 replicate level and intermediate overdispersion of the count data), the *F*-test proposed by
323 Blanquart et al. (2013) can be applied to log-transformed count data to detect local
324 adaptation.

325 *Heterogeneity of trade-offs among fruits and replicate populations*

326 At the final phenotyping step, we compared mean performance of each population using a
327 unilateral Student's t-test (n=30 tubes). Performance measured on the sympatric fruit was
328 compared to that measured on each of the two allopatric fruits. We controlled for multiple
329 comparisons following Benjamini and Hochberg (1995) adjusting the *p*-values accordingly.
330 A corrected *p*-value < 5% indicates a significantly trade-off.

331 *Influence of fruit composition on trade-offs*

332 We used the method of Blanquart et al. (2013) to evaluate the effects of different compounds
333 in the fruits on the evolution of trade-offs, using data on 18 factors (minerals, nutrient content
334 and fiber) reported in Olazcuaga et al. (2019); see Supp. Table S1). For each factor, we
335 considered a model similar to that described in equation 3, with a term Δ_{ij} replacing SA_{ij}. Δ_{ij}
336 was computed as the squared difference between the concentration of the factor in the test
337 fruit *j* and the evolution fruit *i*. For each factor, a p-value for the Δ_{ij} effect was calculated,
338 adjusting for multiple comparisons as above (Benjamini and Hochberg (1995).

339 *Evolution of oviposition stimulation and oviposition preference*

340 For both the intermediate and the final phenotyping steps, we assessed whether oviposition
341 (stimulation or preference) evolved in concert with the evolution of performance by
342 evaluating whether the number of eggs laid in no-choice or in choice conditions was higher
343 on the sympatric fruit than on the allopatric fruits. Because the experimental preference data
344 were discrete counts (number of eggs laid in choice and no-choice environment), we log-
345 transformed them prior to analysis (Miller 1997). We used a *F*-test as described previously

346 (with eq. 3 for a fruit level investigation or eq. 4 for a population level investigation). For the
347 number of eggs laid in choice environment, we added a random effect of arena ($N(0, \sigma^2_{arena})$).

348 Results

349 **Demographic dynamics of experimental populations**

350 By the fifth generation of experimental evolution, populations on blackcurrant, fig, grape,
351 rose hips or tomato were either extinct or close to extinction, with fewer than 30 individuals
352 (4 to 28) present (Fig. 3). Hence, with our experimental setup, adaptation to these fruits was
353 impossible.

354 Experimental populations persisted over the first five generations on cherry, cranberry
355 and strawberry, but some had relatively small population sizes (Fig. 3; mean number of
356 individuals +/- sd for cherry, cranberry and strawberry respectively: 85.0 +/- 62.0, 89.6 +/-
357 58.5 and 133.8 +/- 134.8). Given these small populations sizes, the five replicate populations
358 on each fruit were pooled after the intermediate phenotyping step to reduce the potential for
359 inbreeding depression. Five generations later, these three pooled populations had grown
360 enough (Fig. S2) to be divided into five, three and three replicate populations for cranberry,
361 cherry and strawberry, respectively (with 500 individuals per replicate population; Fig. 2).
362 We focused on these three remaining fruits in the following sections.

363 **Adaptation during experimental evolution**

364 Each generation, we monitored the number of adults that emerged in each tube (Fig. S3).
365 These data revealed that, on the three focal fruits, population size tended to increase through
366 time (significant interaction between the generation and the evolution fruit: LRT $\chi 2 = 64.41$,
367 df = 2, $P < 0.001$) suggesting adaptation. Adaptation could be in response to a variety of
368 factors including fruit medium as well as laboratory rearing environment.

369 During intermediate phenotyping step, performance was higher than during initial
370 phenotyping, but the increase in performance depended on the evolution fruit (Fig. 4;
371 significant interaction between generation and evolution fruit: LRT $\chi 2 = 13.34$, df = 2, $P =$
372 0.001). Only strawberry populations produced more adults on all three test fruits when
373 compared to the initial phenotyping step ($z = 3.64$, $P < 0.001$, Fig. 4).
374 The final phenotyping revealed that the evolution of performance depended on which
375 fruit the population had evolved on, with a significant three-way interaction between
376 generation, evolution fruit and test fruit (LRT $\chi 2 = 16.92$, df = 4, $P = 0.002$, Fig. 4) that could
377 illustrated local adaptation. For example, for populations evolved on strawberry, performance
378 was higher on strawberry than on cherry (41.64 vs. 26.62 adults, respectively). Similarly, for
379 populations evolved on cherry, performance was higher on cherry than on strawberry (32.28
380 vs. 29.67 adults, respectively).

381 **Evolution of trade-offs across fruits and heterogeneity among populations**

382 During the intermediate phenotyping step, no significant trade-offs between sympatric and
383 allopatric fruits were detected in either the number of adults produced or emergence rate (Fig.
384 4, Table 1). For both traits, the proportion of variance explained by the interaction between
385 test fruit and sympatric fruits was consistently lower than the main population effect,
386 indicating that differences in performance among populations were similar across test fruit
387 (Fig. S4). During final phenotyping step, significant trade-offs were detected in performance
388 when performing a statistical test at the population level ($P = 0.010$ and $P = 0.049$, for the
389 emergence rate and number of adults respectively), but not at the evolution fruit level (Fig. 5,
390 Table 1). Both the number of adults and emergence rates were higher in populations
391 measured on the fruit on which they evolved than when measured on other fruits (Fig. S5).
392 The discrepancy we found, at least for emergence rate, between the fruit level and population
393 level statistical tests indicate that, although local adaptation could not be significantly

394 detected on average over the three fruits, some populations of those fruits show clear
395 hallmarks of trade-offs. Among the 11 populations, seven showed trade-offs in performance
396 in a single allopatric fruit, while four populations showed trade-offs in both allopatric fruits
397 (Fig. 6). Finally, the SA effect and the interaction between test fruit and evolution fruits
398 explained more phenotypic variance during the final than during the intermediate
399 phenotyping steps (Fig. S4).

400 **Influence of fruit composition on trade-offs**

401 The composition of the fruits did not explain differences in performance (Table S1). Fiber,
402 zinc and iron had lower *p*-values than other factors (P = 0.131, 0.159 and 0.166 before
403 correction for multiple comparisons, respectively) and thus are reasonable candidate factors
404 for further exploration of the mechanisms driving trade-offs. In agreement with this, we
405 found that, for these three factors, a bigger difference in concentration between sympatric and
406 allopatric fruits was associated with a decrease in the number of adults (e.g., Fig. S6).

407 **Oviposition stimulation and preference**

408 Neither oviposition stimulation nor preference for the evolution fruit evolved during the
409 experiment (during the final phenotyping: $P > 0.075$; Table 1; Fig. 7). Overall the SA effect
410 explained only a small fraction of phenotypic variation when compared with other effects
411 (Fig. S4).

412 Discussion

413 The main goal of our study was to test whether experimental evolution of a generalist
414 phytophagous species could lead to the evolution of trade-offs in performance across fruits.
415 We found that, at least in a subset of populations, adaptation with trade-offs could evolve
416 after multiple generations of experimental evolution. Trade-offs were not apparent early in

417 the experiment (after the first five generations of evolution). In contrast to predictions
418 regarding joint evolution of preference and performance, the evolution of performance did
419 not drive the evolution of preference for the sympatric fruit.

420 **Can populations develop on and adapt to contrasting fruits?**

421 While multigenerational development was possible on a neutral medium before the
422 experiment, experimental populations of *D. suzukii* on five of the eight fruits had gone extinct
423 (or were likely to in the near future) after five generations on fruit media. This result
424 emphasizes that, even though *D. suzukii* is a generalist, some fruits better support survival
425 than others, at least in our experimental conditions. This contrasts with the widespread view
426 that *D. suzukii* successfully exploits many fruit species. Our findings suggest that wild
427 populations may experience source-sink dynamics (Kawecki 1995) and that the use of at least
428 some hosts in the wild may be transient. Some fruits (such as cherry, cranberry and
429 strawberry) could represent source host plants to which population can adapt, while others
430 fruits (such as blackcurrant, fig, grape, rose hips) could represent sink host plants, where
431 multigenerational survival and adaptation are difficult. Although directly extrapolating our
432 results and conclusions to the dynamics of wild populations remains speculative, our findings
433 emphasize the importance of considering the dynamics of host use at a meta-population scale.
434 Moreover, our results show that differences in both environment quality and genetic quality
435 may explain a substantial portion of the variation in performance among environments (Fig
436 S4). It is thus important to test for local adaptation and trade-offs using a method that
437 accounts for both habitat and population quality effects (Blanquart et al. 2013).

438 **Gradual evolution of specialization**

439 Specialization evolves through gradual steps in our experimental setup. The final
440 phenotyping step, but not the intermediate one, showed that adaptation involves trade-offs
441 among alternative environments, indicating that some populations had become specialized to

442 their fruit medium. Also, the portion of variance in performance explained by the SA effect,
443 was higher during the final phenotyping compared to during the intermediate phenotyping.
444 Our results suggest that after an initial phase where performances increase were general
445 (across all environments), later increases of performance on a given host entail a decrease in
446 performance on alternative hosts. Populations first adapt to factors that are common to all
447 three fruits (Griffin et al. 2017), and then adapt to fruit-specific factors potentially through the
448 mobilization of a set of metabolic functions and their specific associated genes or alleles. The
449 mutational effects associated with this second evolutionary phase can be negatively
450 correlated between environments (i.e., antagonistic pleiotropy), explaining the trade-offs that
451 we observed. These trade-offs could also reflect the accumulation of mutations, which can be
452 neutral in the home environment but deleterious when the gene is expressed in other
453 environments (Kawecki 1994). Our experimental design does not allow us to discriminate
454 between these different root causes or to disentangle the contribution of each of their effects.
455 Addressing this question would require a comparison of the fitness of individuals from
456 crosses between different populations to discriminate the respective role of drift and selection
457 in shaping these trade-offs.

458 **Heterogeneity of adaptation among populations and fruits**

459 Interestingly, we found that populations adapt to the same fruit along different evolutionary
460 trajectories. Specifically, performance trade-offs are only evident at the population level, not
461 the environment level. We expect such a heterogeneity in adaptation patterns among
462 populations based on theory (Garland and Rose 2009). Similar patterns have been observed in
463 experimental evolution studies using *D. melanogaster* (e.g., Clark and Fucito 1998; Rand et
464 al. 2010). Although the populations showed similar increase in fitness, they may have taken
465 different adaptive paths, or they may have diverged through genetic drift in other aspects
466 (Wichman et al. 1999; Heffernan and Wahl 2002). Although our test based on sympatric-

467 allopatric contrasts considers allopatric environments as equivalent, different allopatric
468 environments (different fruits) differ in their chemical composition. We suggest that fiber,
469 zinc and iron be explored as potential candidate factors that might influence the evolution of
470 trade-offs among environments. Subtle variation in host-plant chemistry has been shown to
471 affect herbivores adapted to particular host-plant families (e.g., Huang and Renwick 1993)
472 and host-specific chemical compounds are often involved in the specialization process (Dicke
473 2000; McBride 2007; Linz et al. 2013). For *Drosophila* species, several studies have already
474 shown adaptation to macronutrients during experimental evolution (e.g., Kristensen et al.
475 2011). Our results on *D. suzukii* remain focus on only three fruits, which covers a relatively
476 narrow range of variation in composition, and thus composition may play a larger role in
477 nature than we observe here. We develop a new approach to unravel the role of nutrients
478 potentially responsible for host-plant specialization. This method represents a promising step
479 to link studies focusing on the specialization to whole host plants in natural habitats with
480 those focusing on the experimental evolution of specialization to particular nutrients in the
481 laboratory.

482 **Does evolution of performance influence preference for different fruits?**

483 Evolution of performance to a given host can potentially drive the evolution of preference for
484 the sympatric host (e.g., Agrawal 2000). Theory predicts that females may evolve a
485 preference for laying eggs in the host that maximizes their offspring fitness, an evolutionary
486 feature also known as the "Mother knows best" principle (Thompson 1988; Jaenike 1990).
487 Genetic correlations between adult preference and larval performance might be low in our
488 experiment. This is because selection may take many generations either to increase the
489 preference of females to oviposit on a novel host or to increase the fitness of larvae on this
490 novel host (Wiklund 1975; Chew 1977; Legg et al. 1986; Thompson 1988), specifically in
491 generalist species (Roslin and Salminen 2008; Charlery de la Masselière et al. 2017).

492 Accordingly, experimental evidence for the "Mother knows best" principle has been mixed
493 (Futuyma and Moreno 1988; Poore and Steinberg 1999; Gripenberg et al. 2010). Importantly,
494 selection pressure was on performance only and never involved preference (or any kind of
495 choice) in our experimental setup. The absence of evolution of oviposition preference at the
496 end of our experiment hence suggests that preference and performance are not genetically
497 linked in *D. suzukii*. Moreover, while performance and host choice are expected to feedback
498 positively into each other (Whitlock 1996; Berlocher and Feder 2002; Via and Hawthorne
499 2002), it is often argued that phenotypic plasticity in preference is a stronger driver of
500 specialization than the inverse (Pfennig et al. 2010; Snell-Rood 2013). Dedicated
501 experimental evolution focused on preference could foster our understanding of the
502 relationship between host choice and performance in our biological system and beyond.

503 **Position of a species along a generalist-specialist gradient**

504 The position of a species along the gradient from generalist to specialist depends on several
505 factors: the strength of trade-offs across environments, evolutionary factors such as gene flow
506 across environments and factors such as the frequency and quality of environments
507 (Woolhouse et al. 2001; Jasmin and Kassen 2007; Ravigné et al. 2009). Theory predicts that
508 generalism in herbivorous arthropods could be restricted by trade-offs in fitness among
509 different host plants (Futuyma and Moreno 1988; Jaenike 1990; Joshi and Thompson 1995).
510 Our results show that *D. suzukii* has the genetic diversity to adapt locally and restrict its host
511 range and hence specialize with respect to performance. Our study highlights the importance
512 of carefully employing terms such as generalist. In practice, some species are considered
513 generalists because they emerge from a large range of hosts, or because they are able to use a
514 host for a single generation. However, differences among host plants can result from
515 differences in preference or performance, but also from differences in host plant availability,
516 limited dispersal among host plants, or differences in presence enemies among host plants for

517 instance. Here, we illustrate the importance of multi-generational experiments to evaluate the
518 sustainability of host use. For *D. suzukii*, maintaining populations for multiple generations
519 drastically reduces the range of sustainable hosts. It would be of particular interest to
520 investigate host specialization in wild populations of *D. suzukii* and compare with our results
521 (as done in Fellous et al. 2014 with *Tetranychus urticae*). It is possible that *D. suzukii* might
522 temporarily adapt to host plants, that are available for several generations at a time in the
523 field. This could be tested by evaluating performance on the different hosts available
524 throughout the year. In that respect, four *Drosophila* species have already been followed by
525 'capture-mark-recapture' method to test the effect of resource availability, quantitatively
526 assessed thoroughly in either spatial or temporal terms, on their population biology
527 (Breitmeyer and Markow 1998, see Tait et al. 2018 for an example of population dynamic
528 study using *D. suzukii*).

529 **Potential limits of our experimental setting**

530 For three fruits, we temporary pooled populations evolving on the same fruit to alleviate
531 potential effects of inbreeding depression. The demographic trajectories we observed
532 thereafter suggest evolutionary or genetic rescue (Gomulkiewicz and Holt 1995; Whiteley et
533 al. 2015; Hedrick and Garcia-Dorado 2016). Although the data do fit that interpretation well,
534 our experiment was not set up explicitly to study this phenomenon, and we lack the
535 appropriate experimental controls to confirm or infirm this interpretation. While this pooling
536 step most likely allowed adaptation by reducing inbreeding depression, it also can limited our
537 power to study the adaptive process. The observed trade-offs during the final phenotyping
538 step might have evolved in a single replicate population during the five generations before the
539 pooling step or during the five generations of pooling, instead of several times independently
540 in each replicate populations during the 16 later generations. However, the effect of pooling
541 on reducing inbreeding depression is probably stronger than its effect on increasing the

542 frequency of adaptive mutations. Indeed, the five generations of pooling are rather expected
543 to alleviate the fixation of the numerous deleterious alleles located all over the genome than
544 to favor the spread of beneficial alleles more locally confined in the genome. Finally, we
545 observed substantial phenotypic variance across the different replicate populations at the end
546 of our experiment, which supports the view that most of the adaptive processes occurred
547 during the 16 generations of experimental evolution following the pooling step.

548 **Implications for applied research**

549 The evidence of specialization that we found has important implications for research on wild
550 *D. suzukii* pest populations, which damage cultivated fruits (Walsh et al. 2011). Our results
551 indicate that *D. suzukii* can increase its performance when cycling for several generations on
552 the same host. Damage due to this species may therefore increase if the same fruit is
553 cultivated continuously or at least for long periods of time during the year. This might pose
554 serious threats where fruits are cultivated throughout the year (e.g., strawberry in California,
555 USA). Our results further suggest that monocultural practices might foster the development
556 of better adapted (i.e., more damaging) populations than practices favoring spatial and
557 temporal diversity of cultivated hosts. However, adaptation in the wild might also depend on
558 additional ecological factors (e.g., presence of natural enemies) and intricate evolutionary
559 forces (e.g., migration and selection in a heterogeneous environment) than those studied in
560 the laboratory here. It remains crucial to compare host performance and preference patterns
561 across populations sampled in spatially and temporally diverse agricultural landscapes to
562 ascertain this last point.

563 **Conclusions and perspectives**

564 We found that trade-offs across artificial fruit environments can evolve in the generalist
565 herbivorous species, *D. suzukii*, when it develops on the same fruit over several generations
566 without migration. We show that, at least in some fruits, specialization can evolve. Our study

highlights that some species considered to be generalists because they attack a wide range of hosts can quickly specialize. These results call for great care and justification when calling a species a generalist. Our study shows that several cycles in a single environment can reduce performance in other environments (under restricted migration). It would be of particular interest to assess whether signatures of host specialization signatures are also found in wild populations of *D. suzukii* and whether the level of generalism is modulated by the spatial and temporal distribution of host plants in its natural environment. Another stimulating perspective of the present work lies in the deciphering of the genetic basis and physiological pathways involved in the observed specialization pattern. A genomic comparison of the ancestral and our evolved populations should provide insights into the genetic basis of performance trade-offs and more generally of the rapid adaptation from standing genetic variation and of its dynamics in small populations.

References

Agrawal, A. A. 2000. Host-range evolution : adaptation and trade-offs in fitness of mites on alternative hosts. Ecology 81:500–508.

Angilletta, M. J., R. S. Wilson, C. A. Navas, and R. S. James. 2003. Tradeoffs and the evolution of thermal reaction norms. Trends Ecol. Evol. 18:234–240.

Asplen, M. K., G. Anfora, A. Biondi, D. S. Choi, D. Chu, K. M. Daane, P. Gibert, A. P. Gutierrez, K. A. Hoelmer, W. D. Hutchison, R. Isaacs, Z. L. Jiang, Z. Karpati, M. T. Kimura, M. Pascual, C. R. Philips, C. Plantamp, L. Ponti, G. Vetek, H. Vogt, V. M. Walton, Y. Yu, L. Zappala, and N. Desneux. 2015. Invasion biology of spotted wing Drosophila (*Drosophila suzukii*): a global perspective and future priorities. J. Pest Sci. 88:469–494.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects Models using lme4. J. Stat. Softw. 67:1–48.

Bedhomme, S., G. Lafforgue, and S. F. Elena. 2012. Multihost experimental evolution of a plant RNA virus reveals local adaptation and host-specific mutations. Mol. Biol. Evol. 29:1481–1492.

Behmer, S. T. 2009. Insect herbivore nutrient regulation. Annu. Rev. Entomol. 54:165–187.

Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate - a practical and powerful approach to multiple testing. J. R. Stat. Soc. Ser. B Methodol. 287–300.

Berlocher, S. H., and J. L. Feder. 2002. Sympatric speciation in phytophagous insects: moving beyond controversy? Annu. Rev. Entomol. 47:773–815.

Blanquart, F., O. Kaltz, S. L. Nuismer, and S. Gandon. 2013. A practical guide to measuring local adaptation. Ecol. Lett. 16:1195–1205.

Bolnick, D. I., and R. SvanbÃ. 2003. The ecology of individuals: incidence and implications of individual specialization. Am. Nat. 161:30.

607 Bradshaw, A. D. 1984. Ecological significance of genetic variation between populations.
608 Perspect. Plant Popul. Ecol. Ed. Rodolfo Dirzo Jose Sarukhan.
609 Breitmeyer, C. M., and T. A. Markow. 1998. Resource availability and population size in
610 cactophilic Drosophila. Funct. Ecol. 12:14–21.
611 Charlery de la Masselière, M., B. Facon, A. Hafsi, and P.-F. Duyck. 2017. Diet breadth
612 modulates preference - performance relationships in a phytophagous insect
613 community. Sci. Rep. 7:1–9.
614 Chew, F. S. 1977. Coevolution of pierid butterflies and their cruciferous foodplants. II. The
615 distribution of eggs on potential foodplants. Evolution 31:568–579.
616 Clark, A. G., and C. D. Fucito. 1998. Stress tolerance and metabolic response to stress in
617 *Drosophila melanogaster*. Heredity 81:514–527.
618 Cooper, V. S., and R. E. Lenski. 2000. The population genetics of ecological specialization in
619 evolving Escherichia coli populations. Nature 407:736–739.
620 Devictor, V., J. Clavel, R. Julliard, S. Lavergne, D. Mouillot, W. Thuiller, P. Venail, S.
621 Villéger, and N. Mouquet. 2010. Defining and measuring ecological specialization. J.
622 Appl. Ecol. 47:15–25.
623 Dicke, M. 2000. Chemical ecology of host-plant selection by herbivorous arthropods: a
624 multitrophic perspective. Biochem. Syst. Ecol. 28:601–617.
625 Emiljanowicz, L. M., G. D. Ryan, A. Langille, and J. Newman. 2014. Development,
626 reproductive output and population growth of the fruit fly pest *Drosophila suzukii*
627 (*Diptera: Drosophilidae*) on artificial diet. J. Econ. Entomol. 107:1392–1398.
628 Fellous, S., G. Angot, M. Orsucci, A. Migeon, P. Auger, I. Olivieri, and M. Navajas. 2014.
629 Combining experimental evolution and field population assays to study the evolution
630 of host range breadth. J. Evol. Biol. 27:911–919.
631 Filchak, K. E., J. B. Roethele, and J. L. Feder. 2000. Natural selection and sympatric

632 divergence in the apple maggot *Rhagoletis pomonella*. Nature 407:739–742.
633 Fry, J. D. 1996. The evolution of host specialization: are trade-offs overrated? Am. Nat.
634 148:84–107.
635 Fry, J. D. 1990. Trade-offs in fitness on different hosts: evidence from a selection experiment
636 with a phytophagous mite. Am. Nat. 136:569–580.
637 Fukano, Y., and S. Nakayama. 2018. An experimental test of trade-offs associated with the
638 adaptation to alternate host plants in the introduced herbivorous beetle, *Ophraella*
639 *communa*. J. Insect Behav. 31:490–502.
640 Futuyma, D. J., and G. Moreno. 1988. The evolution of ecological specialization. Annu. Rev.
641 Ecol. Evol. Syst. 19:207–233.
642 Garland, T., and M. R. Rose. 2009. Experimental evolution: concepts, methods, and
643 applications of selection experiments. University of California Press; First edition.
644 Gompert, Z., and F. J. Messina. 2016. Genomic evidence that resource-based trade-offs limit
645 host-range expansion in a seed beetle. Evolution 70:1249–1264.
646 Gomulkiewicz, R., and R. D. Holt. 1995. When does evolution by natural selection prevent
647 extinction? Evolution 49:201–207.
648 Grant, P. R., and B. R. Grant. 1995. Predicting microevolutionary responses to directional
649 selection on heritable variation. Evolution 49:241–251.
650 Griffin, P. C., S. B. Hangartner, A. Fournier-Level, and A. A. Hoffmann. 2017. Genomic
651 trajectories to desiccation resistance: convergence and divergence among replicate
652 selected *Drosophila* lines. Genetics 205:871–890.
653 Gripenberg, S., P. J. Mayhew, M. Parnell, and T. Roslin. 2010. A meta-analysis of
654 preference-performance relationships in phytophagous insects. Ecol. Lett. 13:383–
655 393.
656 Hedrick, P. W., and A. Garcia-Dorado. 2016. Understanding inbreeding depression, purging,

657 and genetic rescue. Trends Ecol. Evol. 31:940–952.
658 Heffernan, J. M., and L. M. Wahl. 2002. The effects of genetic drift in experimental
659 evolution. Theor. Popul. Biol. 62:349–356.
660 Hereford, J. 2009. A quantitative survey of local adaptation and fitness trade-offs. Am. Nat.
661 173:579–588.
662 Holt, R. D. 2009. Bringing the Hutchinsonian niche into the 21st century: ecological and
663 evolutionary perspectives. Proc. Natl. Acad. Sci. 106:19659–19665.
664 Huang, X., and J. a. A. Renwick. 1993. Differential selection of host plants by two *Pieris*
665 species: the role of oviposition stimulants and deterrents. Entomol. Exp. Appl. 68:59–
666 69.
667 Ives, A. R. 2019. R^2 for Correlated Data: Phylogenetic Models, LMMs, and GLMMs. Syst.
668 Biol. 68:234–251.
669 Jaenike, J. 1990. Host specialization in phytophagous insects. Annu. Rev. Ecol. Syst. 21:243–
670 273.
671 Jasmin, J.-N., and R. Kassen. 2007. On the experimental evolution of specialization and
672 diversity in heterogeneous environments. Ecol. Lett. 10:272–281.
673 Joshi, A., and J. N. Thompson. 1995. Trade-offs and the evolution of host specialization.
674 Evol. Ecol. 9:82–92.
675 Kassen, R., and G. Bell. 1998. Experimental evolution in *Chlamydomonas*. IV. Selection in
676 environments that vary through time at different scales. Heredity 80:732–741.
677 Kawecki, T. J. 1994. Accumulation of deleterious mutations and the evolutionary cost of
678 being a generalist. Am. Nat. 144:833–838.
679 Kawecki, T. J. 1995. Demography of source-sink populations and the evolution of ecological
680 niches. Evol. Ecol. 9:38–44.
681 Kawecki, T. J., N. H. Barton, and J. D. Fry. 1997. Mutational collapse of fitness in marginal

682 habitats and the evolution of ecological specialisation. J. Evol. Biol. 10:407–429.
683 Kawecki, T. J., and D. Ebert. 2004. Conceptual issues in local adaptation. Ecol. Lett. 7:1225–
684 1241.
685 Kawecki, T. J., R. E. Lenski, D. Ebert, B. Hollis, I. Olivieri, and M. C. Whitlock. 2012.
686 Experimental evolution. Trends Ecol. Evol. 27:547–560.
687 Kenis, M., L. Tonina, R. Eschen, B. van der Sluis, M. Sancassani, N. Mori, T. Haye, and H.
688 Helsen. 2016. Non-crop plants used as hosts by *Drosophila suzukii* in Europe. J. Pest
689 Sci. 89:735–748.
690 Kristensen, T. N., J. Overgaard, V. Loeschcke, and D. Mayntz. 2011. Dietary protein content
691 affects evolution for body size, body fat and viability in *Drosophila melanogaster*.
692 Biol. Lett. 7:269–272.
693 Laukkanen, L., R. Leimu, A. Muola, M. Lilley, J.-P. Salminen, and P. Mutikainen. 2012.
694 Plant chemistry and local adaptation of a specialized folivore. PLOS ONE 7:e38225.
695 Lee, J. C., D. J. Bruck, A. J. Dreves, C. Ioriatti, H. Vogt, and P. Baufeld. 2011. In Focus:
696 Spotted wing drosophila, *Drosophila suzukii*, across perspectives. Pest Manag. Sci.
697 67:1349–1351.
698 Lee, J. C., P. W. Shearer, L. D. Barrantes, E. H. Beers, H. J. Burrack, D. T. Dalton, A. J.
699 Dreves, L. J. Gut, K. A. Hamby, D. R. Haviland, R. Isaacs, A. L. Nielsen, T.
700 Richardson, C. R. Rodriguez-Saona, C. A. Stanley, D. B. Walsh, V. M. Walton, W. L.
701 Yee, F. G. Zalom, and D. J. Bruck. 2013. Trap designs for monitoring *Drosophila*
702 *suzukii* (*Diptera: Drosophilidae*). Environ. Entomol. 42:1348–1355.
703 Legg, D. E., T. C. Schenk, and H. C. Chiang. 1986. European corn borer (*Lepidoptera:*
704 *Pyralidae*) oviposition preference and survival on sunflower and corn. Environ.
705 Entomol. 15:631–634.
706 Linz, J., A. Baschwitz, A. Strutz, H. K. M. Dweck, S. Sachse, B. S. Hansson, and M. C.

Stensmyr. 2013. Host plant-driven sensory specialization in *Drosophila erecta*. Proc. R. Soc. B Biol. Sci. 280:20130626.

Mackenzie, A. 1996. A trade-off for host plant utilization in the black bean aphid, aphis fabae. Evolution 50:155–162.

Magalhães, S., E. Blanchet, M. Egas, and I. Olivieri. 2009. Are adaptation costs necessary to build up a local adaptation pattern? BMC Evol. Biol. 9:182.

Magalhães, S., and M. Matos. 2012. Strengths and weaknesses of experimental evolution. Trends Ecol. Evol. 27:649–650.

McBride, C. S. 2007. Rapid evolution of smell and taste receptor genes during host specialization in *Drosophila sechellia*. Proc. Natl. Acad. Sci. 104:4996–5001.

Messina, F. J. 1985. Insects on Plants: Community Patterns and Mechanisms. D. R. Strong , J. H. Lawton , Richard South-wood. Q. Rev. Biol. 60:239–239.

Messina, F. J., and S. L. Durham. 2013. Adaptation to a Novel Host by a Seed Beetle (*Coleoptera: Chrysomelidae: Bruchinae*): Effect of Source Population. Environ. Entomol. 42:733–742.

Messina, F. J., and S. L. Durham. 2015. Loss of adaptation following reversion suggests trade-offs in host use by a seed beetle. J. Evol. Biol. 28:1882–1891.

Messina, F. J., M. Mendenhall, and J. C. Jones. 2009. An experimentally induced host shift in a seed beetle. Entomol. Exp. Appl. 132:39–49.

Miller, R. G. 1997. Beyond ANOVA: basics of applied statistics. Chapman and Hall/CRC.

Olazcuaga, L., N. O. Rode, J. Foucaud, B. Facon, V. Ravigné, A. Ausset, N. Leménager, A. Loiseau, M. Gautier, A. Estoup, and R. A. Hufbauer. 2019. Oviposition preference and larval performance of *Drosophila suzukii* (*Diptera*: *Drosophilidae*), spotted-wing drosophila: effects of fruit identity and composition. Environ. Entomol. 48:867–881.

Pfennig, D. W., M. A. Wund, E. C. Snell-Rood, T. Cruickshank, C. D. Schlichting, and A. P.

732 Moczek. 2010. Phenotypic plasticity's impacts on diversification and speciation.
733 Trends Ecol. Evol. 25:459–467.
734 Phillips, B., and R. Shine. 2007. When dinner is dangerous: toxic frogs elicit species-specific
735 responses from a generalist snake predator. Am. Nat. 170:936–942.
736 Poisot, T., J. D. Bever, A. Nemri, P. H. Thrall, and M. E. Hochberg. 2011. A conceptual
737 framework for the evolution of ecological specialisation. Ecol. Lett. 14:841–851.
738 Poore, A. G. B., and P. D. Steinberg. 1999. Preference-performance relationships and effects
739 of host plant choice in an herbivorous marine amphipod. Ecol. Monogr. 69:443–464.
740 R Core Team. 2014. R: A language environment for statistical computing.
741 Rand, D. M., D. M. Weinreich, D. Lerman, D. Folk, and G. W. Gilchrist. 2010. Three
742 selections are better than one: clinal variation of thermal QTL from independent
743 selection experiments in *Drosophila*. Evolution 64:2921–2934.
744 Ravigné, V., U. Dieckmann, and I. Olivieri. 2009. Live where you thrive: joint evolution of
745 habitat choice and local adaptation facilitates specialization and promotes diversity.
746 Am. Nat. 174:E141–E169.
747 Reboud, X., and G. Bell. 1997. Experimental evolution in Chlamydomonas . III. Evolution of
748 specialist and generalist types in environments that vary in space and time. Heredity
749 78:507–514.
750 Roslin, T., and J. P. Salminen. 2008. Specialization pays off: Contrasting effects of two types
751 of tannins on oak specialist and generalist moth species. Oikos 117:1560–1568.
752 Simpson, S. J., F. J. Clissold, M. Lihoreau, F. Ponton, S. M. Wilder, and D. Raubenheimer.
753 2015. Recent advances in the integrative nutrition of arthropods. Annu. Rev. Entomol.
754 60:293–311.
755 Snell-Rood, E. C. 2013. An overview of the evolutionary causes and consequences of
756 behavioural plasticity. Anim. Behav. 85:1004–1011.

757 Tait, G., A. Grassi, F. Pfab, C. M. Crava, D. T. Dalton, R. Magarey, L. Ometto, S. Vezzulli,
758 M. V. Rossi-Stacconi, A. Gottardello, A. Pugliese, G. Firrao, V. M. Walton, and G.
759 Anfora. 2018. Large-scale spatial dynamics of *Drosophila suzukii* in Trentino, Italy. J.
760 Pest Sci. 91:1213–1224.
761 Thompson, J. N. 1988. Evolutionary ecology of the relationship between oviposition
762 preference and performance of offspring in phytophagous insects. Entomol. Exp.
763 Appl. 47:3–14.
764 Travisano, M., and R. E. Lenski. 1996. Long-term experimental evolution in Escherichia coli.
765 IV. Targets of selection and the specificity of adaptation. Genetics 143:15–26.
766 Via, S. 1990. Ecological genetics and host adaptation in herbivorous insects: the experimental
767 study of evolution in natural and agricultural systems. Annu. Rev. Entomol. 35:421–
768 446.
769 Via, S., and D. D. J. Hawthorne. 2002. The genetic architecture of ecological specialization:
770 correlated gene effects on host use and habitat choice in pea aphids. Am. Nat. 159:76–
771 88.
772 Walsh, D. B., M. P. Bolda, R. E. Goodhue, A. J. Dreves, J. C. Lee, D. J. Bruck, V. M.
773 Walton, S. D. O'Neal, and F. G. Zalom. 2011. *Drosophila suzukii* (Diptera:
774 Drosophilidae): invasive pest of ripening soft fruit expanding its geographic range and
775 damage potential. J. Integr. Pest Manag. 2:1–7.
776 Whiteley, A. R., S. W. Fitzpatrick, W. C. Funk, and D. A. Tallmon. 2015. Genetic rescue to
777 the rescue. Trends Ecol. Evol. 30:42–49.
778 Whitlock, M. C. 1996. The red queen beats the jack-of-all-trades: the limitations on the
779 evolution of phenotypic plasticity and niche breadth. Am. Nat. 148.
780 Wichman, H. A., M. R. Badgett, L. A. Scott, C. M. Boulianne, and J. J. Bull. 1999. Different
781 trajectories of parallel evolution during viral adaptation. Science 285:422–424.

Wiklund, C. 1975. The evolutionary relationship between adult oviposition preferences and larval host plant range in Papilio machaon L. Oecologia 18:185–197.

Woolhouse, M. E., L. H. Taylor, and D. T. Haydon. 2001. Population biology of multihost pathogens. Science 292:1109–1112.

Tables

Table 1. Tests for trade-offs in performance (number of adults and emergence rate), oviposition stimulation and oviposition preference. Intermediate phenotyping took place after five generations in each environment, final phenotyping took place after 26 generations. Sympatric fruit indicated the fruit that a population was maintained on. See main text for statistical details.

	Intermediate phenotyping		**Final phenotyping**	
	Evolution fruit	**Population**	**Evolution fruit**	**Population**
Performance: number of adults				
P-value	0.812	0.762	0.268	0.049
F-statistic	0.067	0.094	1.844	4.387
df_1, df_2	1, 3	1, 25	1, 3	1, 19
Performance: emergence rate				
P-value	0.952	0.943	0.087	0.010
F-statistic	0.004	0.005	6.328	8.075
df_1, df_2	1, 3	1, 25	1, 3	1, 19
Oviposition stimulation: number of eggs in no choice environment				
P-value	0.580	0.317	0.927	0.903
F-statistic	0.382	1.043	0.010	0.015
df_1, df_2	1, 3	1, 25	1, 3	1, 19
Oviposition preference: number of eggs in a choice environment				
P-value	0.048	0.074	0.109	0.075
F-statistic	4.391	3.250	2.800	3.242
df_1, df_2	1, 21	1, 131	1, 21	1, 109

Figure legends

Figure 1. Effect of the position of phenotypic optima relative to that of a starting population on the likelihood of evolving specialist genotypes. The relative positions of the phenotypic optima in the two dimensions of the fitness landscape determine whether generalist or specialist genotypes can evolve and whether trade-offs are detected. For each environment, the position of the phenotypic optima with maximum fitness are represented by red or blue dots respectively. The position of ancestral genotypes in the fitness landscape is represented by a grey cross. The adaptive response of the ancestral population to selection for two independent environments (red and blue) is given by the red and blue arrows, respectively, with purple corresponding to the overlap of blue and red arrows. The graphs in the right show the fitness estimates in both environment before and after selection. The crosses represent the fitness of different evolved genotypes assayed in environment 1 and environment 2. The red and blue crosses represent the genotypes evolved on the red and blue environment respectively, with purple corresponding to the overlap of blue and red crosses (see main text for details).

Figure 2. Experimental evolution design depicting the different fruit media and the three phenotyping steps. Before each phenotyping, flies were raised in a common garden (neutral "German food") for one generation, represented by a black line.

Figure 3. Population size over the first five generations of experimental selection of each replicate populations reared on different fruit media showing all five replicate populations per fruit.

822

823 **Figure 4.** Performance during the initial phenotyping step (grey fill), the intermediate
824 phenotyping step (light colored fill) and the final phenotyping step (dark colored fill).
825 The numbers of adults per tube emerged on cherry, cranberry and strawberry (x-axis) were
826 measured for flies evolved on cherry (left), cranberry (middle) and strawberry (right).
827

828 **Figure 5.** Performance of populations during the final phenotyping step measured on one
829 fruit and on another fruit (test fruit), for the populations evolved in one of the two fruits
830 (evolution fruit). Performance is measured as the number of adults produced in a tube (A, B
831 and C) and as the emergence rate (D, E and F). A and D: cherry vs. cranberry, B and E:
832 cranberry vs. strawberry, C and F: strawberry vs. cherry. The dot represents the joint mean
833 with 95% CI error-bars for each population.
834

835 **Figure 6.** Differences between performance on sympatric and allopatric fruits during the final
836 phenotyping step for each population maintained on cherry (pink), cranberry (yellow) and
837 strawberry (green). The shape of the symbol indicates the test fruit: cherry (circle), cranberry
838 (triangle) and strawberry (square). For each of the 11 populations, the difference in
839 performance between a given pair of sympatric and allopatric fruits was calculated as the
840 performance on the sympatric fruit minus the performance on the allopatric fruit. Filled
841 colored symbols indicate that the performance of the population is significantly higher on its
842 sympatric fruit (after applying a multiple test correction) and hence that a trade-off occurs in
843 this population.
844

845 **Figure 7.** Proportions of eggs laid on cherry (red), cranberry (yellow) and strawberry (green)
846 in a choice environment experiment including 12 fruits for populations evolved on strawberry

(top), cherry (middle), and cranberry (bottom). The proportions are given for the initial (left), intermediate (middle) and final (right) phenotyping steps. The ranking of fruit in the pie is always the same: apricot, blackberry, blackcurrant, cherry (pink), cranberry (yellow), fig, grape, kiwi, raspberry, rose hips, strawberry (green), tomato (alphabetical order). The oviposition preference of the ancestral population and measured during the initial phenotyping are from Olazcuaga et al. 2019.

Figures

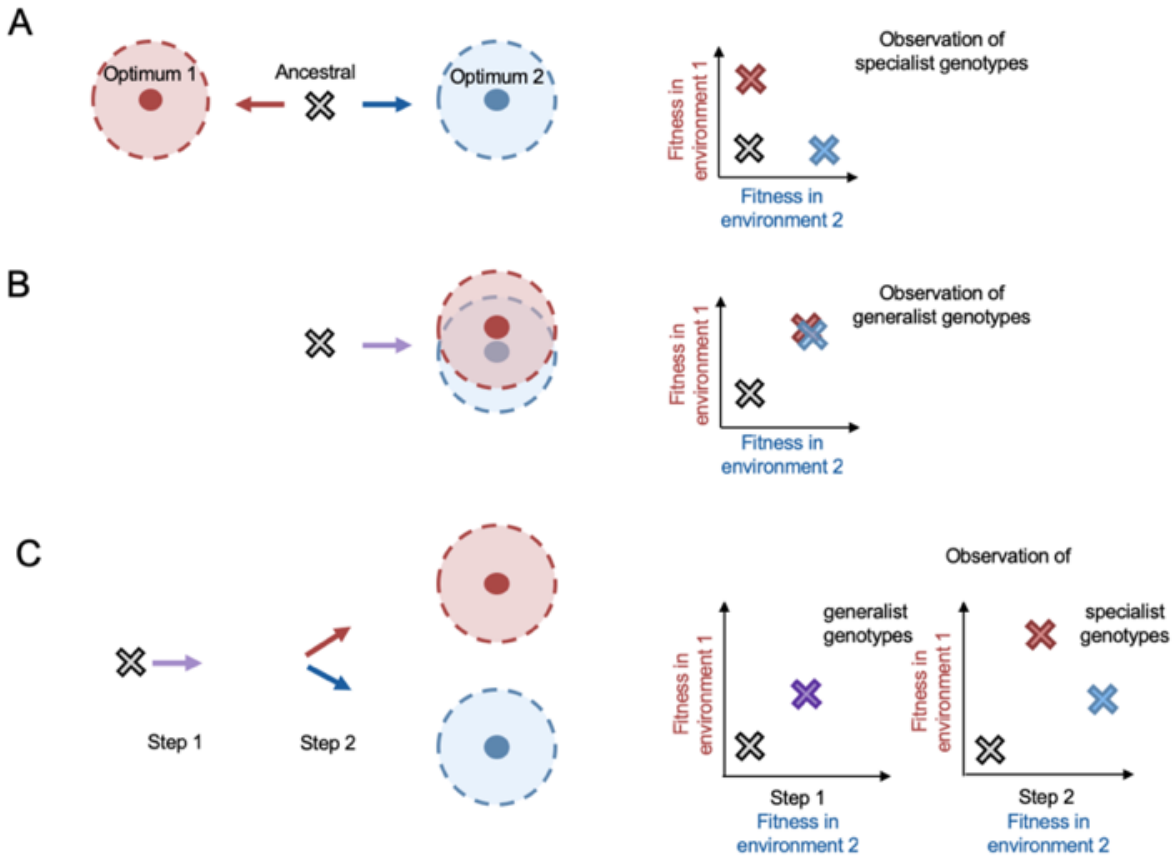


Figure 1.

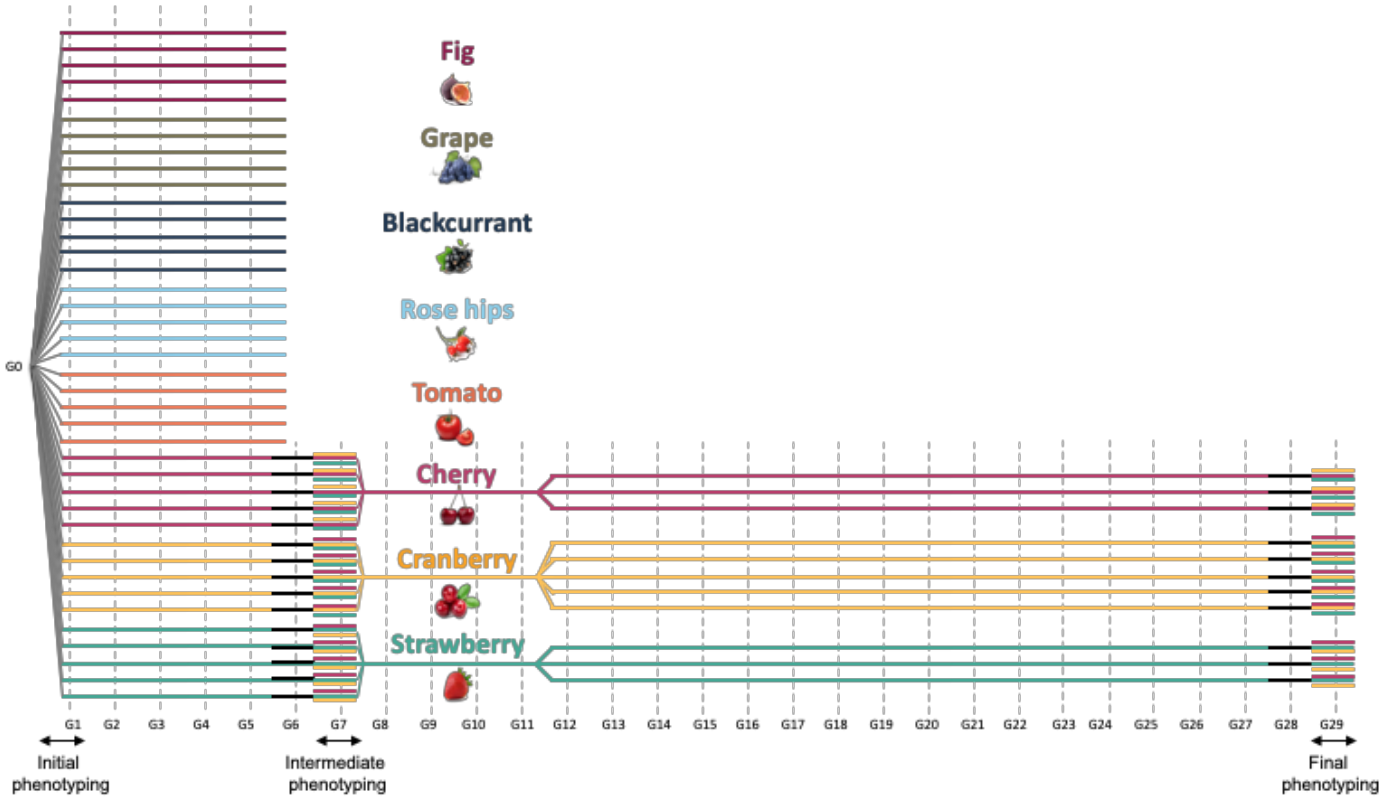


Fig
Grape
Blackcurrant
Rose hips
Tomato
Cherry
Cranberry
Strawberry
G0
G1 G2 G3 G4 G5 G6 G7 G8 G9 G10 G11 G12 G13 G14 G15 G16 G17 G18 G19 G20 G21 G22 G23 G24 G25 G26 G27 G28 G29
Initial phenotyping
Intermediate phenotyping
Final phenotyping

Figure 2.

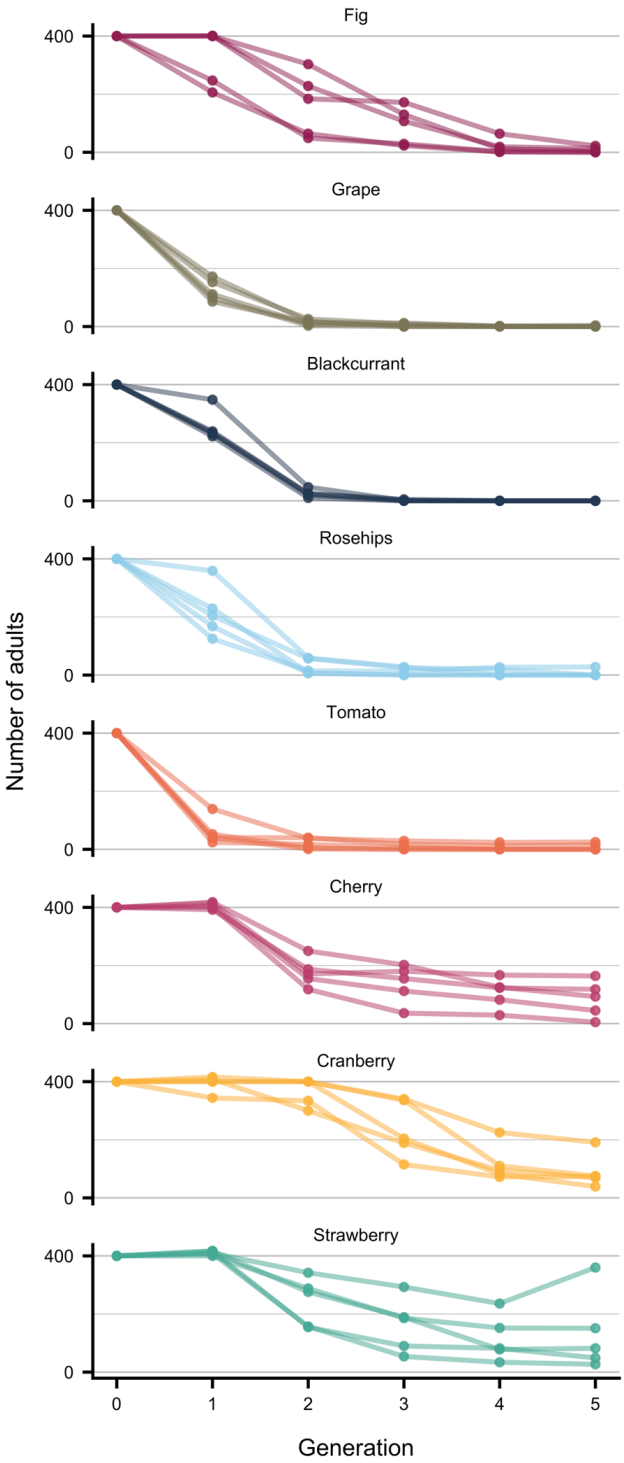


Fig
Grape
Blackcurrant
Rosehips
Tomato
Cherry
Cranberry
Strawberry
400
0
Number of adults
0
1
2
3
4
5
Generation

Figure 3.

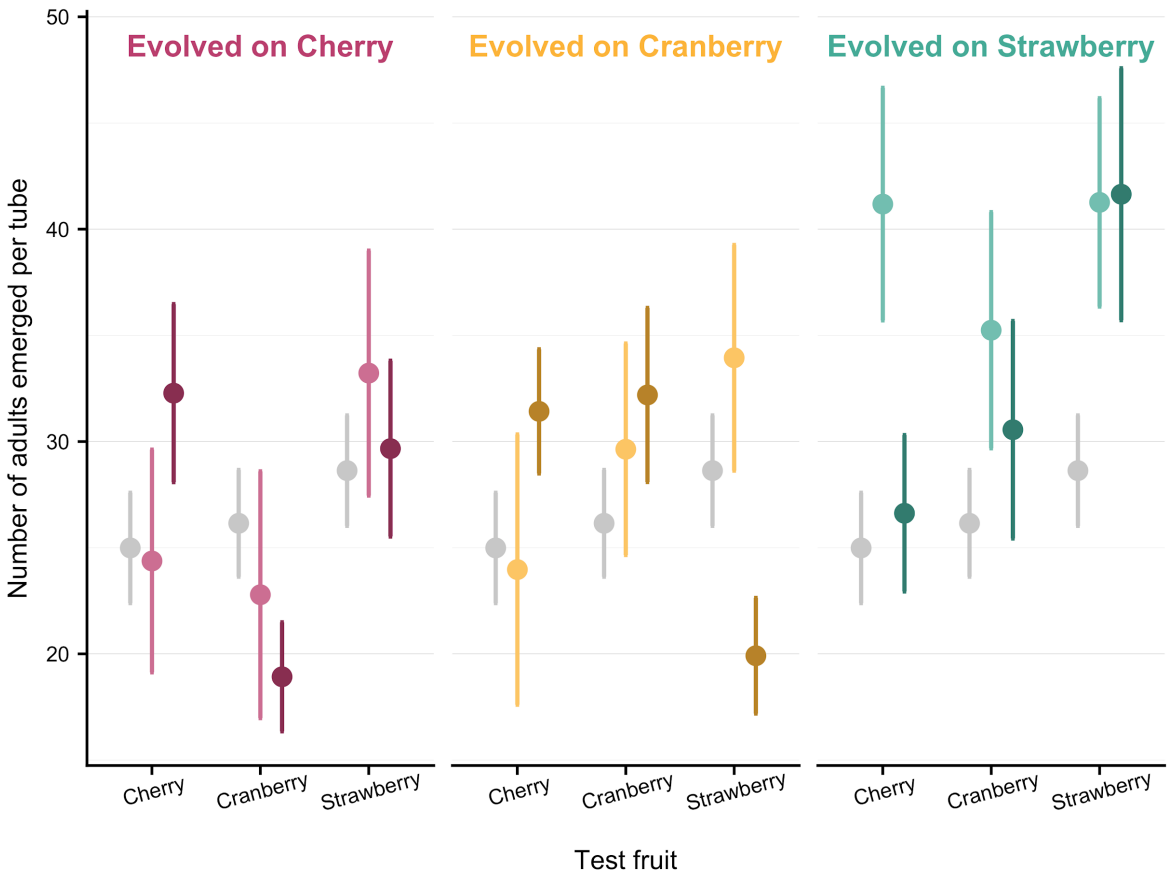

Evolved on Cherry
Evolved on Cranberry
Evolved on Strawberry
Number of adults emerged per tube
50
40
30
20
Cherry
Cranberry
Strawberry
Cherry
Cranberry
Strawberry
Cherry
Cranberry
Strawberry
Test fruit

Figure 4.

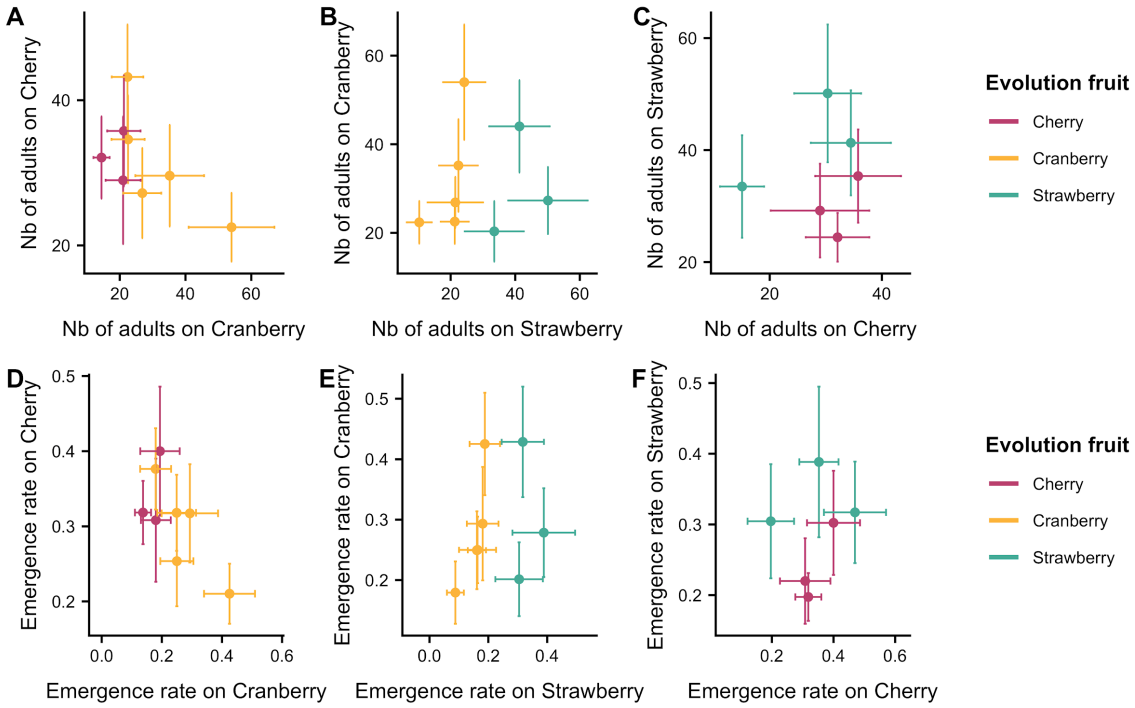
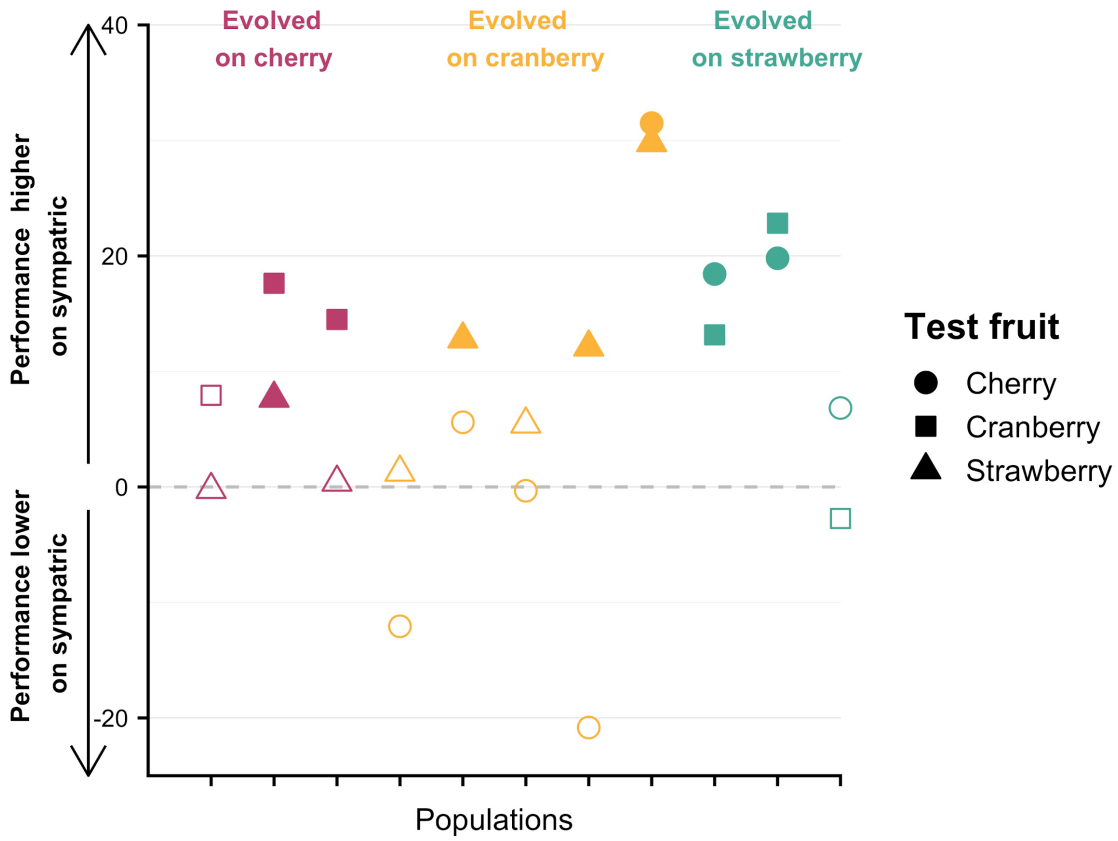

A
Nb of adults on Cherry
Nb of adults on Cranberry
B
Nb of adults on Cranberry
Nb of adults on Strawberry
C
Nb of adults on Strawberry
Nb of adults on Cherry
Evolution fruit
Cherry
Cranberry
Strawberry
D
Emergence rate on Cherry
Emergence rate on Cranberry
E
Emergence rate on Cranberry
Emergence rate on Strawberry
F
Emergence rate on Strawberry
Emergence rate on Cherry
Evolution fruit
Cherry
Cranberry
Strawberry

Figure 5.

887



888

889

890 **Figure 6.**

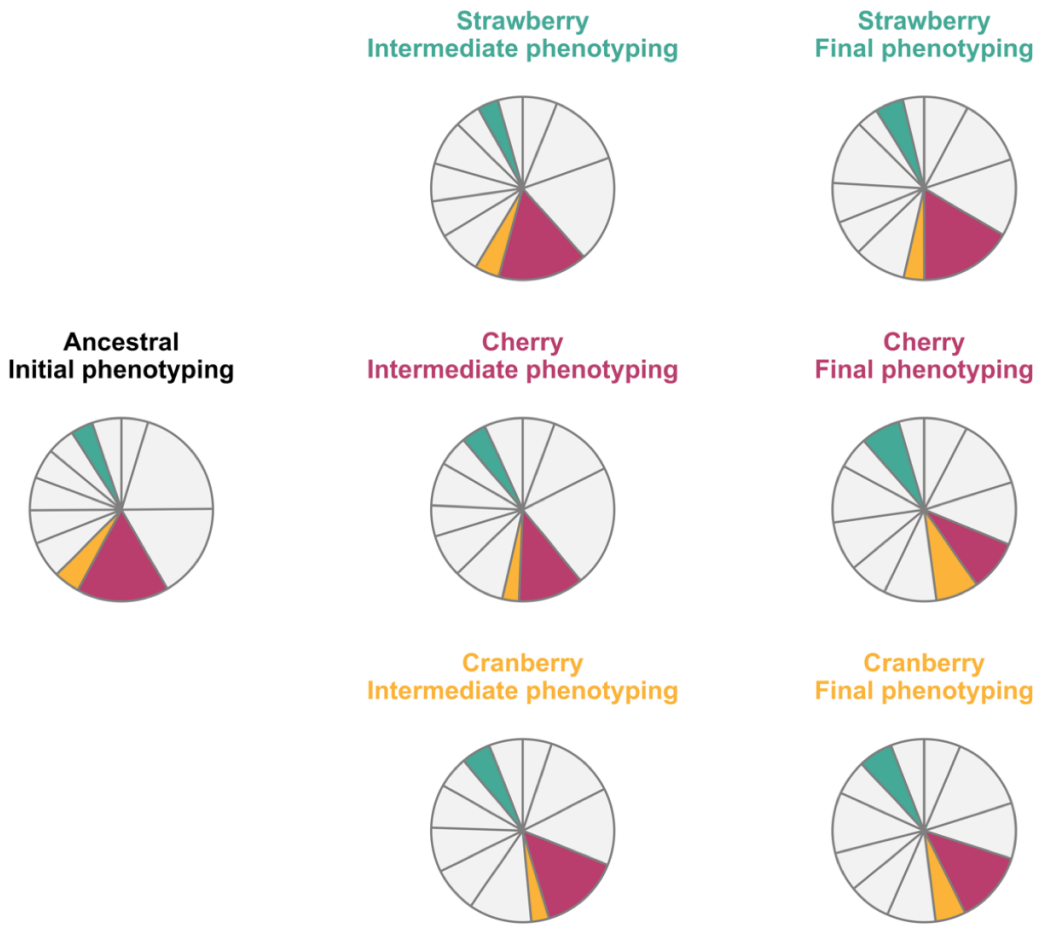

Strawberry
Intermediate phenotyping
Strawberry
Final phenotyping
Ancestral
Initial phenotyping
Cherry
Intermediate phenotyping
Cherry
Final phenotyping
Cranberry
Intermediate phenotyping
Cranberry
Final phenotyping

Figure 7.