

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.

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25 **Key words**

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

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Introduction

Ecological specialization is the process of adaptation to a restricted spectrum of environments originally used by a given species (reviewed in Bolnick and Svanbäck 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. Phenotypic trade-offs appear when such alleles are either neutral or deleterious with respect to fitness in other environments (antagonistic pleiotropy ; Fry 1996). Phenotypic 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

in general (Fry 1996). This challenge has been addressed by performing reciprocal transplant experiments of natural or experimentally evolved populations. In natural populations, reciprocal transplant experiments offer a direct way of revealing the presence of fitness trade-offs and patterns of local adaptation (Bradshaw 1984; Kawecki and Ebert 2004). In such experiments, the fitness of organisms is measured in their evolved environment (also called natal, local, sympatric, source or home environment) and in one or more alternative environments (also called novel, away or allopatric environments; Bradshaw 1984). Reciprocal home site advantage indicates that adaptation to one environment involves performance trade-offs with other environments (Kawecki and Ebert 2004), or that alleles favorable in one environment are simply neutral in other environments (Fry 1996). An important question in evolutionary biology is to know whether trade-offs lead to specialization or whether the evolution of specialization leads to the evolution of trade-offs. The order of such processes is difficult if not impossible to discern through the direct study of natural populations (Kawecki et al. 2012; Magalhães and Matos 2012). Experimental evolution aids in establishing the order and causality of evolutionary processes such as the evolution of organisms in response to selection pressures induced by an environment over time (Magalhães and Matos 2012).

Although fitness trade-offs across environments are expected predicted by theory (Futuyma and Moreno 1988; though see Fry 1996), experimental evidence for their evolution in experimental populations is mixed (Hereford 2009). Experimental evolution of organisms ranging from bacteria, viruses, insects and plants in different abiotic and biotic environments have found trade-offs in some cases (Fry 1990; Mackenzie 1996; Bedhomme et al. 2012; Messina and Durham 2015; Gompert and Messina 2016), but not others (Magalhães et al. 2009; Messina et al. 2009; Laukkanen et al. 2012; Messina and Durham 2013). The lack of 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.

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

Methods

Field sampling and laboratory maintenance

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

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

Fruit media

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

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

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

Experimental evolution

Experimental populations of flies were established in March 2017 by placing adults from the 9th generation after collection from the field, on eight different fruit media (400 adults per fruit population; Fig. 2). No populations were maintained on the neutral media, and a fruit media control was not feasible, because the host plants used by the population sampled in the wild was unknown, as wild adult individuals were collected using traps late in the season when crops were no longer grown in the field. Five replicate populations were established per fruit (40 populations in total). Each population consisted of 20 tubes of 20 flies. For each tube, we put 20 flies that had emerged as adults six days earlier into a tube with a single fruit medium (approximately 10 ml) to mate and oviposit. At this stage of adult development, all adults would be ready to oviposit (Emiljanowicz et al. 2014). The sex ratio of the adults was neither controlled nor measured. After 18 hours, adults were removed. After 15 days, adults of the new generation were counted in every single tube. With this measure, we could keep track of the population size of each population, over the course of the experiment. 400 individuals per population were kept to produce the next generation, on a 21 day cycle. Each generation, adults were anesthetized using CO₂ and mixed to produce 20 tubes of 20 flies and were placed on fresh medium. If fewer than 400 individuals emerged, less than 20 tubes were made but always with 20 adults each. Populations were randomly distributed among eight racks (100 tubes per rack, composed of five populations of 20 tubes each) and randomly arranged spatially in a climate chamber. The 40 populations were reared in two temporal blocks separated by two days.

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

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

Phenotyping

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

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

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

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

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

Statistical analyses

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

Adaptation during experimental evolution

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

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

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

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

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

comparison, we analyzed the number of adults y_{ijkl} measured on the three test fruits i ($i=1,...,3$), for a population evolved on the fruit j ($j=1,...,3$) at generation k ($k=1$ and 7 or 29 for the first and second comparison, respectively), and in each tube l . y_{ijkl} was assumed to follow a Poisson distribution with parameter λ_{ijkl} which was modelled as follows:

$$\begin{aligned} \log(\lambda_{ijkl}) = & \text{test fruit}_i + \text{evolution fruit}_j + \text{generation}_k + \\ & \text{test fruit:evolution fruit}_{ij} + \text{test fruit:generation}_{ik} + \\ & \text{generation:evolution fruit}_{jk} + \\ & \text{test fruit:evolution fruit:generation}_{ijk} + \text{obs}_{ijkl} \end{aligned} \quad (2),$$

where fixed effects included the effect of the test fruit (test fruit_{*i*}) the fruit on which the populations evolved (evolution fruit_{*j*}), the effect of generation (generation_{*k*}) and all the interactions between those terms. To account for overdispersion, we added an observation-specific random effect obs_{*ijkl*}, assumed to be normally distributed with mean zero and variance σ^2_{obs} .

Detection of trade-offs across fruits

We tested for ‘sympatric–allopatric’ (SA) contrasts following Blanquart et al. (2013). Briefly, this method compares the performance of populations measured on their host of origin (their sympatric fruit) to that of populations measured on alternative hosts (all allopatric fruits) while controlling for variation in intrinsic quality among environments and for variation in genetic background among populations (e.g., due to differences in inbreeding depression). Detecting local adaptation with this method is equivalent to detecting trade-offs in performance across fruits.

For both the intermediate and final phenotyping steps, we analyzed performance y_{ijkl} (log-transformed number of adults produced and emergence rate), measured on the test fruit i ($i=1,...,3$), for a population evolved on fruit j ($j=1,...,3$), in the replicate population k (with

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

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

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 ($\text{evolution fruit}_{ij}$). 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 df_1 and df_2 . 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:

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

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

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

Heterogeneity of trade-offs among fruits and replicate populations

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

Influence of fruit composition on trade-offs

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

Evolution of oviposition stimulation and oviposition preference

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

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

Results

Demographic dynamics of experimental populations

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

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

Adaptation during experimental evolution

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

During intermediate phenotyping step, performance was higher than during initial phenotyping, but the increase in performance depended on the evolution fruit (Fig. 4; significant interaction between generation and evolution fruit: LRT $\chi^2 = 13.34$, $df = 2$, $P = 0.001$). Only strawberry populations produced more adults on all three test fruits when compared to the initial phenotyping step ($z = 3.64$, $P < 0.001$, Fig. 4).

The final phenotyping revealed that the evolution of performance depended on which fruit the population had evolved on, with a significant three-way interaction between generation, evolution fruit and test fruit (LRT $\chi^2 = 16.92$, $df = 4$, $P = 0.002$, Fig. 4) that could illustrated local adaptation. For example, for populations evolved on strawberry, performance was higher on strawberry than on cherry (41.64 vs. 26.62 adults, respectively). Similarly, for populations evolved on cherry, performance was higher on cherry than on strawberry (32.28 vs. 29.67 adults, respectively).

Evolution of trade-offs across fruits and heterogeneity among populations

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

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

Influence of fruit composition on trade-offs

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

Oviposition stimulation and preference

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

Discussion

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

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

Can populations develop on and adapt to contrasting fruits?

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

Gradual evolution of specialization

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

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

Heterogeneity of adaptation among populations and fruits

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

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

Does evolution of performance influence preference for different fruits?

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

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

Position of a species along a generalist-specialist gradient

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

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

Potential limits of our experimental setting

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

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

Implications for applied research

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

Conclusions and perspectives

We found that trade-offs across artificial fruit environments can evolve in the generalist herbivorous species, *D. suzukii*, when it develops on the same fruit over several generations 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. sukukii* 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.

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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				
<i>P</i> -value	0.812	0.762	0.268	0.049
<i>F</i> -statistic	0.067	0.094	1.844	4.387
df ₁ , df ₂	1, 3	1, 25	1, 3	1, 19
Performance: emergence rate				
<i>P</i> -value	0.952	0.943	0.087	0.010
<i>F</i> -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				
<i>P</i> -value	0.580	0.317	0.927	0.903
<i>F</i> -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				
<i>P</i> -value	0.048	0.074	0.109	0.075
<i>F</i> -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

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.

853

854

Figures

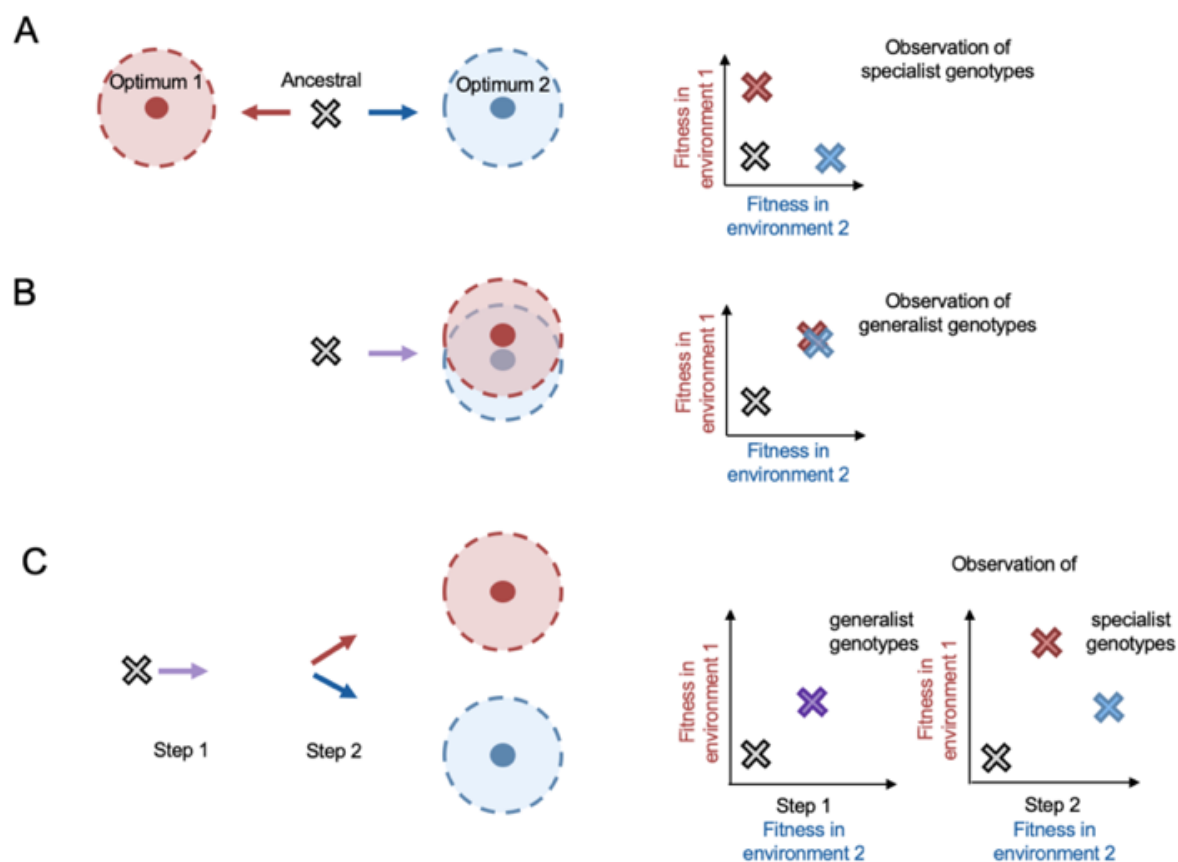


Figure 1.

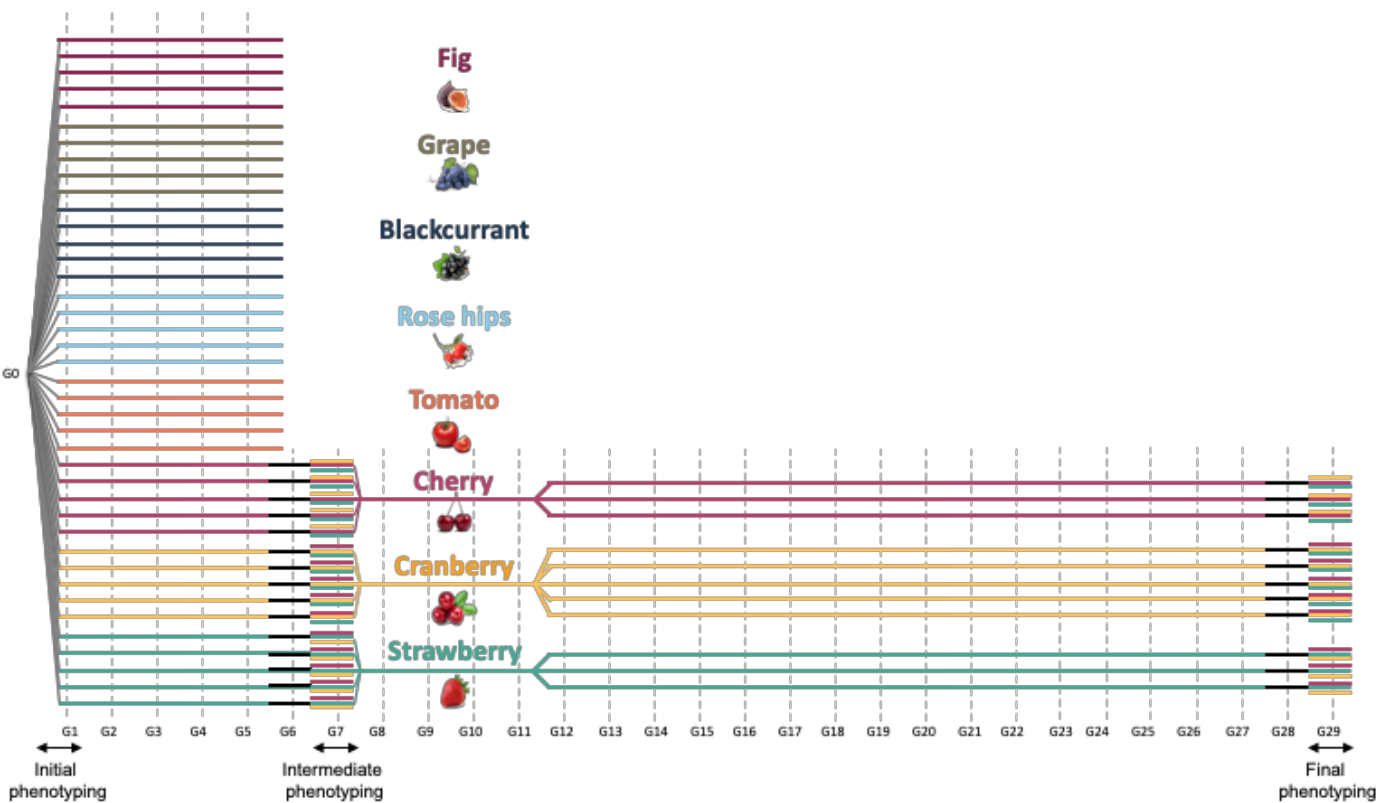


Figure 2.

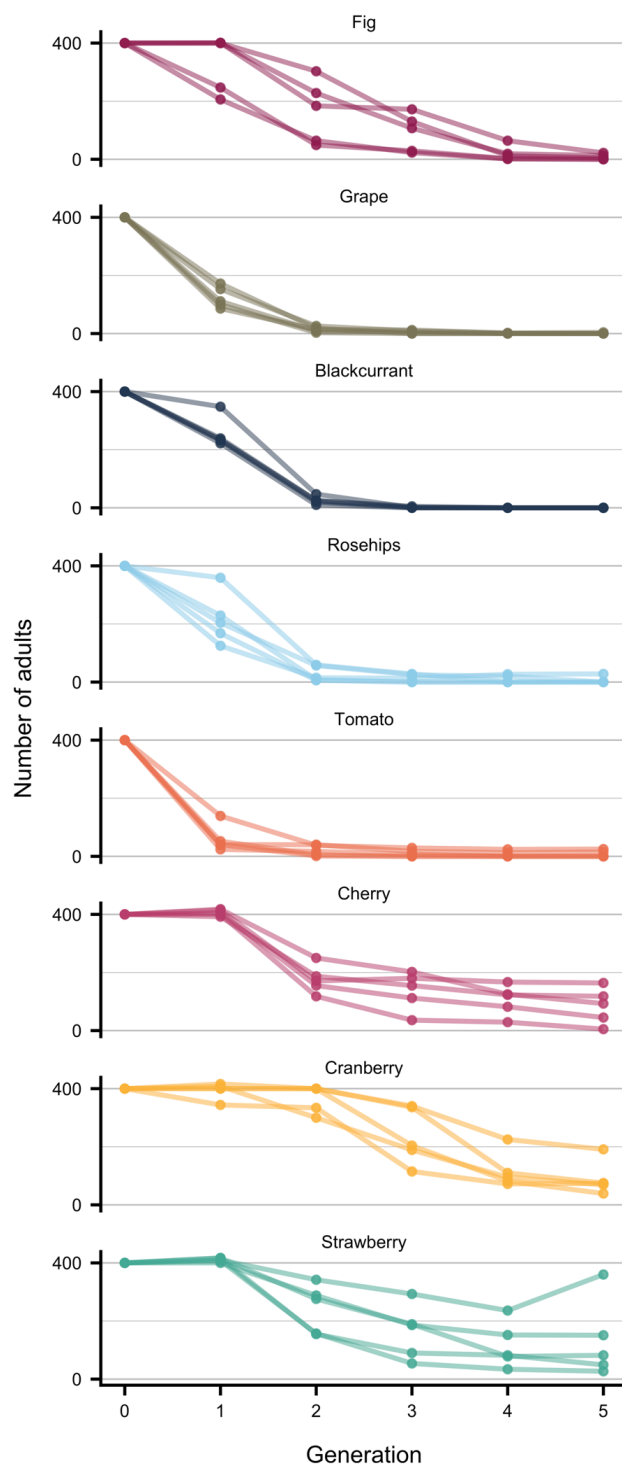


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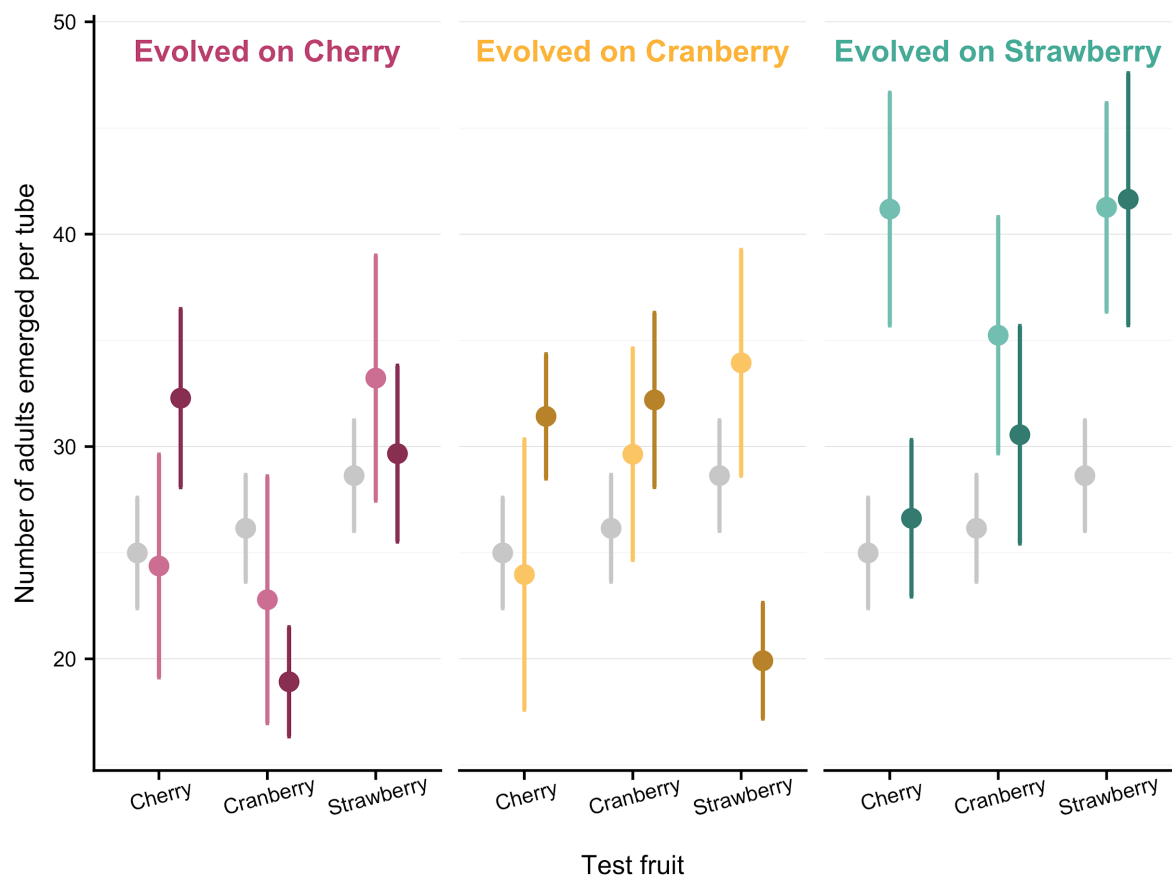


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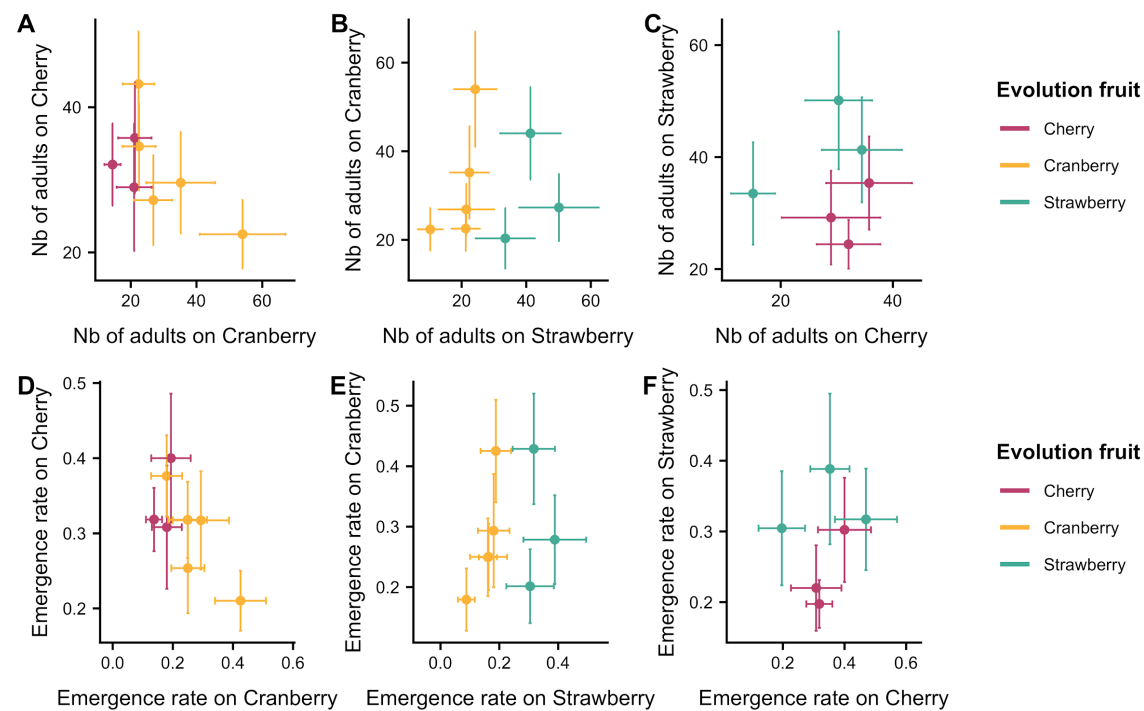


Figure 5.

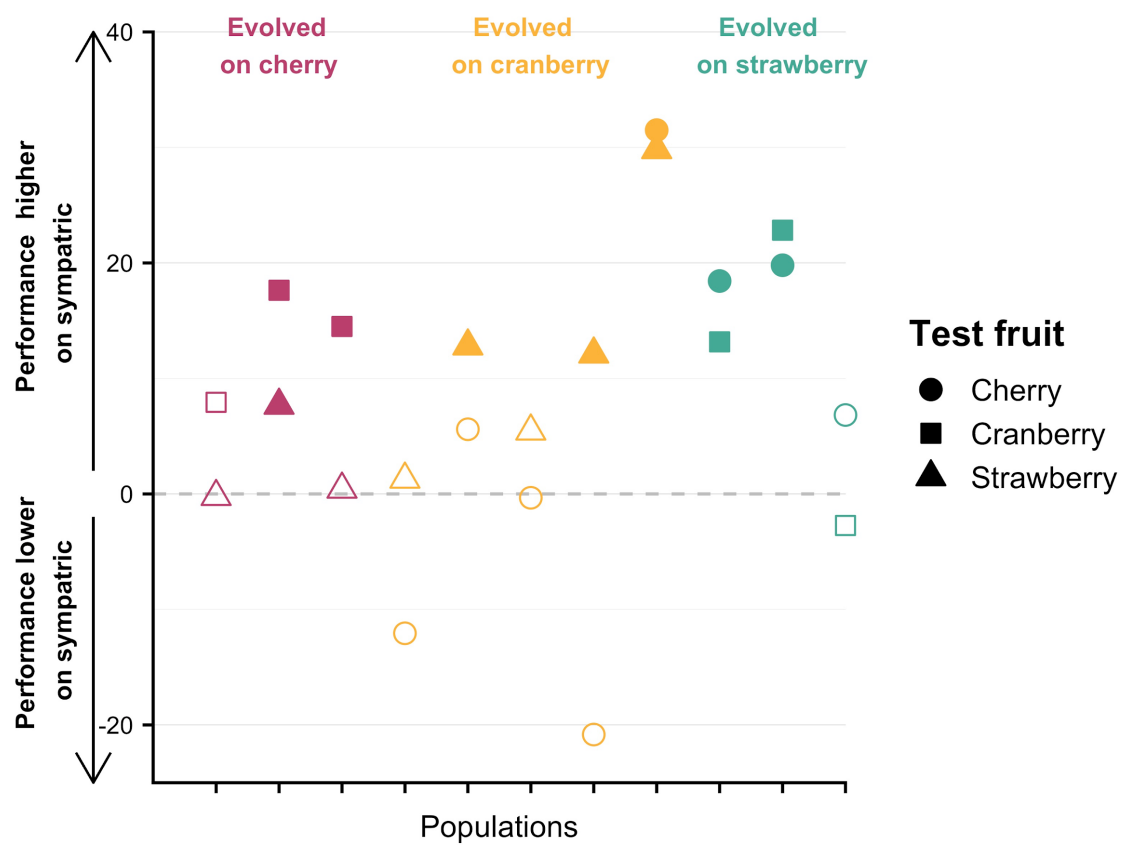
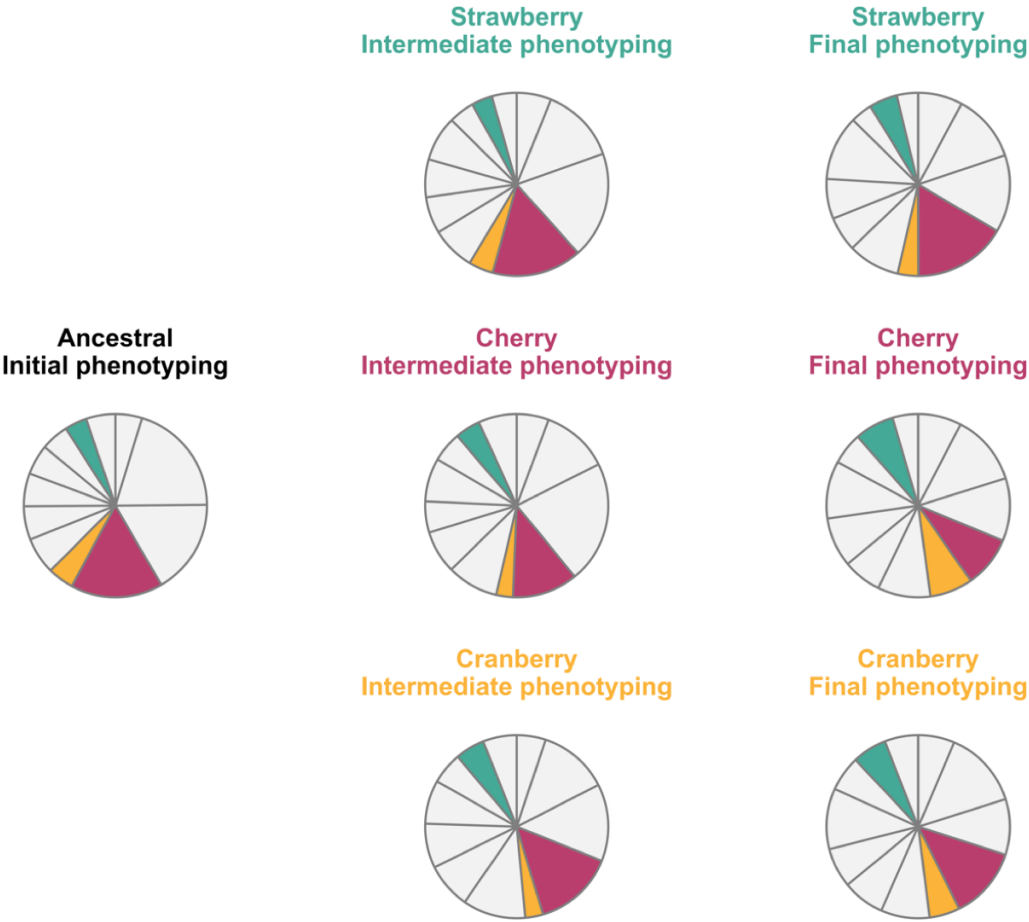


Figure 6.

891



892

893 **Figure 7.**

894