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

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50 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.

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Key words

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

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Introduction

28

29 Ecological specialization is the process of adaptation to a restricted spectrum of environments

30 originally used by a given species (reviewed in Bolnick and Svanbà 2003; Holt 2009;

31 Ravigné et al. 2009; Devictor et al. 2010; Poisot et al. 2011). This process is widely

32 recognized as a major determinant of the emergence and maintenance of biodiversity

33 (Futuyma and Moreno 1988). It can be the first step in the evolution of host races and even

34 speciation (e.g., Filchak et al. 2000). The process of ecological specialization leads to a

35 pattern of increased fitness in a local environment and decreased fitness in other

36 environments (Futuyma and Moreno 1988). When distinct trait values are optimal in different

37 environments (for example, the ability of plants to tolerate salts or metals in the soil), fitness

38 trade-offs across environments can emerge. Trade-offs can be measured phenotypically as a

39 decrease in fitness in an environment associated with an increase in fitness in another

40 environment (Angilletta et al. 2003). Genetically, adaptation to a subset of environments (i.e.,

41 specialization) is due to an increase in the presence or frequency of alleles favorable in those

42 environments. Phenoptypic trade-offs appear when such alleles are either neutral or

43 deleterious with respect to fitness in other environments (antagonistic pleiotropy ; Fry 1996).

44 Phenothypic trade-offs also arise when alleles that are neutral in the focal environment (and

45 thus not selected against and can accumulate over time) are deleterious in other environments

46 (Kawecki et al. 1997).

47 Darwin's finches provide one of the most famous illustrations of trade-offs leading to

48 ecological specialization (Grant and Grant 1995). Species or populations with small beaks

49 can open small seeds but not large seeds and reciprocally for species or populations with

50 large beaks. Environmental changes in the size and toughness of the seeds available results in

51 the evolution of both beak shape and beak size in these finches. While the inability of a bird

52 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).

78   Trade-offs are likely to evolve and be detected when populations adapt to environments with

79   distinct phenotypic optima (Travisano and Lenski 1996; Cooper and Lenski 2000; Fig. 1A).

80   Using this reasoning, trade-offs are unlikely to evolve when the environments are similar

81   (Fig. 1B). For example, in the context of plant-insect interactions, the host plant determine

82   the environment to which the herbivorous insects must adapt. If plant species share secondary

83   (defensive) compounds, they may not represent substantially different selective environments

84   (Agrawal 2000; Fukano and Nakayama 2018). Even when the environments to which

85   populations are adapting differ, they might differ in similar ways from the ancestral

86   environment, so that organisms adapting to one of the environments will also adapt to the

87   other environment, increasing their fitness in both environments (Fig. 1C; Reboud and Bell

88   1997; Kassen and Bell 1998). For both these scenarios (Figs. 1B & 1C), trade-offs can

89   ultimately evolve over multiple generations as mutations accumulate that are neutral in the

90   home environment but deleterious elsewhere. Other more technical reasons may also explain

91   the failure to detect trade-offs, including low statistical power and measurement of traits not

92   involved in trade-offs (Phillips and Shine 2007). Finally, the multidimensionality of trade-

93   offs makes them inherently difficult to measure (Poisot et al. 2011).

94   Herbivorous arthropods represent one of the most diverse taxa on Earth and many are

95   important pests (Messina 1985). They are therefore often studied in the context of the

96   evolution of specialization (Via 1990). Theory predicts that polyphagy and hence generalism

97   in herbivorous arthropods will be restricted by fitness trade-offs when attacking distinct host

98   plants (Futuyma and Moreno 1988; Jaenike 1990; Joshi and Thompson 1995) that differ in

99   their physical or chemical defenses, nutrient profiles, phenologies and other traits (Behmer

100   2009; Simpson et al. 2015). However, even when host plants differ strongly, experimental

101   evolution does not consistently find evidence of theoretically predicted trade-offs. For

102   instance, Magalhães et al. (2009) did not find any trade-offs across host plants in spider mites

103   that had evolved on distinct hosts for 25 generations.

104   Studying the evolution of specialization in herbivorous arthropods can strengthen our

105   current understanding of when and why trade-offs evolve. In herbivorous insects, preference

106   of adult females for different hosts and performance of juveniles on those hosts are two

107   important fitness components. Theory shows that both preference (also called habitat choice)

108   and performance are important drivers of the evolution of ecological specialization (Ravigné

109   et al. 2009). Because the evolution of preference can reinforce specialization through habitat

110   sorting, by decreasing migration between environments, preference and performance are

111   expected to be positively correlated (Jaenike 1990). Such a correlation means that females

112   should choose to lay eggs onto hosts that are suitable for their offspring, an idea called the

113   "Mother Knows Best" hypothesis (Thompson 1988; Jaenike 1990). Several experimental

114   studies have found support for this hypothesis in a variety of taxa (Gripenberg et al. 2010).

115   During experimental evolution of performance, preference might hence co-evolve, for

116   example towards higher preference for the fruit a population was reared on, especially when

117   preference and performance are genetically linked (Via and Hawthorne 2002).

118   *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), the spotted wing drosophila,

119   represents an excellent biological model to study the potential for specialization and the

120   evolution of trade-offs in a generalist species. It is a generalist vinegar fly that has invaded

121   several regions of the world (Lee et al. 2011). *Drosophila suzukii* uses more than 80 host

122   plant species, including wild (e.g., rose hips and blackberries), ornamental (e.g., *Phytolacca*

123   *americana*) and crop host plants (e.g., cherries and strawberries, Kenis et al. 2016).

124   Moreover, spotted wing drosophila is a major pest of berries and stone fruits (Asplen et al.

125   2015). A better understanding the potential adaptations of this species to different hosts is

126   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.

Methods

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Field sampling and laboratory maintenance

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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.

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.

Statistical analyses

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

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

298    $k=1,..,14$ or $k=1,..,11$ for the intermediate and final phenotyping steps, respectively), and in

299   each tube l. y_{ijkl} was fitted with a linear model as follows:

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

301   where fixed effects included the effect of the test fruit (test fruit$_i$), the effect of the population

302   (population$_k$) and the fruit on which the populations evolved (evolution fruit$_j$). SA_{ij} is the

303   sympatric vs. allopatric effect that measures local adaptation. ε_{ijkl} is a random error term

304   assumed to be normally distributed with a mean of zero and variance σ^2_{res}. To test for local

305   adaptation (and hence trade-offs), we used a two-way ANOVA to compute a F-test statistics

306   (eq. D1 in Blanquart et al. 2013). Statistical significance was assessed against the expected

307   Fisher-Snedecor distribution with degrees of freedom df1 and df2. The proportion of variance

308   explained by each effect was calculated with the coefficient of determination (R^2) using the

309   "rr2" package (Ives 2019).

310   The previous test detects local adaptation providing that replicate populations adapt at

311   similar rates. Since each population can follow a distinct evolutionary path, we also tested for

312   local adaptation at the population level. We performed a similar statistical analysis

313   considering the interaction between the replicate population k and the test fruit i (instead of

314   the interaction between the fruit on which the population evolved j and the test fruit i as

315   above) using the following model:

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

317   Because the experimental performance data were discrete counts (number of adults

318   emerged), we log-transformed them prior to analysis (Miller 1997). We performed a set of

319   computer simulations mimicking our experimental setup to evaluate the performance of the

320   F-test when applied to log-transformed count data. Such simulations are detailed in the

321   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 ($N(0, \sigma^2_{arena})$).

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 $\chi2 = 64.41$,

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 $\chi2 = 13.34$, 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 $\chi2 = 16.92$, 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).

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.

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).

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.

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-

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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

567   highlights that some species considered to be generalists because they attack a wide range of

568   hosts can quickly specialize. These results call for great care and justification when calling a

569   species a generalist. Our study shows that several cycles in a single environment can reduce

570   performance in other environments (under restricted migration). It would be of particular

571   interest to assess whether signatures of host specialization signatures are also found in wild

572   populations of *D. suzukii* and whether the level of generalism is modulated by the spatial and

573   temporal distribution of host plants in its natural environment. Another stimulating

574   perspective of the present work lies in the deciphering of the genetic basis and physiological

575   pathways involved in the observed specialization pattern. A genomic comparison of the

576   ancestral and our evolved populations should provide insights into the genetic basis of

577   performance trade-offs and more generally of the rapid adaptation from standing genetic

578   variation and of its dynamics in small populations.

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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

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Figure legends

799

800 **Figure 1.** Effect of the position of phenotypic optima relative to that of a starting population

801 on the likelihood of evolving specialist genotypes. The relative positions of the phenotypic

802 optima in the two dimensions of the fitness landscape determine whether generalist or

803 specialist genotypes can evolve and whether trade-offs are detected. For each environment,

804 the position of the phenotypic optima with maximum fitness are represented by red or blue

805 dots respectively. The position of ancestral genotypes in the fitness landscape is represented

806 by a grey cross. The adaptive response of the ancestral population to selection for two

807 independent environments (red and blue) is given by the red and blue arrows, respectively,

808 with purple corresponding to the overlap of blue and red arrows. The graphs in the right show

809 the fitness estimates in both environment before and after selection. The crosses represent the

810 fitness of different evolved genotypes assayed in environment 1 and environment 2. The red

811 and blue crosses represent the genotypes evolved on the red and blue environment

812 respectively, with purple corresponding to the overlap of blue and red crosses (see main text

813 for details).

814

815 **Figure 2.** Experimental evolution design depicting the different fruit media and the three

816 phenotyping steps. Before each phenotyping, flies were raised in a common garden (neutral

817 "German food") for one generation, represented by a black line.

818

819 **Figure 3.** Population size over the first five generations of experimental selection of each

820 replicate populations reared on different fruit media showing all five replicate populations per

821 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

847   (top), cherry (middle), and cranberry (bottom). The proportions are given for the initial (left),

848   intermediate (middle) and final (right) phenotyping steps. The ranking of fruit in the pie is

849   always the same: apricot, blackberry, blackcurrant, cherry (pink), cranberry (yellow), fig,

850   grape, kiwi, raspberry, rose hips, strawberry (green), tomato (alphabetical order). The

851   oviposition preference of the ancestral population and measured during the initial

852   phenotyping are from Olazcuaga et al. 2019.
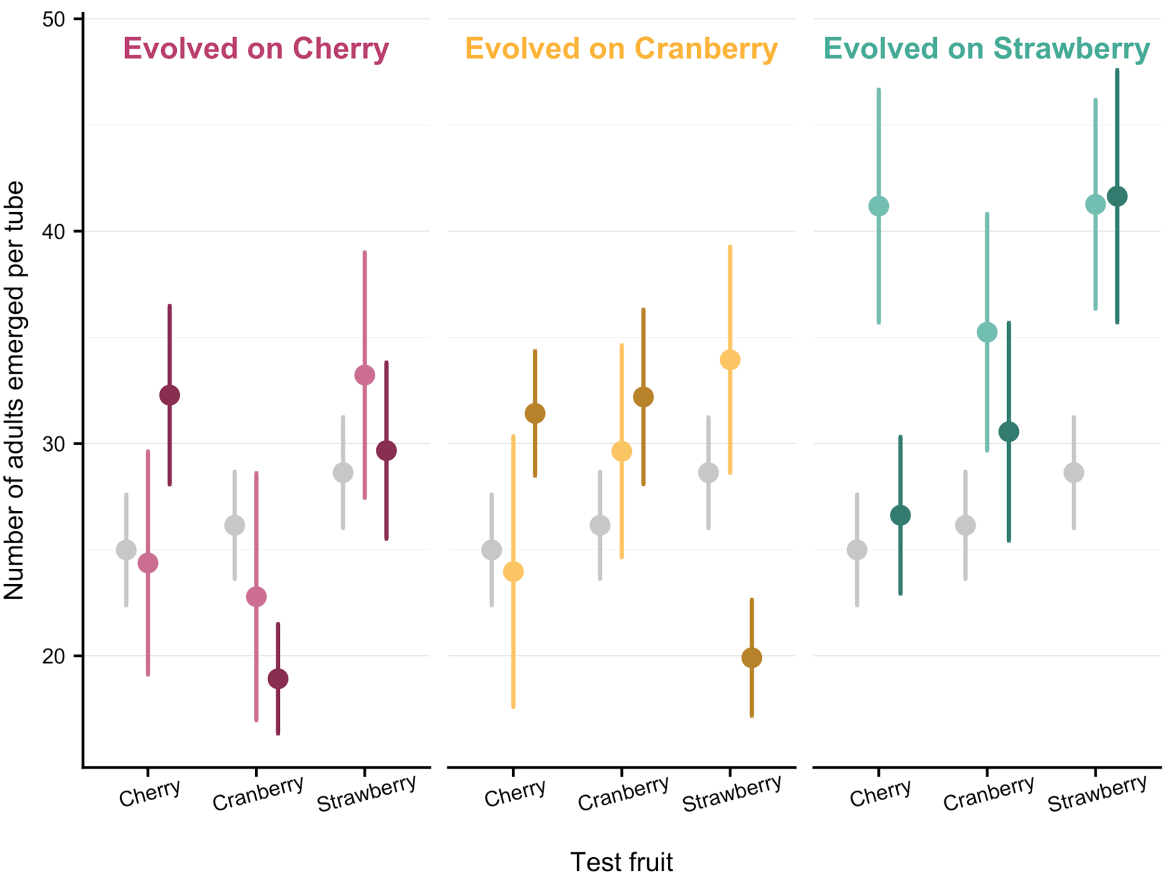
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Figures

Figure 1.

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864 **Figure 2.**

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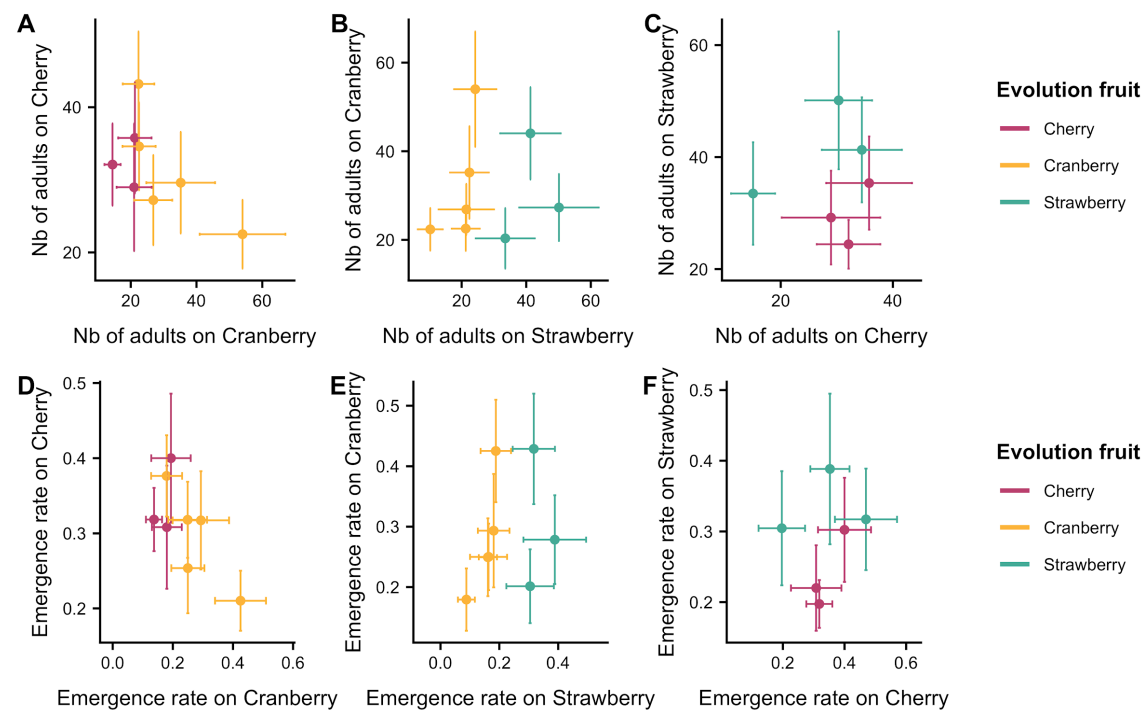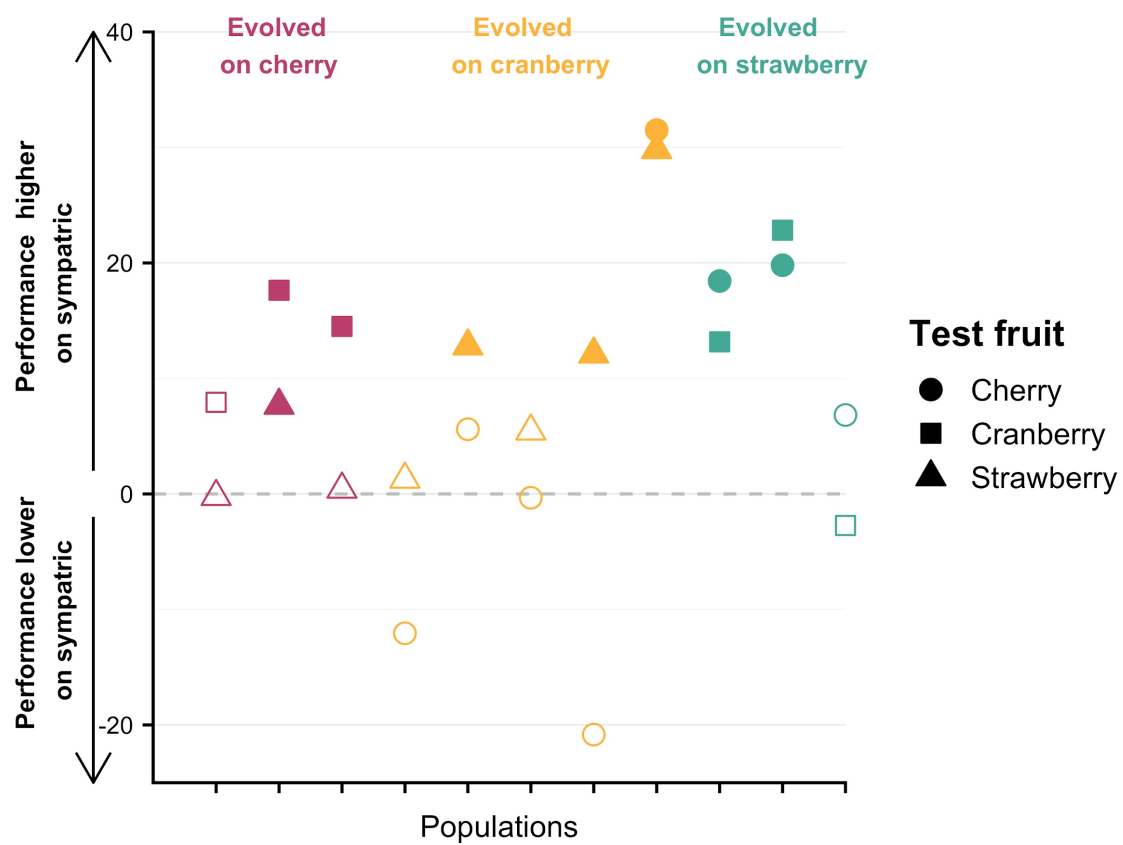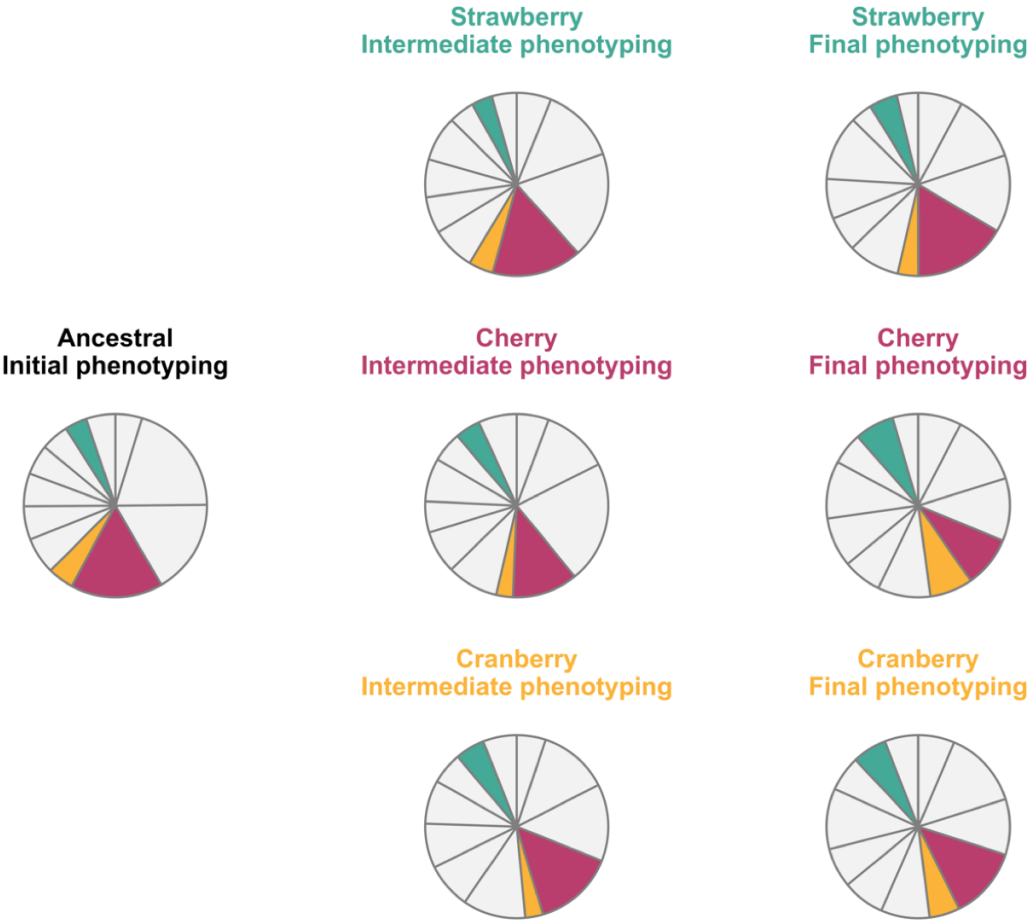875

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Figure 5.

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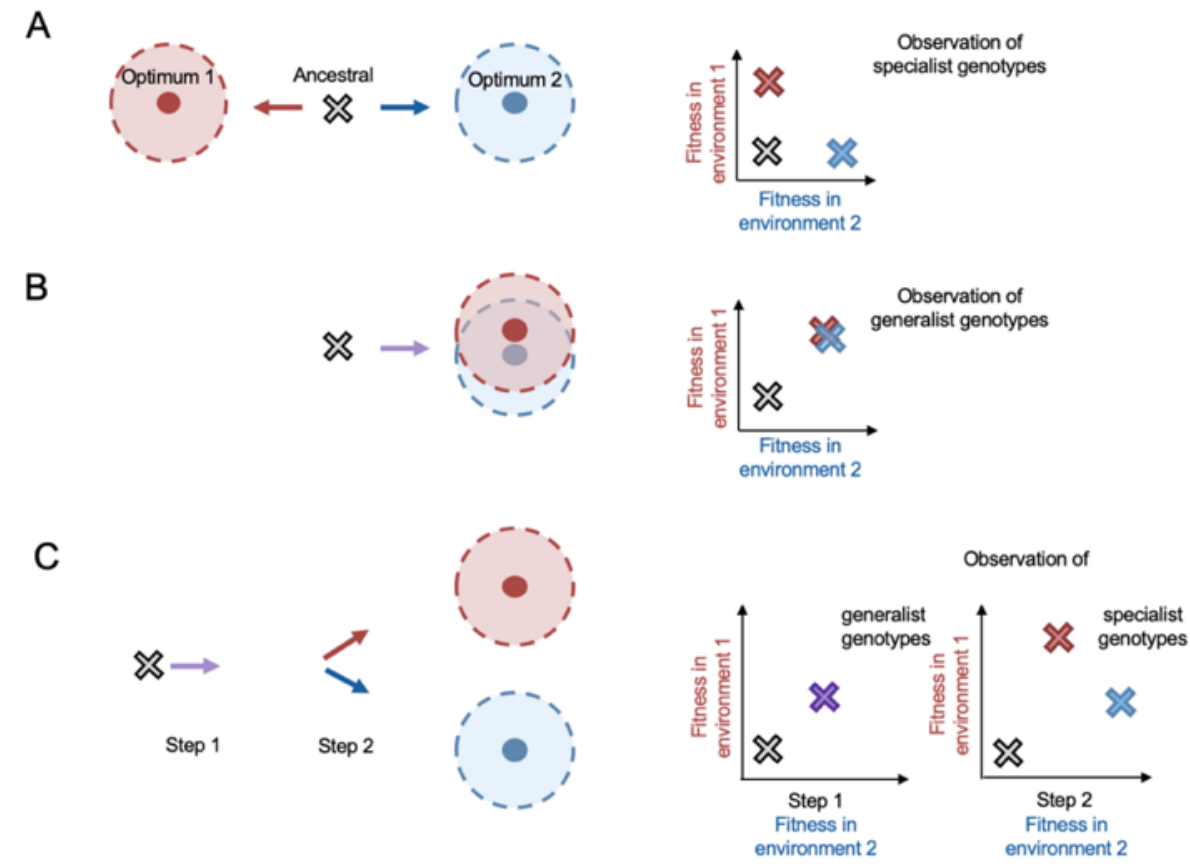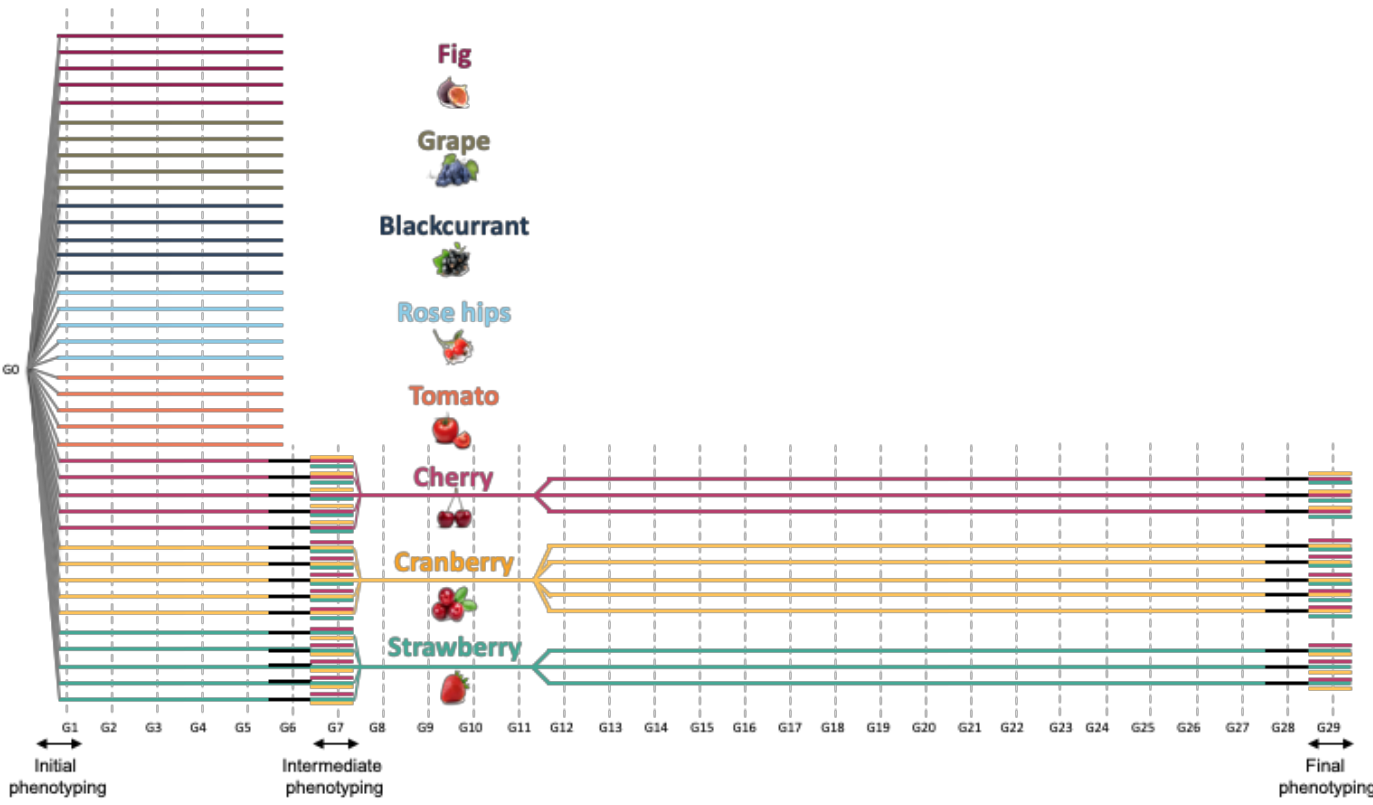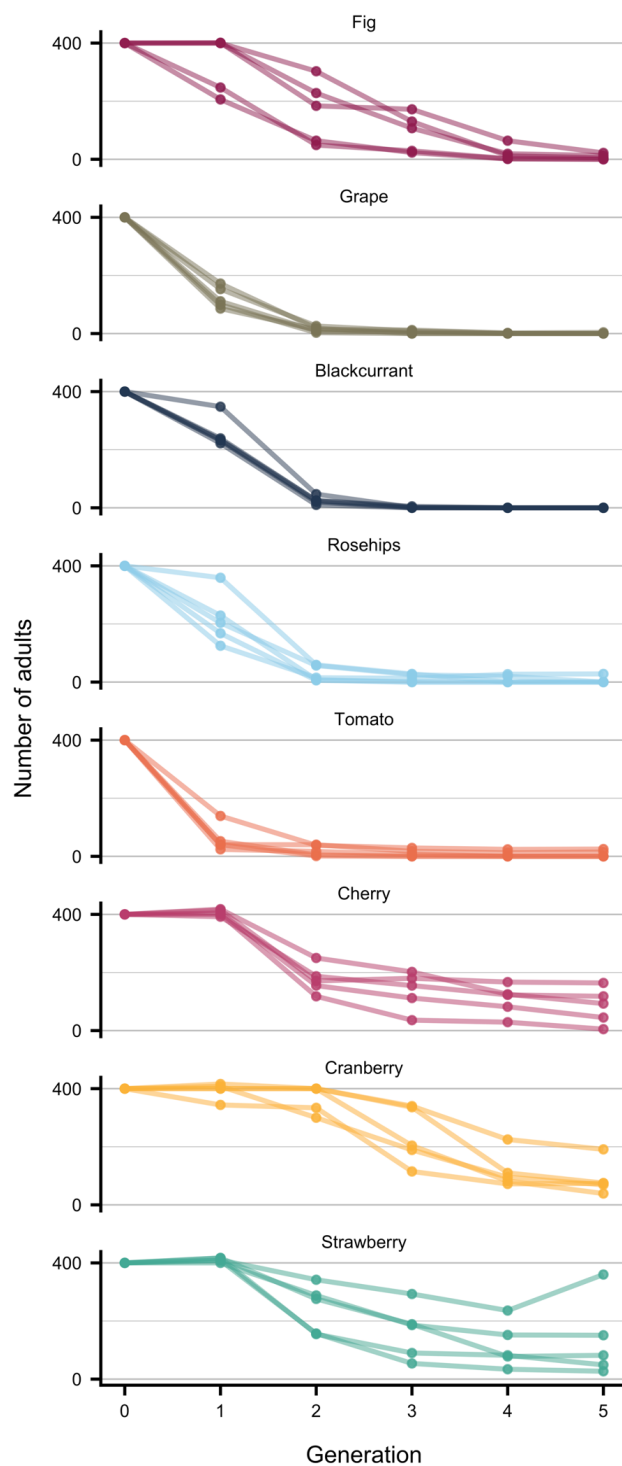
890 **Figure 6.**

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893   **Figure 7.**

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