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Author contributions

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Abstract (<250 words)

The process of local adaptation involves differential changes in fitness over time across different environments. While experimental evolution studies have extensively tested for patterns of local adaptation at a single time point, there is relatively little research that examines fitness more than once during the time course of adaptation. We allowed replicate populations of the fruit pest *Drosophila suzukii* to evolve in one of eight different fruit media. After five generations, populations with the highest initial levels of maladaptation had mostly gone extinct, whereas experimental populations evolving on cherry, strawberry and cranberry media had survived. We measured the fitness of each surviving population in each of the three fruit media after five and after 26 generations of evolution. After five generations, adaptation to each medium was associated with increased fitness in the two other media. This was also true after 26 generations, except when populations that evolved on cranberry medium developed on cherry medium. These results suggest that, in the theoretical framework of a fitness landscape, the fitness optima of cherry and cranberry media are the furthest apart. Our results show that studying how fitness changes across several environments and across multiple generations provides insights into the dynamics of local adaptation that would not be evident if fitness were analyzed at a single point in time. By allowing a qualitative mapping of an experimental fitness landscape, our approach will improve our understanding of the ecological factors that drive the evolution of local adaptation in *D. suzukii*.

84

85 **Keywords (4 to 10)**

86 Adaptation, experimental evolution, fitness landscape, fitness trade-off, specialization,

87 reciprocal transplant experiment

88

Introduction

Adaptation to local environmental conditions plays a central role in the maintenance of biodiversity (Levins 1968; Felsenstein 1976; Gillespie and Turelli 1989). In particular, when selection is divergent across environments, if migration is low enough, rare genotypes can accumulate in environments where they have the highest fitness (Deakin 1966; Gillespie 1975; Svardal et al. 2015). Despite the importance of understanding this process of local adaptation, its investigation in natural populations has been limited by a number of experimental and conceptual factors (Rausher 1988; Fry 1996; Hansen et al. 2006; Barghi et al. 2020). In particular, disentangling natural selection from genetic drift requires accurate fitness estimates that are relevant to natural environments, a notoriously difficult task (Fry 1996; Forister et al. 2012). Heterogeneity in environmental conditions and lack of knowledge regarding the starting populations represent additional challenges to studying the process of adaptation in natural populations (Barghi et al. 2020).

Experimental evolution under laboratory conditions represents a powerful alternative to studying the dynamics of local adaptation in the field (Fry 2003; Kawecki et al. 2012). The approach consists of allowing replicated experimental populations to evolve in different environments and performing reciprocal transplant experiments to study fitness changes in each environment. Here, we call the environment in which a population is evolving the selective environment. Reciprocal transplant experiments following experimental evolution can reveal whether fitness changes in the selective environment (i.e., “direct fitness reponses” sensu Bennet et al 1992) are greater than fitness changes in other environments (i.e., “correlated fitness responses” sensu Bennet et al 1992). These experiments also can be used to test directly for a pattern of local adaptation (Blanquart et al 2013) by investigating whether populations have higher mean fitness in the selective environment (in this context also often called the sympatric environment) than in other environments (the non-selective or allopatric

114 environments). To emphasize that selective environments determine the selective pressures at
115 the origin of the process of local adaptation, we will hereafter solely use the terms selective
116 and alternative environments.

117 Evidence for the evolution of local adaptation has been mixed in experimental evolution
118 studies over a single time scale (for reviews see Fry 1996; Kassen 2002; Hereford 2009; Jasmin
119 and Zeyl 2013; Bono et al. 2017; Bergh et al. 2018). For example, adaptation to one
120 environment can be associated with an increase (e.g., Bennett et al. 1990; Magalhães et al.
121 2009; Messina et al. 2009; Laukkanen et al. 2012; Messina and Durham 2013), a decrease (e.g.,
122 Fry 1990; Mackenzie 1996; Turner and Elena 2000; Agudelo-Romero et al. 2008; Bedhomme
123 et al. 2012; Messina and Durham 2015; Gompert and Messina 2016) or no change (e.g., Fry
124 2001) in fitness in other environments. These contrasting results are not actually contradictory
125 if interpreted in the light of evolutionary theory, particularly that based on invasion fitness and
126 evolutionary branching (Svardal et al. 2015) or based on fitness landscapes theory and Fisher's
127 geometric model (Martin and Lenormand 2015). If a population has split into subpopulations
128 that experience two different environments with low migration, whether or not adaptation to
129 the selective environment leads to an increase in fitness in the alternative environment will
130 depend upon how similar those environments are to each other and to the ancestral environment
131 (Fig. 1). Specifically, whether adaptation to the selective environment leads to adaptation to
132 other environments is highly dependent on i) the (mal)adaptation of the ancestral population to
133 the two selective environments and ii) the amount of time a population has spent in the selective
134 environment. If the phenotypes that maximize fitness in the two new environments are similar
135 to each other, but relatively different from the optimal phenotype in the ancestral environment,
136 adaptation will increase in both environments regardless of how long a population has
137 experienced an environment (Fig. 1A). In contrast, if the optimal phenotypes in the two new
138 environments are different from each other, but more similar to each other than they are to the

optimal phenotype in the ancestral environment, whether or not adaptation to the selective environment leads to an increase in fitness in the alternative environment will depend on when fitness is measured because selection in the two environments changes over time from being convergent to divergent (Fig. 1B). During the first few generations, adaptive evolution will shift the mean phenotype in each environment in a similar direction, so that adaptation increases in both environments (step 1 in Fig. 1B); but over time, those mean phenotypes will diverge, resulting in adaptation in one environment and maladaptation in the other environment (step 2 in Fig. 1B). This scenario shown in Fig. 1B is often not explicit in theoretical models (e.g., Levins 1962), which often focus on predicting the outcome of evolution rather than the dynamics of evolution (Roughgarden 1979). Consistent with the scenario shown in Fig. 1B, a temporal reversal in the association between adaptation in a given selective environment and fitness changes in alternative environments has been observed in experimental populations of yeast (Jasmin and Zeyl 2013) and bacteria (Satterwhite and Cooper 2015; Schick et al. 2015). Also consistent with Fig. 1B is the observation that negative associations between the rate of adaptation in an environment and the rate of fitness decline in other environments are more likely to be found in experimental evolution studies performed over about 10,000 generations than in studies performed over fewer generations (Bono et al. 2017).

In all, we found only a handful of studies that evaluated adaptation and correlated responses over more than one time period to test whether selection patterns change from convergent to divergent (spider mite: Magalhães et al. 2009; yeast: Jasmin and Zeyl 2013; bacteria: Satterwhite and Cooper 2015; Schick et al. 2015; virus: Agudelo-Romero et al. 2008). Most of them considered adaptation to different environments of microorganisms grown from a single clone to different environments (Jasmin and Zeyl 2013; Satterwhite and Cooper 2015; Schick et al. 2015). However, many natural populations of interest are macro-organisms, in which population sizes are smaller and generation times longer than for experimental micro-

164 organisms. In such populations, standing genetic variation can be expected to play a larger role
165 in adaptation to a new environment than *de novo* mutations (Barrett and Schluter 2008, Bailey
166 & Bataillon 2016). Although providing an important jumping off point, microbial studies
167 provide an incomplete picture of the process of local adaptation from standing genetic
168 variation. The spider mite study addressed this issue using genetically diverse spider mite
169 populations, and found a positive association between adaptation to a given environment and
170 fitness changes in other environments after both 15 and 25 generations (Magalhães et al. 2009).
171 This result suggests that populations adapted to two new environments whose fitness optima
172 were close to each other, as represented in Fig. 1A. Studies that investigate adaptation and
173 correlated responses to selection in genetically diverse populations adapting to a wide variety
174 of environments such that some are likely to be different from each other and from the ancestral
175 environment (Fig. 1B) are deeply needed.

176 The spotted wing drosophila, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae),
177 represents an attractive biological model to address this issue. First, growth media made of
178 different fruits can be used to represent environments with different fitness optima (Olazcuaga
179 et al. 2019). When comparing natural *D. suzukii* populations sampled in different fruits,
180 Olazcuaga (2019) recently found that emergence rates were significantly higher on fruit media
181 corresponding to the fruit from which each population originated, than on fruit media
182 corresponding to alternative fruits. This suggests that the standing genetic variation that
183 segregates in natural populations is sufficient to adapt to different fruit media during
184 experimental evolution in the laboratory. In addition, *D. suzukii* presents several advantages
185 for experimental evolution including short generation time, small size, and relative ease of
186 maintenance of large populations over many generations in the laboratory. These
187 characteristics greatly facilitate a straightforward estimation of population mean fitness across
188 different environments, using the same protocol as the one used for experimental evolution.

Consequently, this biological system does not rely on phenotypic traits that might be only loosely associated with fitness in experimentally evolving populations. Finally, a better understanding of the potential of *D. suzukii* to adapt to the fruits of different host plants is of agronomic interest, as this species is a major pest of berries and stone fruits (Asplen et al. 2015).

In this study, we maintained *D. suzukii* populations on different fruit media and investigated adaptation to those different media as well as fitness changes on alternative media using reciprocal transplant experiments after five and 26 generations of evolution. We specifically addressed the following questions: 1) Are populations able to adapt to all selective environments at the same rate (i.e., are direct fitness responses positive and of the same magnitude across environments)? 2) Are fitness changes in selective and alternative environments of the same sign and magnitude across replicate populations evolving on the same fruit medium? 3) Is there a reversal over time in the direction of the association between fitness changes in selective and alternative environments?

Materials and methods

Field sampling

To initiate a laboratory population, approximately 1,000 *D. suzukii* flies (more than 500 females and 460 males) were collected using baited cup traps (Lee et al. 2013) at six sampling sites within 10 km of Montpellier, France in September and October 2016. At this time of the year, most of the fruit crops cultivated over large areas (e.g., cherries and strawberries) are no longer available. Based on the literature (Poyet et al. 2015), we estimate that flies have potentially emerged from more than 18 wild (i.e., non crop) host plants from nine families available in the sampling area in October (Table S1). Thus our sampled individuals likely emerged from a large range of host plants rather than a few specific host plants, enabling us to

establish a laboratory population likely to harbor alleles that could potentially provide adaptation to a large variety of host plants.

Laboratory maintenance

As we did not want to create a population adapted to a particular fruit, flies were maintained for nine generations in 10 ml vials with standard laboratory fly food (consisting of sugar, dry yeast, minerals and antifungal solution; Backhaus et al. 1984), prior to the start of the experiment. This maintenance on a protein-rich medium likely selected for a combination of phenotypic traits different from those selected on host fruits (which are likely to be poor in proteins). Each generation, newly emerged adults were mixed across vials and randomly distributed into groups of 20 adults in 100 new vials to maintain a large panmictic population (i.e., ~2000 individuals) and as much genetic variation as possible. We chose this approach rather than using isofemale lines to maintain genetic variation because linkage disequilibrium in synthetic populations recomposed by crossing isofemale lines decreases additive genetic variance (Kessner and Novembre 2015) and the subsequent response to selection. In addition, selection among isofemales due to different degrees of inbreeding depression could reduce genetic diversity at loci responsible for adaptation to different fruits. Our large population of flies was maintained with discrete generations over two-week cycles (21°C, 65%, 16:8 day/night light cycle; see Olazcuaga et al. 2019 for details). Experimental evolution and phenotyping were performed under the same temperature, humidity and light conditions.

Fruit media

To investigate the dynamics of fitness changes in selective and alternative environments, we reared replicate populations on media made using fruit purees. To standardize generation time and fitness estimation protocol across environments, we compare emergence rates across fruit media (Fig. 4 in Olazcuaga et al. 2019). We chose eight fruit media where our population exhibited similar emergence rates (blackcurrant, cherry, cranberry, fig, grape, strawberry, rose

237 hips, tomato), and which correspond to host fruits attacked to varying degrees in the field
238 (Walsh et al. 2011; Cini et al. 2012; Bellamy et al. 2013; Steffan et al. 2013; Kenis et al. 2016;
239 Kanda et al. 2019).

240 To limit variation within each environment throughout the experiment and to directly
241 compare fruits that ripen at different times of the year, we used media made with frozen fruit
242 purees rather than whole fruits (recipe available in Olazcuaga et al. 2019). We hence assume
243 that temporal variation within each environment is minimal so that we can compare fitness
244 across phenotyping steps. We also assume that fruit media differ in their biochemical properties
245 and select for different phenotypic optima. This assumption seems reasonable, as adaptation to
246 some of these media varies across natural populations and matches the fruit from which they
247 originated (Olazcuaga 2019).

248 **Experimental evolution experiment**

249 The three phases of the evolution experiment are summarized in Fig. 2. During phase 1, we
250 established experimental populations by deriving replicate populations from our base
251 population and placing them on each of eight different fruit media (Fig. 2). Five replicate
252 populations were established per fruit (40 populations in total). Each population consisted of
253 400 adults (20 vials of 20 flies), which corresponds to a reasonably large population size to
254 limit genetic drift (Woodworth et al. 2002). Populations were maintained on a 21-day cycle.
255 For each vial, we placed 20 six-day-old flies into a vial filled with 10 ml of a single fruit
256 medium to mate and oviposit. At this stage of adult development, all adult females should be
257 ready to oviposit (Emiljanowicz et al. 2014). The sex ratio of the adults was neither controlled
258 nor measured. While not controlling sex ratio increased variation in the total number of eggs
259 among tubes, it made it possible to handle more flies, increase replication, and minimize
260 additional stress on the flies due to manipulation (for instance a longer time of CO_2 anesthesia).
261 The sex ratio did not evolve over the course of the experiment (Olazcuaga 2019). After 18

hours, adults were removed. After 15 days, we anesthetized emerging adults from each replicate population using CO_2, mixed them across vials to produce 20 groups of 20 flies, each placed in a vial with 5 ml of the same fruit medium. Adults could mature for 6 days before starting the next cycle. At most 400 individuals per population were kept to produce the next generation. If fewer than 400 individuals emerged, less than 20 vials were made but always with 20 adults each. Populations were randomly distributed among eight racks (100 vials per rack, composed of five populations of 20 vials each) and randomly arranged spatially in a climate chamber. The 40 populations were reared in two temporal blocks separated by two days.

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 per population (4 to 28 individuals; see Appendix S1, Fig. S1). Experimental populations persisted over the first five generations on cherry, cranberry and strawberry, but some of the replicates had relatively small population sizes (mean of the population size after five generations on fruit ± sd for populations on cherry, cranberry and strawberry: 85.0 ± 62.0, 89.6 ± 58.5 and 133.8 ± 134.8, respectively). During phase 2 (seven generations after the start of the experiment), the five replicate populations on each fruit were pooled together to counteract inbreeding depression that we assumed to be the main driver of the decline in population size (1840, 420 and 2220 individuals pooled for cranberry, cherry and strawberry respectively, with demographic and genetic stochasticity likely being responsible for differences across fruit media).

During phase 3 (11 generations after the start of the experiment), the three pooled populations had recovered (3040, 1560 and 1600 individuals for cranberry, cherry and strawberry respectively) and were divided into five, three and three replicate populations for cranberry, cherry and strawberry, respectively (with 500 individuals per replicate population;

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 as described above for the first phase. Populations were randomly distributed among four racks (75 vials per rack i.e., three populations of 25 vials). The 11 populations were reared in a single temporal block. To estimate Malthusian fitness at each generation, we counted the number of adults that emerged from each vial (except during the pooling step where we counted the total number of adults across all vials and computed an average fitness across vials).

To reduce experimental burden, we compared the fitness of evolved populations with that of the ancestral population rather than to that of an evolved control population. As fruit media were based on the same stock of frozen puree, we assume the variation in experimental conditions across phenotyping steps to be small. Moreover, experiments using inbred lines show that variation among replicates within a single phenotyping assay is as large as variation between phenotyping assays several generations apart (Olazcuaga 2019). In addition, because the combination of host fruits used by the population sampled in the wild was unknown, no single fruit medium could represent an appropriate control for our experiment. Finally, using a control population maintained on standard medium (as recommended by Fry 2003) was likely inappropriate. Our ancestral population was likely adapted to our rearing schedule (discrete generations over three weeks) but might not have been entirely adapted to the standard medium. As a result, control populations maintained on standard medium would have likely diverged from the ancestral population over the course of the experiment. Small populations would have experienced genetic drift while large populations would have experienced selection.

Estimation of fitness changes in selective and alternative environments using reciprocal transplant experiments

We estimated the average fitness of each population in each of the three fruit media (cherry, cranberry and strawberry) during the initial, intermediate and final phenotyping steps (respectively corresponding to one, seven and 29 generations after the start of the experiment; Fig. 2), after one generation in a common garden (standard laboratory medium to standardize maternal environmental effects; Fry 2003). Thus, populations had evolved for five and 26 generations in each selective fruit medium when their fitness was estimated during the intermediate and final phenotyping steps respectively. In phenotyping we used the same protocol as that used to maintain experimental populations and estimated fitness over the 21-day life cycle (number of adults emerging that descended from the 20 initial adults from the previous generation). We also counted the number of eggs in vial so that our fitness measure corresponded to the product of number of eggs and egg-to-adult viability. During the initial phenotyping step, the average fitness of the base population in each fruit medium was estimated using 100 vials (two temporal blocks). The average fitness of each evolved population was estimated in each fruit medium using between two and 32 vials during the intermediate phenotyping step and 30 vials during the final phenotyping step (three and six temporal blocks, respectively).

Statistical analyses

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

Fitness change in selective environments

To investigate the temporal dynamics of adaptation, we used census data recorded each generation during the three phases of the experiment. To avoid environmental effects (including maternal effects), we excluded data from generations where individuals or their

parents developed in standard medium. As we used discrete generations, we computed the average Malthusian fitness of each population at the the *i*th generation as: $m_i = log(\frac{\overline{N_i}}{20})$, where $\overline{N_i}$ is the average number of emerging adults per tube over one life cycle. For each generation and each population, we considered the average fitness across vials and used the number of vials counted as a weight in the analyses. To test for differences in the rate of adaptation among fruit media, we fitted the following linear model on average fitness m_{ijkl}:

$$m_{ijkl} = generation_i + phase_j + selective\_fruit_k + generation{:}selective\_fruit_{ik} + phase{:}selective\_fruit_{jk} + \underline{generation{:}selective\_fruit_{ik}} + \varepsilon_{ijkl} \quad (1),$$

where fixed effects included the effect of the *i*th generation as a covariate (*generation*$_i$, with *i*=2,..,27), the effect of the *j*th phase of experimental evolution (*phase*$_j$, with either *j*=1,...,3), the effect of the *k*th selective fruit (*selective_fruit*$_k$, with *i*=1,..,3 for cherry, cranberry and strawberry respectively), the interaction between the *generation* and *selective_fruit* effects, the interaction between the *phase* and *selective_fruit* effects. Random effects included the interaction between the *i*th generation and the *k*th *selective_fruit* (mean of zero and variance $\sigma^2_{gen\ fruit}$) to control for potential batch effects among vials of the same fruit medium cooked across generations. A random error (ε_{ijkl} mean of zero and variance σ^2_{res}) accounted for the variation among populations evolving on the same fruit medium. We compared several simpler models derived from this full model that included none, one or more of the effects described above), while keeping random effects in all models. All the models tested are listed in Table 1. Due to their partial redundancy, the *generation* and *phase* effects were never tested together. Models were ranked according to their corrected Akaike's information criterion (AICc; Hurvich and Tsai 1989) using the *MuMIn* package (Barton 2009). For each model, we computed ΔAICc value as the difference between the AICc of that model and the best fit model. Best competing models with AICc differences lower than two were considered as strongly supported by the

data, except when they differed by a single degree of freedom and had essentially the same log-likelihood (Burnham and Anderson 2002).

Finally, a complementary analysis was performed on phases 1 and 3 separately to test whether the rate of adaptation differed across replicate populations evolving on the same fruit medium (see Appendix S1).

Comparison across populations of the direction of fitness changes in selective and alternative environments

To quantify the direction and magnitude of fitness changes in selective and alternative fruit media, we combined the data of the three phenotyping steps and fitted the following negative binomial model (log link) on the number of adults, n_{ijkl}, that emerged from each tube:

$$n_{ijkl} = \text{test\_fruit}_i + \text{phenotyping\_step}_j + \text{selective\_fruit}_k + \text{test\_fruit:phenotyping\_step}_{ij} + \text{test\_fruit:selective\_fruit}_{ik} + \text{test\_fruit:phenotyping\_step}_{jk} + \text{phenotyping\_step:selective\_fruit}_{jk} + \text{population}_l \quad (2),$$

where fixed effects included the effect of the *i*th test fruit medium (*test_fruit*$_i$, with i=1, 2 and 3, for cherry, cranberry and strawberry respectively), the effect of the *j*th phenotyping step (*phenotyping_step*, with either j=1, 2 and 3 for the initial, intermediate and final phenotyping steps, respectively), the effect of the *k*th selective fruit medium (*selective_fruit*$_k$, with k=1, 2 and 3, for cherry, cranberry and strawberry respectively), and their two-way or and three-way interactions and a random population effect (population with mean of zero and variance σ^2_{pop}). We used the *glmer.nb* function of the *lme4* package and computed the 95% confidence interval of each parameter with 1,000 simulations using the *bootMer* function (Bates et al. 2015). Similarly, we used a negative binomial model and a binomial model to respectively investigate changes in fecundity (corresponding to count data) and in egg-to-adult viability (corresponding to proportion data) between phenotyping steps.

Correlation between fitness changes in selective and alternative environments

To investigate whether fitness changes in selective environments were associated with positive or negative fitness changes in alternative environments, we used two approaches: i) for populations from each pair of environments, we tested whether fitness changes in selective and alternative environments were in the same or in different directions and ii) we tested whether fitness changes in selective fruit media were significantly greater than fitness changes in alternative fruit media. First, for each pair of environments and for the intermediate and final phenotyping steps separately, we estimated the correlation coefficient between the average fitness change of populations in their selective medium and their average fitness change in each of the two alternative media using the sum of number of tubes of selective and fruit media as weight and estimated its 95% confidence interval using the *sjstats* package (Lüdecke 2018). For each pair of fruit media, we estimated the difference between the correlation coefficients estimated during the intermediate and final phenotyping steps and tested whether the 95% confidence interval of this difference included zero (Zou 2007). To illustrate the relationship between fitness change in selective and alternative fruit media, we estimated the intercept and the slope of the regression of fitness change in alternative environments over fitness change in selective environments using a Major Axis regression in the *lmodel2* package (Legendre 2014). This method requires the existence of variation across replicate populations and assumes that the correlation between fitness changes in selective and alternative environments is independent of the selective environment.

Second, to test whether fitness changes in selective fruit media were significantly greater than fitness changes in alternative fruit media, we used 'sympatric–allopatric' (SA) contrasts (Blanquart et al. 2013), a test that controls for variation among populations (e.g., due to different levels of inbreeding) and among environments (e.g., due to differences in

environment quality). To this end, we fitted the following linear model on the average fitness change relative to the ancestor, s_{ijk}, for the intermediate and final phenotyping steps separately:

$$s_{ijk} = \text{test\_fruit}_i + \text{population}_j + \text{test\_fruit:selective\_fruit}_{ik} + SA_{ik} + \varepsilon_{ijk} \quad (3),$$

where fixed effects included the effect of the *i*th test fruit medium (*test_fruit*$_i$, with i=1,...,3, for cherry, cranberry and strawberry respectively), the effect of the *j*th population (*population*$_j$, with either j=1,...,14 or j=1,...,11 for the intermediate and final phenotyping steps, respectively), the interaction between the *i*th test fruit and the *k*th selective fruit medium on which the *j*th population evolved (*selective_fruit*$_k$, with k=1,...,3, for cherry, cranberry and strawberry respectively), a sympatric vs. allopatric effect that measures local adaptation (SA_{ik}) and a random error (ε_{ijkl} mean of zero and variance σ^2_{res}). We did not include a *selective_fruit* effect, which is already statistically accounted for by the *population* fixed effect. For each population, we used the inverse of the variance of each estimate ($se(s_{ijk})^2$) as a weight in the analyses. To test for a pattern of local adaptation, we used a two-way ANOVA and computed a F-test with the appropriate degrees of freedom (eq. D1 in the supplementary information of Blanquart et al. 2013). We performed a set of computer simulations showing 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 count data (see Appendix S3).

Results

Temporal fitness change in each selective fruit medium

Fitness change in each selective fruit medium is shown for the three phases of the experiment in Fig. 3 and Fig. S1. During phase 1, fitnesses were initially negative in all eight fruit media and differed significantly across fruit media (ΔAICc > 28.3 for models 14 and 15 without a fruit effect, Tables S2), but not across populations evolving on the same fruit medium (ΔAICc = 9.48 for the model 12 including a population effect, Table S2). Temporal changes in fitness

differed across fruits (ΔAICc = 2.12 for the model 10 without an interaction between the fruit and generation effects, Table S2). Populations with the lowest initial fitness (Table S3) went extinct by the fifth generation due to demographic effects, so that subsequent fitness changes could only be measured on cherry, strawberry and cranberry media.

Between the start and the end of the experiment (generations 2 and 27 respectively), the average number of adult flies emerging from each tube increased from 8.8 to 35.8, from 19.4 to 33.6 and from 12.2 to 42.3 for cherry, cranberry and strawberry respectively. The corresponding increase observed between phases 1 and 2 were 123%, 93% and 45% and those observed between phases 1 and 3 were 230%, 176% and 145% for cherry, cranberry and strawberry respectively. This increase in fitness across phases had high support (ΔAICc > 27.6 for models 4 to 8 without this effect, Table 1). Support for fitness changes across the three fruit media was strong, while support for differences in temporal fitness increase among fruit media was low (ΔAICc = 3.44 for the model 2 without a fruit effect and ΔAICc = 5.85 for the model 3 with an interaction between the phase and fruit effects, Table 1), indicating that adaptation rates were similar across fruit media. During phase 3, support for fitness changes among populations with each fruit medium was not very strong (ΔAICc = 1.69 for the model 3 including a population effect, Table S4), suggesting that variation in adaptation rate among populations evolving on the same fruit medium tended to be low.

Changes in fecundity, egg-to-adult viability and fitness in selective and alternative media

Average fecundity increased on the selective environments between the initial and intermediate phenotyping (Table S5, Fig. S3A top panel). In contrast, in egg-to-adult viability were unchanged or decreased modestly between the initial and intermediate phenotyping step, Table S6, Fig. S3B top panel). Changes in fecundity and in egg-to-adult viability were negatively correlated (Fig. S6A). Fitness tended to increase between the first and intermediate genotyping step, with a significant increase for populations that evolved on strawberry and

were measured on cherry (large confidence intervals are likely due to a lack of statistical power, Table 2, Fig. 4).

Between the initial and final phenotyping steps, no consistent pattern was apparent in average fecundity on the three selective fruits (Table S5, Fig. S3A bottom panel). Egg-to-adult viability increased for populations measured on cherry medium, but did not change consistently or significantly on other media (Table S6, Fig. S4A bottom panel). Changes in fecundity and in egg-to-adult viability were negatively correlated as for the first time-step comparison (Fig. S6B bottom panel). Fitness tended to increase, but not significantly so (Table 2, Fig. 4).

Correlation between fitness changes in selective and alternative environments

During the intermediate phenotyping step, the increase in fitness in each selective fruit medium was associated with an increase in fitness in the two other fruit media (Fig. 5A-C). This pattern was also seen for some fruit combinations during the final phenotyping step: increases in fitness in selective fruit media were associated with increases in fitness in other fruit media for the pairs strawberry/cranberry and strawberry/cherry (Fig. 5E-F). The confidence intervals of the difference in correlation coefficients between the intermediate and final phenotyping steps overlapped with zero, indicating that the correlation coefficients did not differ significantly. In contrast, increased fitness on cherry or cranberry selective medium were associated with decreased fitness in the reciprocal medium (Fig. 5D). The confidence interval of the difference in correlation coefficients between the intermediate and final phenotyping steps was positive and did not overlap with zero, indicating that the correlation coefficient during the final phenotyping step was lower than that during the intermediate phenotyping step (Fig. 5D). These results remained unchanged when considering only populations that evolved on cranberry (estimate of correlation difference=1.21 [0.32, 1.86]), but not when considering only populations that evolved on cherry medium (estimate of correlation difference=0.55 [-0.71, 1.67]), probably due to a lack of power (see Discussion). The negative correlation in fitness

change between cherry and cranberry media was primarily driven by changes in egg-to-adult viability in populations that evolved in cranberry medium, and not by changes in their fecundity (Fig. S4-S5).

Finally, we did not detect a significant pattern of local adaptation during either the intermediate or the final phenotyping steps ($F_{1,3} < 0.01$, $P = 0.97$ and $F_{1,3} = 2.89$, $P = 0.19$ respectively), indicating that fitness changes in selective fruit media were not significantly greater than fitness changes in alternative fruit media.

Discussion

In the present study, we aimed at quantifying fitness changes in selective and alternative environments of *D. suzukii* experimental populations evolving on each of eight different selective fruit media. Due to the almost complete extinction of populations on five fruit media, we could only estimate fitness changes for populations evolving on cherry, cranberry or strawberry media. After five generations, fecundity had consistently increased in both selective and alternative media, resulting in positive fitness changes across the three fruit media. After 26 generations, both fecundity and egg-to-adult viability had changed compared to the ancestral population. Adaptation to each selective medium was associated with an increase in fitness in alternative media, except for populations that evolved on cranberry when measured on cherry (we had low power to detect the same effect in populations that evolved on cherry medium when measured on cranberry medium). Indeed, egg-to-adult viability on cherry and cranberry media were negatively correlated for populations that evolved on cranberry. These results suggest that cranberry and cherry media might exert very different selective pressures on egg-to-adult viability.

Relative importance of selection, genetic drift and pleiotropy in shaping fitness changes in selective and alternative environments

During the first five generations of evolution in phase 1, we found weak evidence for the adaptation of populations evolving on each of the eight fruit media, which could suggest the absence of genetic variation in adaptive alleles or the absence of selection (i.e., that adaptive alleles did not increase in frequency in these populations). This interpretation might be overly simplistic for two reasons. First, Olazcuaga (2019) recently found that local adaptation to different fruit occurs over less than four generations in natural populations, which suggests that genetic variation in adaptive alleles present in natural populations likely persisted in our lab population. Second, we observed that the fitness of experimental populations evolving on cherry, cranberry and strawberry media increased significantly following the pooling step and in the subsequent generations (Fig. 3), which indicates that natural selection is present in our experiment.

Furthermore, two different processes could account for the increase in fitness after the pooling step. First, the increased fitness might be due to heterosis following the masking of mildly deleterious alleles that independently increase in frequency in replicate populations during phase 1 (low population sizes likely increased genetic drift during phase 1 so that mildly deleterious mutations became selectively neutral). Second, the increase in fitness might be due to the combination of different adaptive alleles that independently increase in frequency in replicate populations evolving on the same fruit medium during phase 1 (Barghi et al. 2019). Note, that the occurrence of only one of the two processes during phase 1 would likely have resulted in a fitness change (a decrease in fitness with increase in frequency of deleterious alleles or an increase in fitness with an increase in adaptive alleles). As we did not detect such a change, both processes likely occurred concurrently during phase 1; the negative fitness effect associated with the increase in frequency of mildly deleterious alleles across the genome might

have been counterbalanced by the positive fitness effects associated with the increase in frequency of a few large-effect mutations, as observed in Stewart et al. (2017) and Koch and Guillaume (2020). Finally, we did not detect a pattern of local adaptation during the intermediate phenotyping step, which suggests that beneficial mutations that increased in frequency might not have been fruit-specific. However, this result might also be due to our low statistical power, so that this interpretation remains speculative. Genomic data could help in estimating the extent of genetic drift.

During phase 3, the fitness changes of populations evolving on the same fruit medium tended to be in the same direction, while their fitness changes in each of the two other fruit media also tended to be in the same direction. Fitness changing in a consistent way across replicate populations is likely the result of natural selection, as genetic drift would result in the evolution of fitness increasing or decreasing at random across replicate populations. Furthermore, the convergent evolution of fitness changes in alternative fruit media suggests that the same trait(s) might have been selected independently in replicate populations adapting to the same fruit medium. An alternative explanation would be that this pattern arose through the independent evolution of different phenotypic traits in replicate populations (due to selection or to genetic drift). This would require these different traits to have the exact same pleiotropic fitness effects across fruit media. The precise relationship between a given phenotypic trait and fitness is often environment-specific, so that this alternative hypothesis appears quite unlikely. Genomic data would be necessary to thoroughly assess and quantify convergence among replicate populations at the molecular level (e.g., following Barghi et al. 2019).

Finally, the demographic trajectories we observed after the temporary pooling suggest the occurrence of evolutionary or genetic rescue (Gomulkiewicz and Holt 1995; Whiteley et al. 2015; Hedrick and Garcia-Dorado 2016). However, although the data do fit that

552 interpretation well, our experiment was not set up explicitly to study this phenomenon, and we
553 lack the appropriate experimental controls to confirm or infirm this interpretation.

554 **Fitness in selective and alternative environments shed light on the hypothetical fitness**
555 **landscape**

556 The main focus of our study was to use distinct environments to test for a reversal over time in
557 the direction of the association between fitness changes in selective and alternative
558 environments. Such a reversal was observed for populations that evolved on cranberry when
559 measured on cherry, confirming our predictions based on fitness landscapes theory and Fisher's
560 geometric model (Martin & Lenormand 2015).

561 More generally, our results can be used in an interpretation of the theoretical fitness
562 landscape represented by different environments. The increase in fitness in both selective and
563 alternative environments observed during the intermediate phenotyping step clearly indicates
564 that the ancestral population was initially maladapted to each of the three selective fruit media
565 in a similar way (Figs. 1 & 6). Alleles favored early in the experiment likely increased fitness
566 across the three selective media, as adaptation occurred mostly through an increase in fecundity
567 that was of similar magnitude across replicate populations and across fruit media (Fig. S3A).
568 Given that the initial rate of adaptation was similar across the three fruit media, the distance
569 between the ancestral population and the phenotypic optimum of each of the three fruit media
570 was likely similar (this interpretation parsimoniously assumes that the levels of adaptive
571 genetic variation to each of the three fruit media were similar in the ancestral population and
572 that the intensity of selection was also similar across fruit media). Adaptation occurred through
573 changes in both fecundity and egg-to-adult viability that depended on the population or on the
574 selective fruit (Fig. S3). Eventually, populations that evolved on cranberry, exhibited reduced
575 fitness in one of the alternate environments (cherry), which suggests that the phenotypic optima
576 of these two fruit media lie the furthest away from each other in the fitness landscape (Fig. 6).

577 This pattern was primarily driven by changes in egg-to-adult viability in populations that
578 evolved in cranberry. We had low power to detect similar changes in populations that evolved
579 in the cherry medium. This example (Fig. 6) illustrates how examining direct and correlated
580 responses to selection over time can help visualize the fitness landscapes organisms experience
581 in different environments.

582 **Limits of our experimental approach**

583 The fitness optima of the five fruit media where populations went extinct were likely further
584 away than the fitness optima of the three fruit media where populations survived.
585 Consequently, we could not assess fitness changes across fruit environments that exert the most
586 divergent selective pressures (i.e., cherry, cranberry, and strawberry vs. the five other fruits).
587 Hence, population extinctions have limited our power to detect a reversal in fitness correlations
588 among fruit media. Despite the likely closer proximity of the fitness optima of the three
589 remaining fruit media (cherry, cranberry, and strawberry), we nevertheless detected a reversal
590 in the fitness correlation between cherry and cranberry media.

591 Fitness changes could be caused by temporal variation in environmental conditions in
592 the laboratory (e.g., change of quality of frozen fruit purees). However, temporal environmental
593 variation would be unlikely to result in adaptation to selective media and thus unlikely to
594 explain our findings.

595 While the temporary pooling of replicate populations most likely facilitated further
596 adaptation by reducing inbreeding depression, it might have limited our power of inference
597 regarding the variation in fitness changes among replicate populations. Indeed, changes in
598 fitness of replicate populations in each selective medium or in the two alternative media tended
599 to be in the same direction during phase 3. This pattern might be due to the co-ancestry of
600 replicate populations. In other words, the observed fitness changes might have evolved in a
601 single replicate population during phase 1 or phase 2, rather than several times independently

602 in each replicate population during the 16 later generations. We consider this hypothesis to be
603 unlikely for four reasons. First, the fitness of replicate populations evolving on each fruit
604 increased significantly between the intermediate and final phenotyping steps, when populations
605 evolved independently. This demonstrates that the observed fitness changes are partly
606 independent of each other. Second, at the end of phase 3, fitness changes in selective or
607 alternative environments varied among replicate populations evolving on the same fruit
608 medium, which supports the view that fitness changes are partially independent. Third, the
609 simulations of unequal contributions of replicate populations to the pool during phase 2 show
610 that the pattern of reversal in the association between fitness changes in selective and
611 alternative environments (Fig. 5D) cannot be explained by the pooling step (see Appendix S4,
612 Fig. S10 and S11). Fourth, the effect of pooling on reducing inbreeding depression is probably
613 stronger than its effect on increasing the frequency of adaptive mutations. Indeed, we expect
614 the five generations of pooling would mask the effect of the numerous deleterious alleles
615 located all over the genome but would not favor the spread of beneficial alleles confined to
616 particular loci within the genome.

617 **Recommendations for using fitness landscapes to interpret selection experiments**

618 We can make several recommendations for future studies that aim to track and predict the
619 evolutionary trajectories of experimental populations evolving in contrasted environments.
620 First, measuring fitness rather than fitness proxies allows for a standard comparison among
621 populations reared on different (i.e., selective and alternative) environments. For example,
622 fitness can be used to compare populations evolving with different maintenance schemes (for
623 example, egg-to-adult viability over two weeks versus two months). Second, when using
624 experimental evolution to study fitness changes in selective and alternative environments, the
625 likely position of the ancestral population in the fitness landscape relative to the phenotypic
626 optima of selective environments should be considered with care. For example, many studies

627 of ecological specialization using experimental evolution find positive instead of negative
628 fitness correlated responses in alternative environments (Futuyma 2008). As explained in the
629 introduction, these results can be explained by two alternative and mutually exclusive
630 hypotheses (Fig. 1). On the one hand, if the environments have the same fitness optima,
631 additional generations of experimental evolution would still result in positive correlation in
632 fitness across environments (Fig. 1A). On the other hand, if the environments have different
633 fitness optima, the level of maladaptation of the ancestral population matters. When starting
634 from an ancestral population similarly maladapted to the two environments, additional
635 generations of experimental evolution would result in negative fitness correlation across
636 environments (Fig. 1B), as illustrated in our study. Following other studies (e.g., Fragata et al.
637 2019), we emphasize that fitness landscape theory represents a powerful framework for
638 studying the process of local adaptation using experimental evolution. Third, how long it takes
639 for experimental evolution to show a reversal in the direction of fitness changes across
640 environments depends on the level of adaptive genetic variation in the ancestral population.
641 Negative correlations in fitness can evolve over short time scales when initial levels of standing
642 adaptive genetic variation are high, as exemplified in our study. In contrast, negative
643 correlations in fitness likely evolve more slowly in studies based on de novo mutation, as
644 illustrated by Bono et al 2017.

645 **Conclusion and perspectives**

646 We found temporal adaptation in *D. suzukii* experimental populations evolving in three
647 different selective environments. Adaptation to each fruit was associated with an increase in
648 fitness in the two other fruits with the exception of populations that evolved either on cherry or
649 on cranberry medium. Our results show that the temporal study of fitness changes in selective
650 and alternative environments across multiple generations allows a better characterization of the
651 dynamics of local adaptation compared to typical cross-sectional studies performed over a

652 single generation. This framework could improve our understanding of the ecological factors
653 that drive the evolution of local adaptation.
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662 Data availability statement

663 Data code for our analyses are available at Dryad https://doi.org/10.5061/dryad.crjdfn33t
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667 # References

668 Agudelo-Romero, P., F. de la Iglesia, and S. F. Elena. 2008. The pleiotropic cost of host-
669 specialization in Tobacco etch potyvirus. Infect. Genet. Evol. J. Mol. Epidemiol.
670 Evol. Genet. Infect. Dis. 8:806–814.
671 Asplen, M. K., G. Anfora, A. Biondi, D. S. Choi, D. Chu, K. M. Daane, P. Gibert, A. P.
672 Gutierrez, K. A. Hoelmer, W. D. Hutchison, R. Isaacs, Z. L. Jiang, Z. Karpati, M. T.
673 Kimura, M. Pascual, C. R. Philips, C. Plantamp, L. Ponti, G. Vetek, H. Vogt, V. M.
674 Walton, Y. Yu, L. Zappala, and N. Desneux. 2015. Invasion biology of spotted wing
675 drosophila (*Drosophila suzukii*): a global perspective and future priorities. J. Pest Sci.
676 88:469–494.
677 Backhaus, B., E. Sulkowski, and F. Schlote. 1984. A semi-synthetic, general-purpose
678 medium for Drosophila melanogaster. Dros Inf Serv.
679 Barghi, N., J. Hermisson, and C. Schlötterer. 2020. Polygenic adaptation: a unifying
680 framework to understand positive selection. Nat. Rev. Genet. 21:769–781. Nature
681 Publishing Group.
682 Barghi, N., R. Tobler, V. Nolte, A. M. Jakšić, F. Mallard, K. A. Otte, M. Dolezal, T. Taus, R.
683 Kofler, and C. Schlötterer. 2019. Genetic redundancy fuels polygenic adaptation in
684 Drosophila. PLOS Biol. 17:e3000128. Public Library of Science.
685 Barrett, R. D. H., and D. Schluter. 2008. Adaptation from standing genetic variation. Trends
686 Ecol. Evol. 23:38–44.
687 Barton, K. 2009. MuMIn : multi-model inference, R package version 0.12.0. Httpr-Forg.-
688 Proj.
689 Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models
690 using lme4. J. Stat. Softw. 67:1–48.
691 Bedhomme, S., G. Lafforgue, and S. F. Elena. 2012. Multihost experimental evolution of a

692 plant RNA virus reveals local adaptation and host-specific mutations. Mol. Biol. Evol.
693 29:1481–1492.
694 Bellamy, D. E., M. S. Sisterson, and S. S. Walse. 2013. Quantifying host potentials: indexing
695 postharvest fresh fruits for spotted wing drosophila, *Drosophila suzukii*. PLoS ONE 8.
696 Bennett, A. F., K. M. Dao, and R. E. Lenski. 1990. Rapid evolution in response to high-
697 temperature selection. Nature 346:79–81.
698 Blanquart, F., O. Kaltz, S. L. Nuismer, and S. Gandon. 2013. A practical guide to measuring
699 local adaptation. Ecol. Lett. 16:1195–1205.
700 Bono, L. M., L. B. Smith, D. W. Pfennig, and C. L. Burch. 2017. The emergence of
701 performance trade-offs during local adaptation: insights from experimental evolution.
702 Mol. Ecol. 26:1720–1733.
703 Burke, M. K., G. Liti, and A. D. Long. 2014. Standing Genetic Variation Drives Repeatable
704 Experimental Evolution in Outcrossing Populations of Saccharomyces cerevisiae.
705 Mol. Biol. Evol. 31:3228–3239. Oxford Academic.
706 Burnham, K. P., and D. R. Anderson. 2002. Model Selection and Multimodel Inference: A
707 Practical Information-Theoretic Approach. 2nd ed. Springer-Verlag, New York.
708 Cini, A., C. Ioriatti, and G. Anfora. 2012. A review of the invasion of *Drosophila suzukii* in
709 Europe and a draft research agenda for integrated pest management. Bull. Insectology
710 65:149–160.
711 Deakin, M. A. B. 1966. Sufficient conditions for genetic polymorphism. Am. Nat. 110:690–
712 692.
713 Emiljanowicz, L. M., G. D. Ryan, A. Langille, and J. Newman. 2014. Development,
714 reproductive output and population growth of the fruit fly pest *Drosophila suzukii*
715 (*Diptera: Drosophilidae*) on artificial diet. J. Econ. Entomol. 107:1392–1398.
716 Felsenstein, J. 1976. The theoretical population genetics of variable selection and migration.

717 Annu. Rev. Genet. 10:253–280.

718 Forister, M., A. L. Dyer, S. M. Singer, O. J. Stireman, and J. T. Lill. 2012. Revisiting the
719 evolution of ecological specialization, with emphasis on insect-plant interactions.
720 Ecology 93:981–991.

721 Fragata, I., A. Blanckaert, M. A. Dias Louro, D. A. Liberles, and C. Bank. 2019. Evolution in
722 the light of fitness landscape theory. Trends Ecol. Evol. 34:69–82.

723 Fry, J. D. 2003. Detecting ecological trade-offs using selection experiments. Ecology
724 84:1672–1678.

725 Fry, J. D. 2001. Direct and correlated responses to selection for larval ethanol tolerance in
726 Drosophila melanogaster. J. Evol. Biol. 14:296–309.

727 Fry, J. D. 1996. The evolution of host specialization: are trade-offs overrated? Am. Nat.
728 148:84–107.

729 Fry, J. D. 1990. Trade-offs in fitness on different hosts: evidence from a selection experiment
730 with a phytophagous mite. Am. Nat. 136:569–580.

731 Futuyma. 2008. Sympatric speciation: norm or exception? Pp. 136–148 *in* Specialization,
732 Speciation, and Radiation: the evolutionary biology of herbivorous insects. K. J.
733 University of California Press, Berkeley, California, USA.

734 Gillespie, J. H. 1975. The Role of Migration in the Genetic Structure of Populations in
735 Temporarily and Spatially Varying Environments. I. Conditions for Polymorphism.
736 Am. Nat. 109:127–136. The University of Chicago Press.

737 Gillespie, J. H., and M. Turelli. 1989. Genotype-environment interactions and the
738 maintenance of polygenic variation. Genetics 121:129–138. Genetics.

739 Gompert, Z., and F. J. Messina. 2016. Genomic evidence that resource-based trade-offs limit
740 host-range expansion in a seed beetle. Evolution 70:1249–1264.

741 Gomulkiewicz, R., and R. D. Holt. 1995. When does evolution by natural selection prevent

742 extinction? Evolution 49:201–207.

743 Hansen, T. F., A. J. R. Carter, and C. Pélabon. 2006. On Adaptive Accuracy and Precision in
744 Natural Populations. Am. Nat. 168:168–181. The University of Chicago Press.

745 Hedrick, P. W., and A. Garcia-Dorado. 2016. Understanding Inbreeding Depression, Purging,
746 and Genetic Rescue. Trends Ecol. Evol. 31:940–952.

747 Hereford, J. 2009. A quantitative survey of local adaptation and fitness trade-offs. Am. Nat.
748 173:579–588.

749 Hurvich, C. M., and C.-L. Tsai. 1989. Regression and time series model selection in small
750 samples. Biometrika 76:297–307. Oxford Academic.

751 Jasmin, J.-N., and C. Zeyl. 2013. Evolution of pleiotropic costs in experimental populations.
752 J. Evol. Biol. 26:1363–1369.

753 Kanda, Y., K. NAMIKAWA, and H. WATABE. 2019. Biology of the Spotted-Wing
754 Drosophila suzukii (Diptera: Drosophilidae) in Hokkaido, Northern Japan. III.
755 Breeding on Blueberries. J. Hokkaido Univ. Educ. 71:6.

756 Kassen, R. 2002. The experimental evolution of specialists, generalists, and the maintenance
757 of diversity. J. Evol. Biol. 15:173–190.

758 Kawecki, T. J., R. E. Lenski, D. Ebert, B. Hollis, I. Olivieri, and M. C. Whitlock. 2012.
759 Experimental evolution. Trends Ecol. Evol. 27:547–560.

760 Kenis, M., L. Tonina, R. Eschen, B. van der Sluis, M. Sancassani, N. Mori, T. Haye, and H.
761 Helsen. 2016. Non-crop plants used as hosts by *Drosophila suzukii* in Europe. J. Pest
762 Sci. 89:735–748.

763 Kessner, D., and J. Novembre. 2015. Power analysis of artificial selection experiments using
764 efficient whole genome simulation of quantitative traits. Genetics 199:991–1005.

765 Koch, E. L., and F. Guillaume. 2020. Additive and mostly adaptive plastic responses of gene
766 expression to multiple stress in Tribolium castaneum. PLOS Genet. 16:e1008768.

767 Public Library of Science.

768 Laukkanen, L., R. Leimu, A. Muola, M. Lilley, J.-P. Salminen, and P. Mutikainen. 2012.

769 Plant chemistry and local adaptation of a specialized folivore. PLOS ONE 7:e38225.

770 Lee, J. C., P. W. Shearer, L. D. Barrantes, E. H. Beers, H. J. Burrack, D. T. Dalton, A. J.

771 Dreves, L. J. Gut, K. A. Hamby, D. R. Haviland, R. Isaacs, A. L. Nielsen, T.

772 Richardson, C. R. Rodriguez-Saona, C. A. Stanley, D. B. Walsh, V. M. Walton, W. L.

773 Yee, F. G. Zalom, and D. J. Bruck. 2013. Trap designs for monitoring *Drosophila*

774 *suzukii* (Diptera: Drosophilidae). Environ. Entomol. 42:1348–1355.

775 Legendre, P. 2014. lmodel2: Model II Regression. R package version 1.7-2.

776 Levins, R. 1968. Evolution in changing environments: some theoretical explorations.

777 Princeton University Press.

778 Levins, R. 1962. Theory of fitness in a heterogeneous environment. I. The fitness set and

779 adaptive function. Am. Nat. 96:361–373.

780 Lüdecke, D. 2018. sjstats: Statistical Functions for Regression Models. Zenodo.

781 Mackenzie, A. 1996. A trade-off for host plant utilization in the black bean aphid, aphis

782 fabae. Evolution 50:155–162.

783 Magalhães, S., E. Blanchet, M. Egas, and I. Olivieri. 2009. Are adaptation costs necessary to

784 build up a local adaptation pattern? BMC Evol. Biol. 9:182.

785 Martin, G., and T. Lenormand. 2015. The fitness effect of mutations across environments:

786 Fisher's geometrical model with multiple optima. Evolution 69:1433–1447.

787 Messina, F. J., and S. L. Durham. 2013. Adaptation to a novel host by a seed beetle

788 (Coleoptera: Chrysomelidae: Bruchinae): effect of source population. Environ.

789 Entomol. 42:733–742.

790 Messina, F. J., and S. L. Durham. 2015. Loss of adaptation following reversion suggests

791 trade-offs in host use by a seed beetle. J. Evol. Biol. 28:1882–1891.

792 Messina, F. J., M. Mendenhall, and J. C. Jones. 2009. An experimentally induced host shift in
793 a seed beetle. Entomol. Exp. Appl. 132:39–49.
794 Olazcuaga, L. 2019. Adaptive responses of Drosophila suzukii, a generalist invasive species.
795 Supagro/INRA ; Ecole Doctorale GAIA, Université de Montpellier, France.
796 Olazcuaga, L., N. O. Rode, J. Foucaud, B. Facon, V. Ravigné, A. Ausset, N. Leménager, A.
797 Loiseau, M. Gautier, A. Estoup, and R. A. Hufbauer. 2019. Oviposition preference
798 and larval performance of *Drosophila suzukii* (Diptera: Drosophilidae), spotted-wing
799 drosophila: effects of fruit identity and composition. Environ. Entomol. 48:867–881.
800 Poyet, M., V. Le Roux, P. Gibert, A. Meirland, G. Prevost, P. Eslin, and O. Chabrerie. 2015.
801 The wide potential trophic niche of the asiatic fruit fly *Drosophila suzukii*: The key of
802 its invasion success in temperate Europe? PLoS ONE 10.
803 R Core Team. 2014. R: A language environment for statistical computing.
804 Rausher, M. D. 1988. Plant-Insect Interfaces. Ecology 69:295–296.
805 Roughgarden, J. 1979. Theory of Population Genetics and Evolutionary Ecology: An
806 Introduction. P. 266 *in*. MacMillan Publishing Co, New York.
807 Satterwhite, R. S., and T. F. Cooper. 2015. Constraints on adaptation of Escherichia coli to
808 mixed-resource environments increase over time. Evolution 69:2067–2078.
809 Schick, A., S. F. Bailey, and R. Kassen. 2015. Evolution of Fitness Trade-Offs in Locally
810 Adapted Populations of Pseudomonas fluorescens. Am. Nat. 186:S48–S59. The
811 University of Chicago Press.
812 Steffan, S. A., J. C. Lee, M. E. Singleton, A. Vilaire, D. B. Walsh, L. S. Lavine, and K.
813 Patten. 2013. Susceptibility of cranberries to *Drosophila suzukii* (Diptera:
814 Drosophilidae). J. Econ. Entomol. 106:2424–2427.
815 Stewart, G. S., M. R. Morris, A. B. Genis, M. Szűcs, B. A. Melbourne, S. J. Tavener, and R.
816 A. Hufbauer. 2017. The power of evolutionary rescue is constrained by genetic load.

817 Evol. Appl. 10:731–741.

818 Svardal, H., C. Rueffler, and J. Hermisson. 2015. A general condition for adaptive genetic
819 polymorphism in temporally and spatially heterogeneous environments. Theor. Popul.
820 Biol. 99:76–97.

821 Turner, P. E., and S. F. Elena. 2000. Cost of Host Radiation in an RNA Virus. Genetics
822 156:1465–1470.

823 Van den Bergh, B., T. Swings, M. Fauvart, and J. Michiels. 2018. Experimental Design,
824 Population Dynamics, and Diversity in Microbial Experimental Evolution. Microbiol.
825 Mol. Biol. Rev. 82. American Society for Microbiology.

826 Walsh, D. B., M. P. Bolda, R. E. Goodhue, A. J. Dreves, J. C. Lee, D. J. Bruck, V. M.
827 Walton, S. D. O'Neal, and F. G. Zalom. 2011. *Drosophila suzukii* (Diptera:
828 Drosophilidae): invasive pest of ripening soft fruit expanding its geographic range and
829 damage potential. J. Integr. Pest Manag. 2:1–7.

830 Whiteley, A. R., S. W. Fitzpatrick, W. C. Funk, and D. A. Tallmon. 2015. Genetic rescue to
831 the rescue. Trends Ecol. Evol. 30:42–49.

832 Woodworth, L. M., M. E. Montgomery, D. A. Briscoe, and R. Frankham. 2002. Rapid
833 genetic deterioration in captive populations: Causes and conservation implications.
834 Conserv. Genet. 3:277–288.

835 Zou, G. Y. 2007. Toward using confidence intervals to compare correlations. Psychol.
836 Methods 12:399–413. American Psychological Association, US.

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Tables

Table 1: Results of the AICc model selection for comparing temporal dynamics of adaptation across the three selective fruits. All models included *generation:selective_fruit* as a random effect.

Model	Effects	df	logLik	AICc	ΔAICc
(1)	*Phase* + *Selective_fruit*	7	-122.68	259.80	0.00
(2)	*Phase*	5	-126.51	263.30	3.44
(3)	*Phase* × *Selective_fruit*	11	-121.28	265.70	5.85
(4)	*Generation* + *Selective_fruit*	6	-137.59	287.50	27.69
(5)	*Generation*	4	-140.17	288.50	28.67
(6)	*Generation* × *Selective_fruit*	8	-136.99	290.60	30.76
(7)	Null Model	3	-155.00	316.10	56.26
(8)	*Selective_fruit*	5	-153.12	316.50	56.64

861 **Table 2: Estimate of the change in fitness between the initial and either the intermediate**
862 **or final phenotyping steps based on the negative binomial model (log scale).**

Phenotyping step	Selective fruit	Test fruit	Estimate	95% confidence interval
Intermediate	Cherry	Cherry	-0.02	[-0.44 ; 0.37]
		Cranberry	-0.17	[-0.61 ; 0.24]
		Strawberry	0.15	[-0.32 ; 0.54]
	Cranberry	Cherry	-0.01	[-0.46 ; 0.42]
		Cranberry	0.18	[-0.26 ; 0.52]
		Strawberry	0.25	[-0.17 ; 0.61]
	Strawberry	Cherry	0.46	[0.06 ; 0.81]
		Cranberry	0.27	[-0.08 ; 0.65]
		Strawberry	0.33	[-0.02 ; 0.68]
Final	Cherry	Cherry	0.26	[-0.12 ; 0.6]
		Cranberry	-0.33	[-0.68 ; 0.05]
		Strawberry	0.03	[-0.37 ; 0.38]
	Cranberry	Cherry	0.25	[-0.11 ; 0.6]
		Cranberry	0.18	[-0.16 ; 0.52]
		Strawberry	-0.38	[-0.75 ; -0.06]
	Strawberry	Cherry	0.02	[-0.36 ; 0.41]
		Cranberry	0.12	[-0.24 ; 0.51]
		Strawberry	0.37	[-0.07 ; 0.74]
Overall variance among replicate populations			0.14	[0.01 ; 0.17]

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Figure legends

Figure 1. Changes in fitness in selective and alternative environments depend on the level of adaptation of the ancestral population and on the relative positions of the phenotypic optima. In a two-dimensional fitness landscape, phenotypic optima for environments 1 and 2 (red and blue crosses respectively) are either (A) closed to each other or (B) distant from each other. In the left panels, the ancestral population, the population evolving in environments 1 and the population evolving in environments 2 are represented by grey, red and blue ellipses, respectively. In the right panels, the fitness changes relative to the ancestral population (step 0) in the selective environment or in the alternative environment are represented for step 1 and 2, respectively. Open and closed ellipses respectively correspond to early (step 1) and late (step 2) adaptation.

Figure 2. Experimental evolution design depicting the different fruit media and the three phenotyping steps.
Before each phenotyping step, populations spend one generation in a common environment (standard laboratory fly medium), represented by a black line. For each phenotyping step and each population, we measured fitness in each of the three fruit media with an average of 9 (range: 2-32) and 30 vials for the intermediate and final phenotyping steps, respectively. During the intermediate phenotyping step, fitness on alternative environment was not measured for one replicate population evolving on cherry due to small population size.

Figure 3. Temporal dynamics of the mean fitness of populations evolved on (A) cherry, (B) cranberry or (C) strawberry during the three phases of the experimental evolution. Malthusian fitness (solid line) was estimated for each of the three phases of the experiment. To avoid the confoundings of maternal effects, data from generations where individuals or their parents developed in standard medium were removed. Error bars represent standard deviation among tubes. The level of significance is provided above horizontal bars (**: P-value < 0.01; ***: P-value < 0.001).

Figure 4. Change in fitness (A) between the initial and intermediate phenotyping steps and (B) between the initial and final phenotyping steps for each population x environment combination. Replicate populations are ordered following their fitness change on selective fruit medium (symbols with thick outline). The color indicates the selective environment, as shown at the top of each group of populations. The shape of the symbol indicates the test fruit as shown in the key to the right. Error bars represent 95% confidence intervals.

Figure 5. Relationship between the changes in fitness on selective and alternative fruit media between the intermediate and initial phenotyping step (A, B and C) and the final and initial phenotyping step (D, E and F). Solid lines represent fitted major axis slopes. Error bars represent 95% confidence intervals. Correlation coefficients are represented by $\boldsymbol{\rho}$ symbols. Values in brackets show 95% confidence intervals. Difference in correlation coefficients between the intermediate and final phenotyping steps for cherry vs. cranberry: 1.05 [0.37,

1.69], cranberry vs. strawberry: -0.06 [-0.65, 0.58] and strawberry vs. cherry: -0.06 [-0.60, 0.60].

Figure 6. Hypothetical fitness landscape to help in the interpretation of our results. For each environment, the position of the phenotypic optimum providing maximal fitness is represented by a cross. The positions of populations and genotypes within populations are respectively represented by ellipses and closed circles. We hypothesized that adaptation during phase 1 was masked by the increase in frequency of mildly deleterious mutations due to genetic drift (see Discussion), populations during the intermediate phenotyping step are represented closer to the fruit media optima than the ancestral population, although no significant fitness change was detected.

Figure

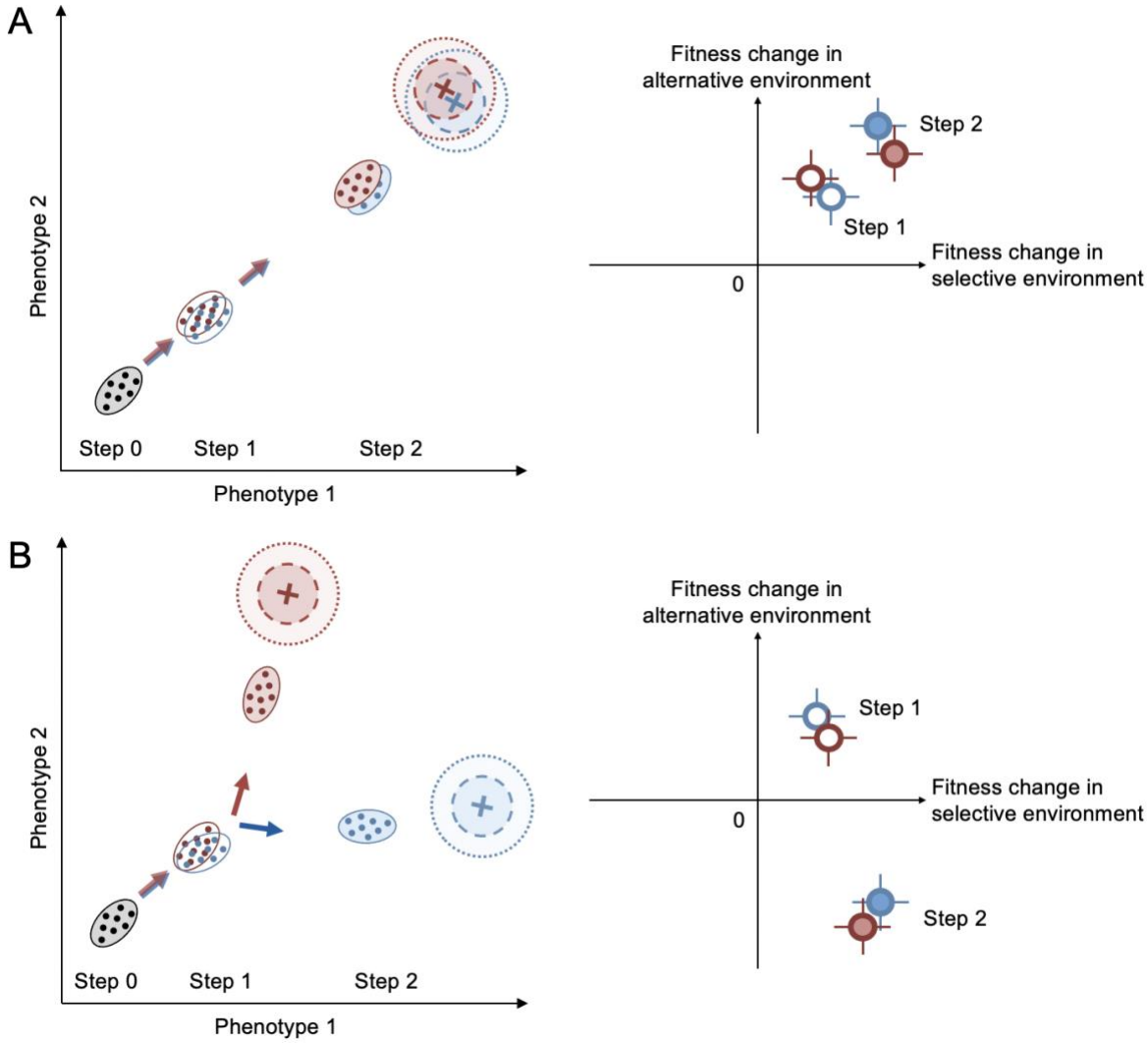
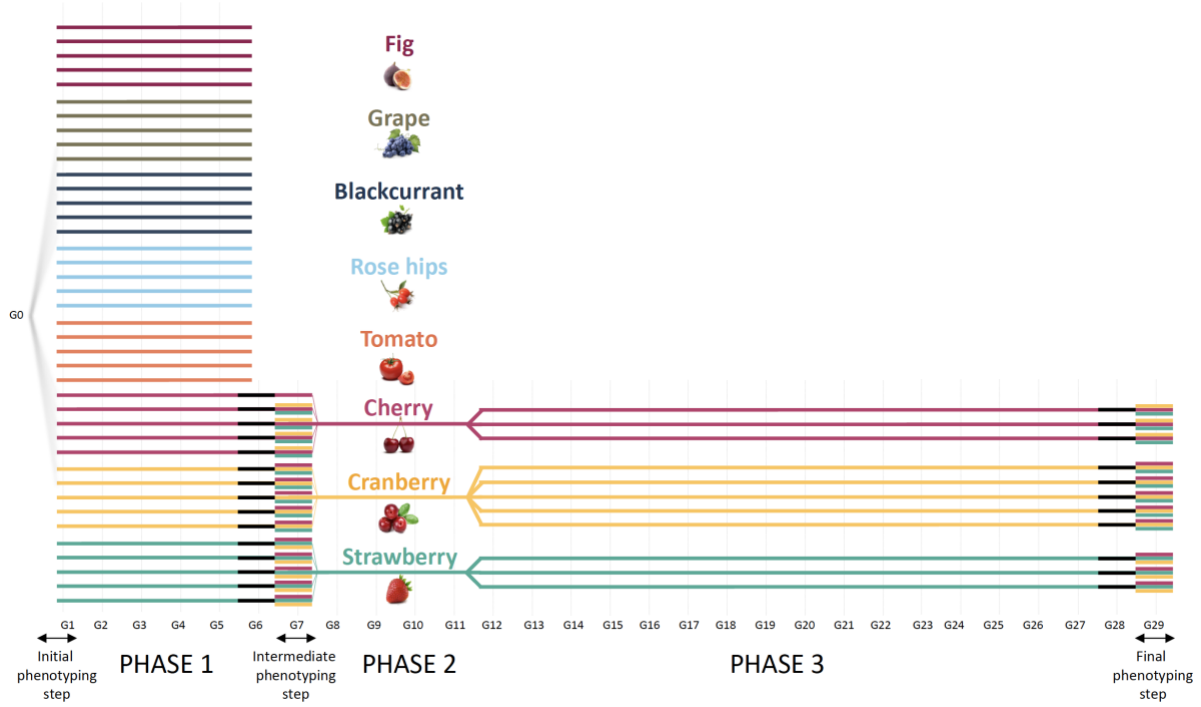


Figure 1.



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931 **Figure 2.**

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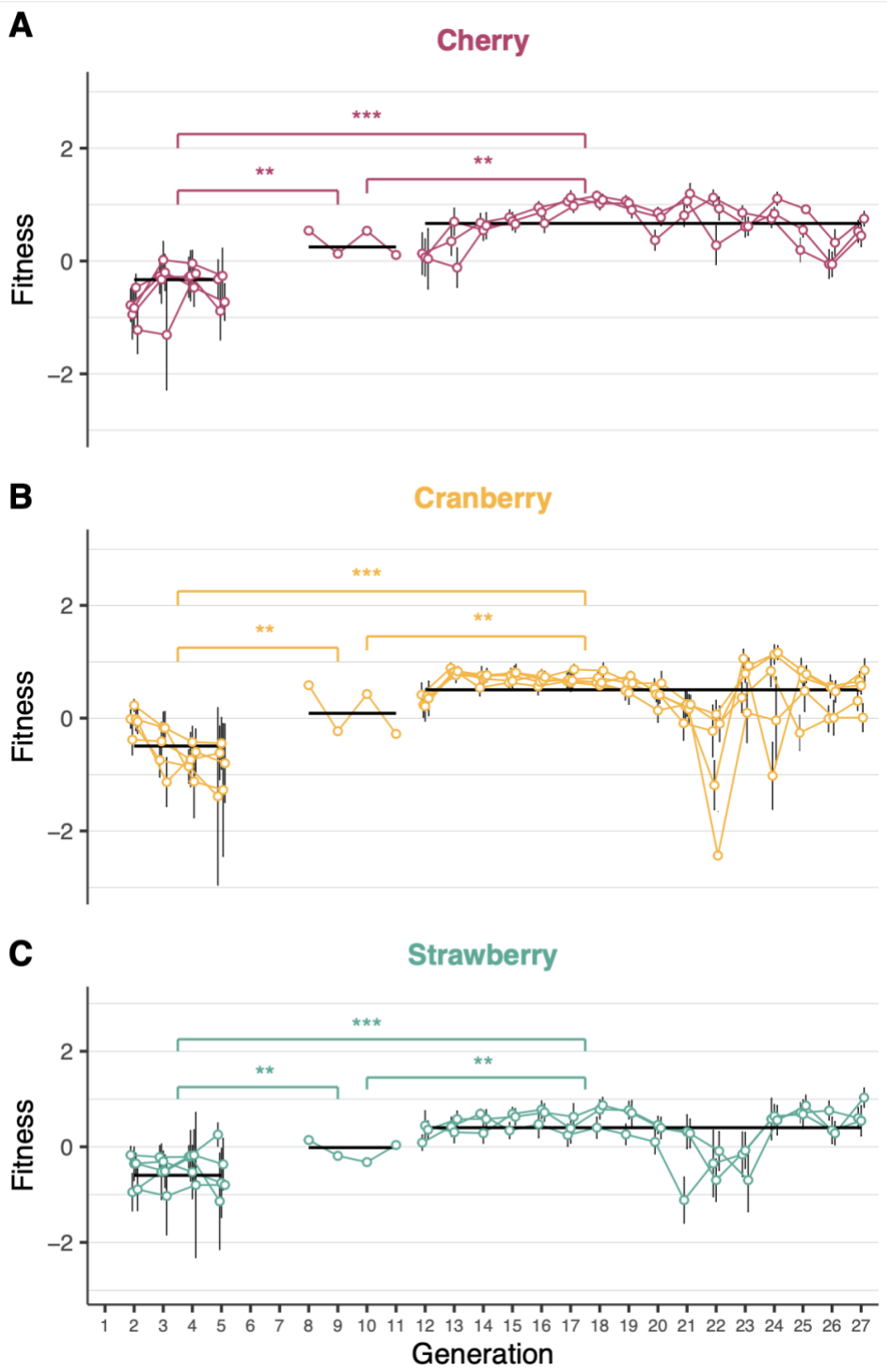

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937 **Figure 3.**

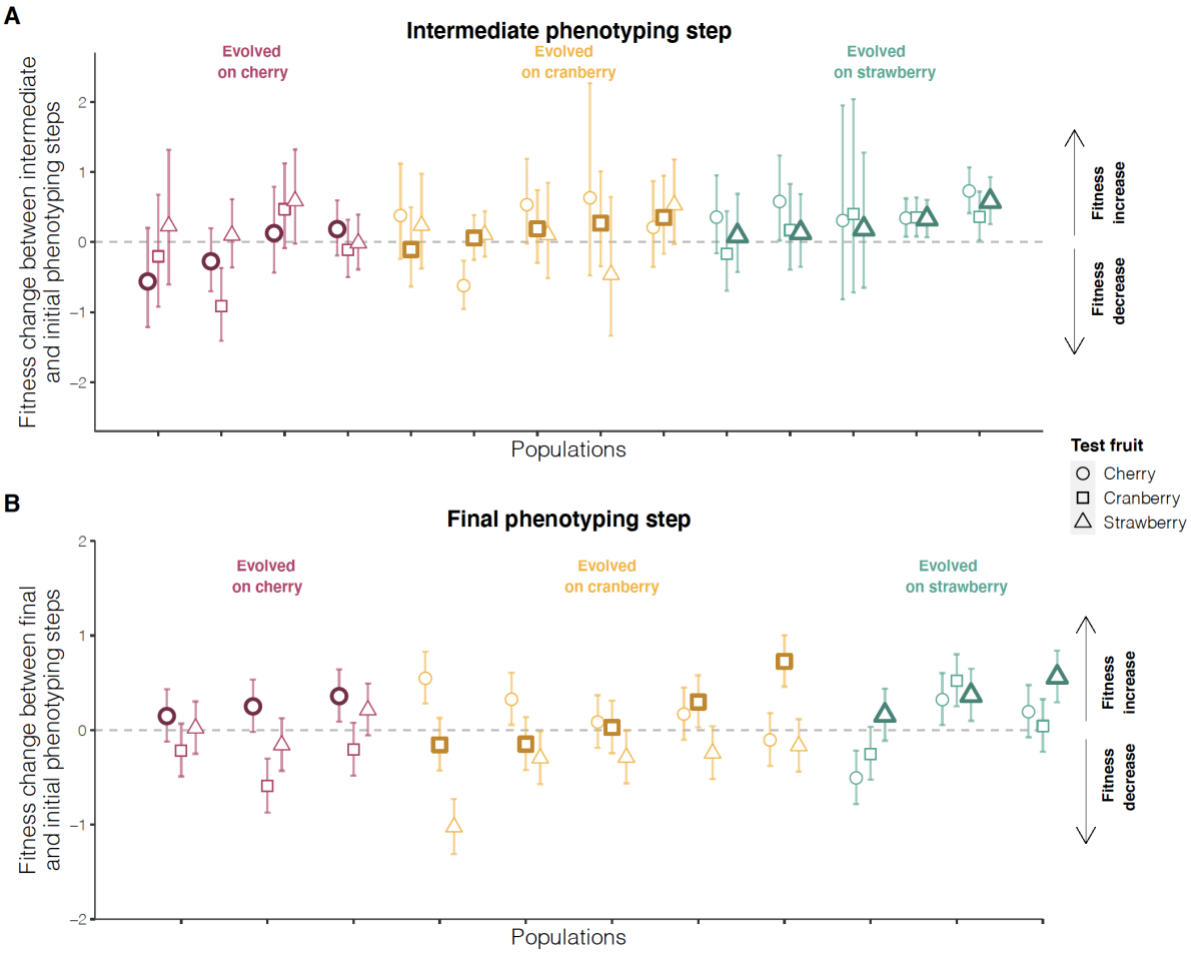

Figure 4.

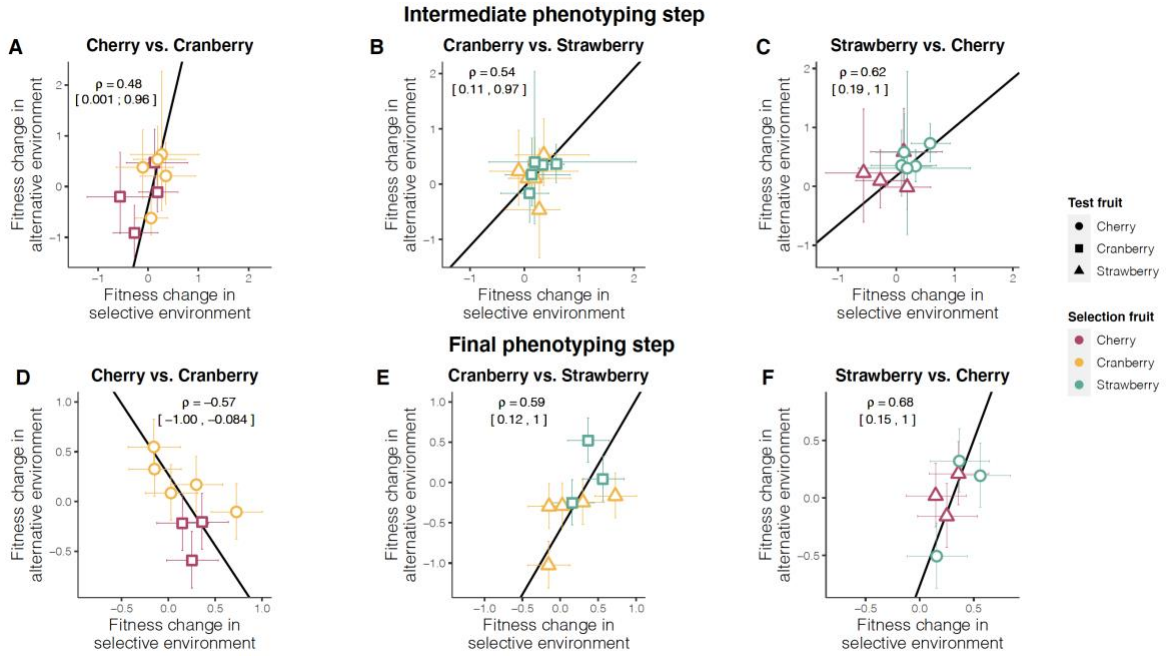

Figure 5.

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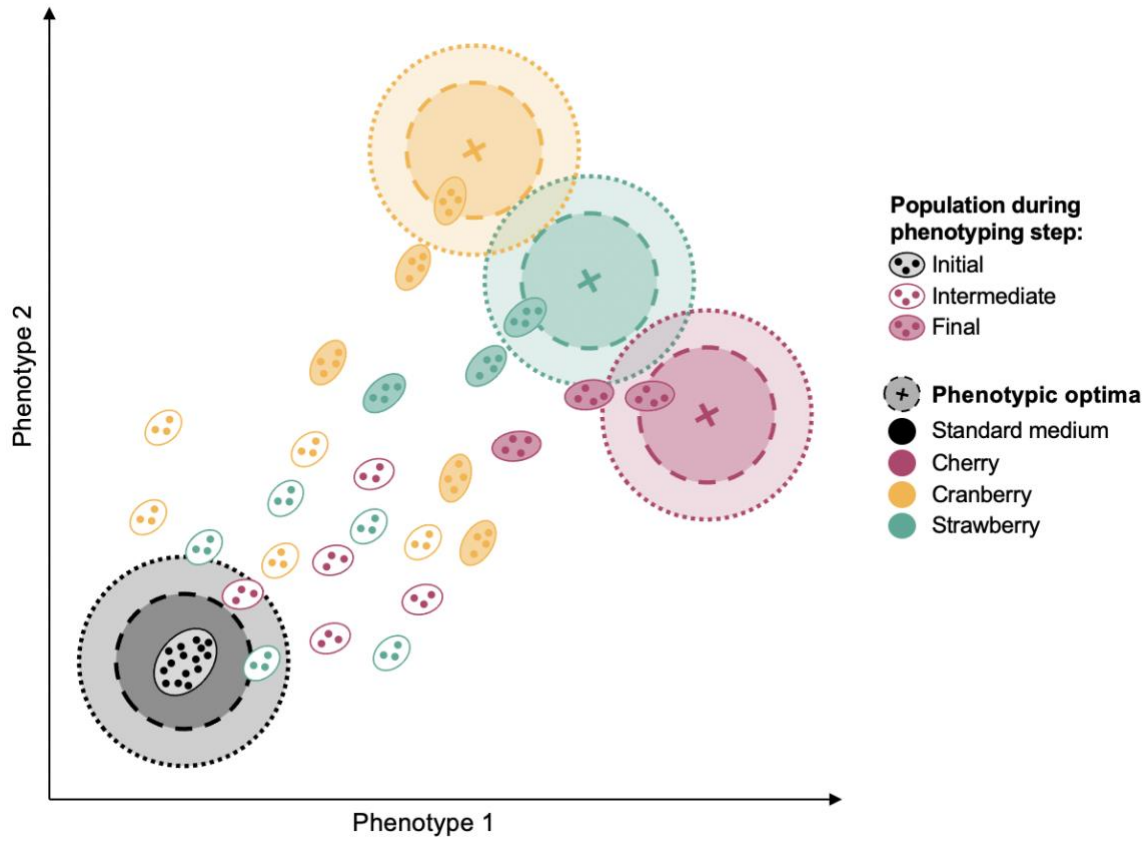

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951 **Figure 6.**
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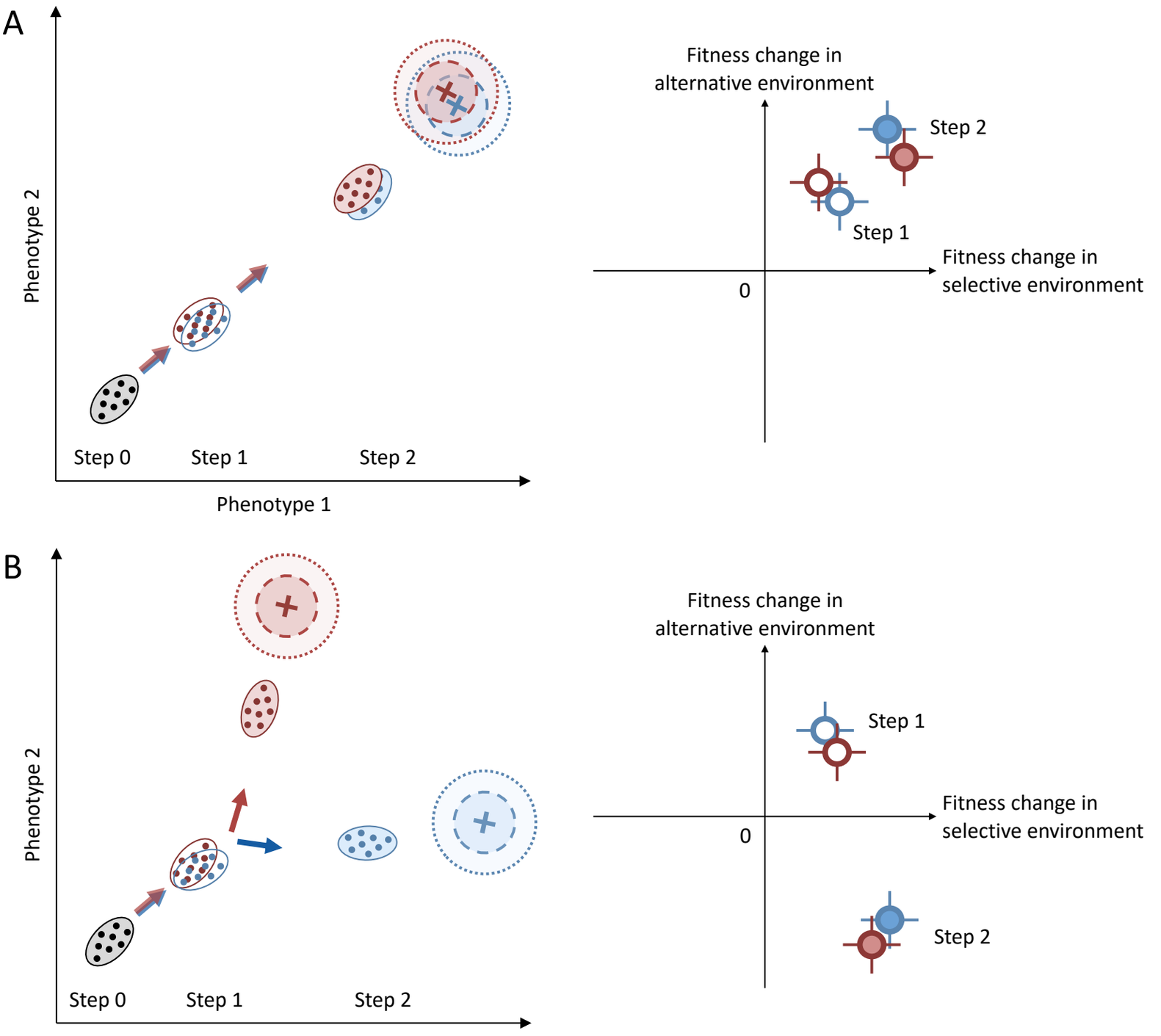

A
Phenotype 2
Step 0
Step 1
Step 2
Phenotype 1
Fitness change in
alternative environment
Step 2
Step 1
Fitness change in
selective environment
0
B
Phenotype 2
Step 0
Step 1
Step 2
Phenotype 1
Fitness change in
alternative environment
Step 1
Fitness change in
selective environment
0
Step 2

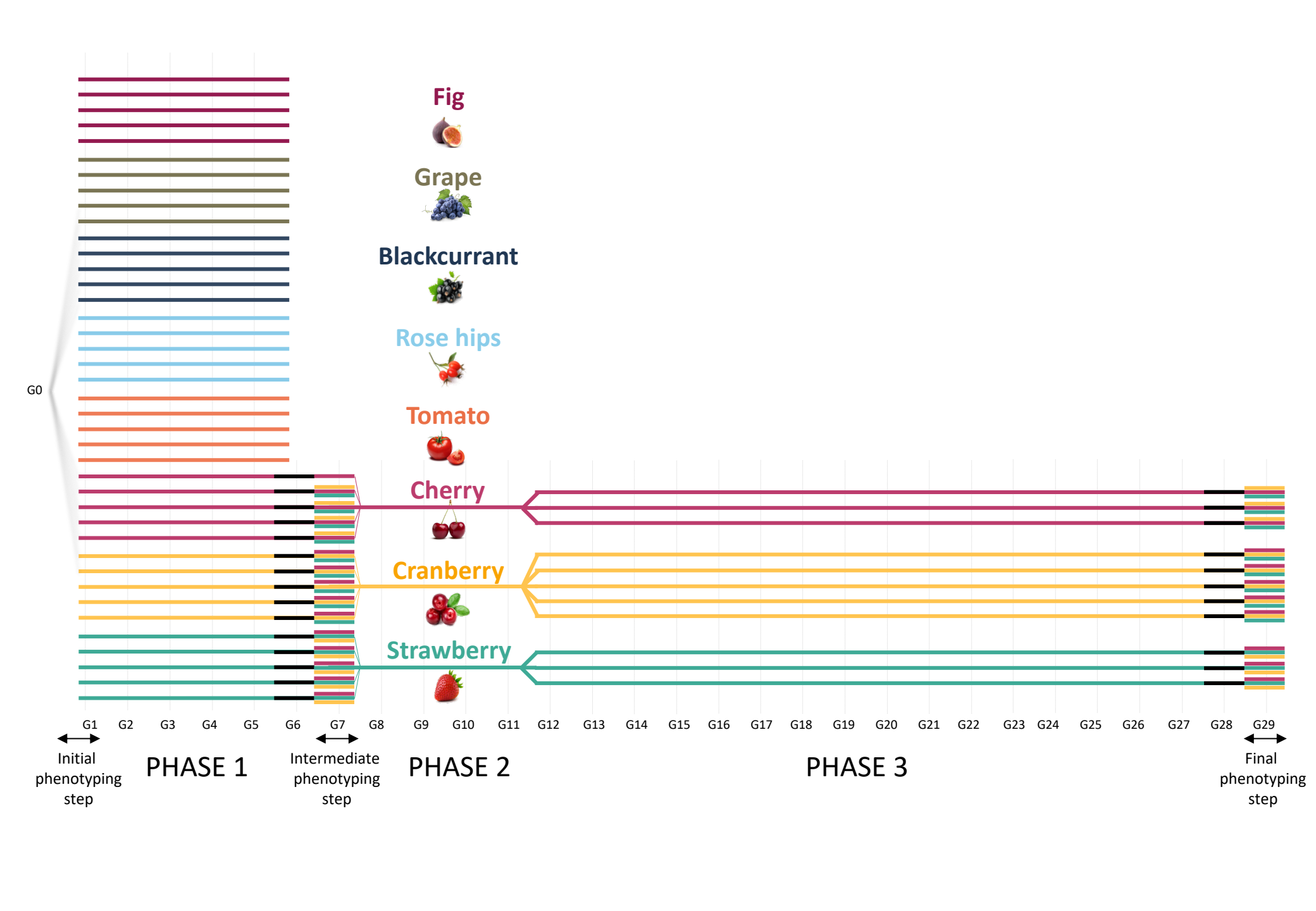
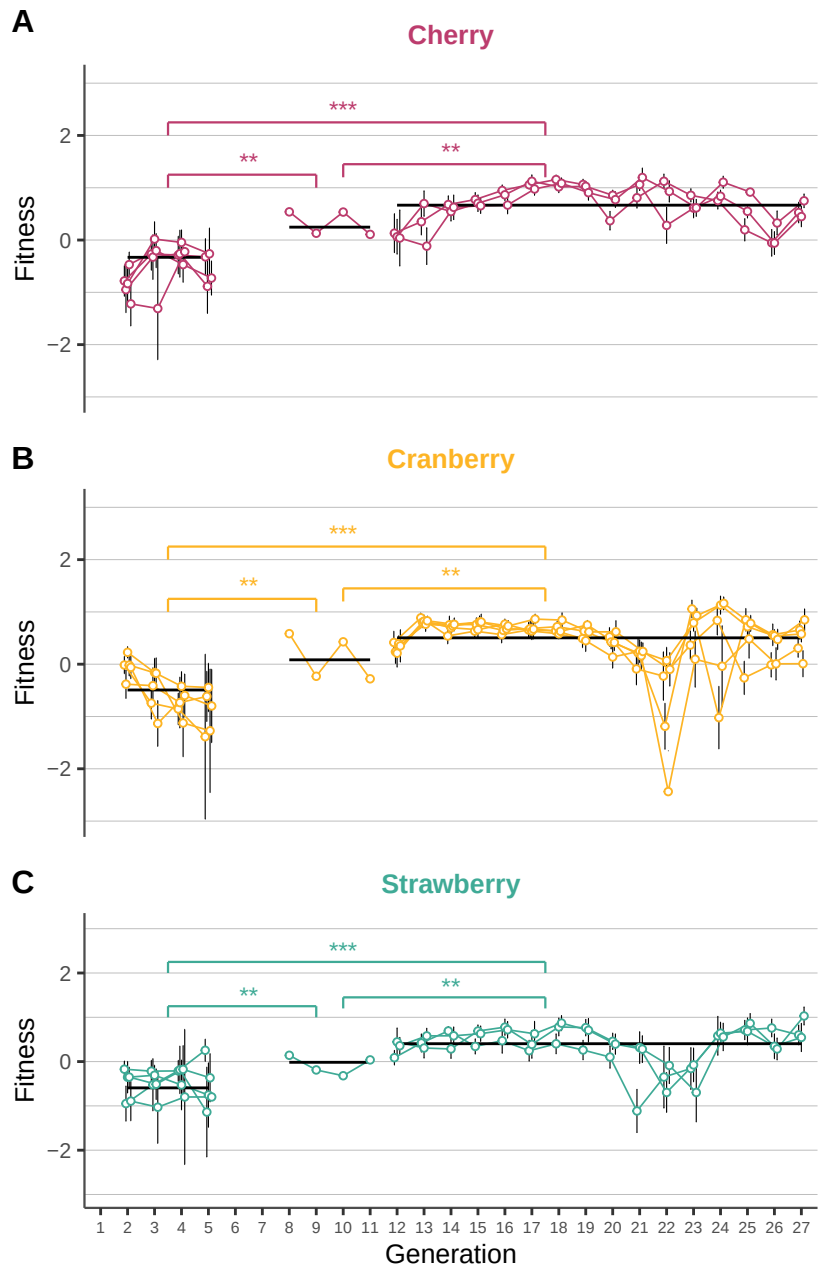
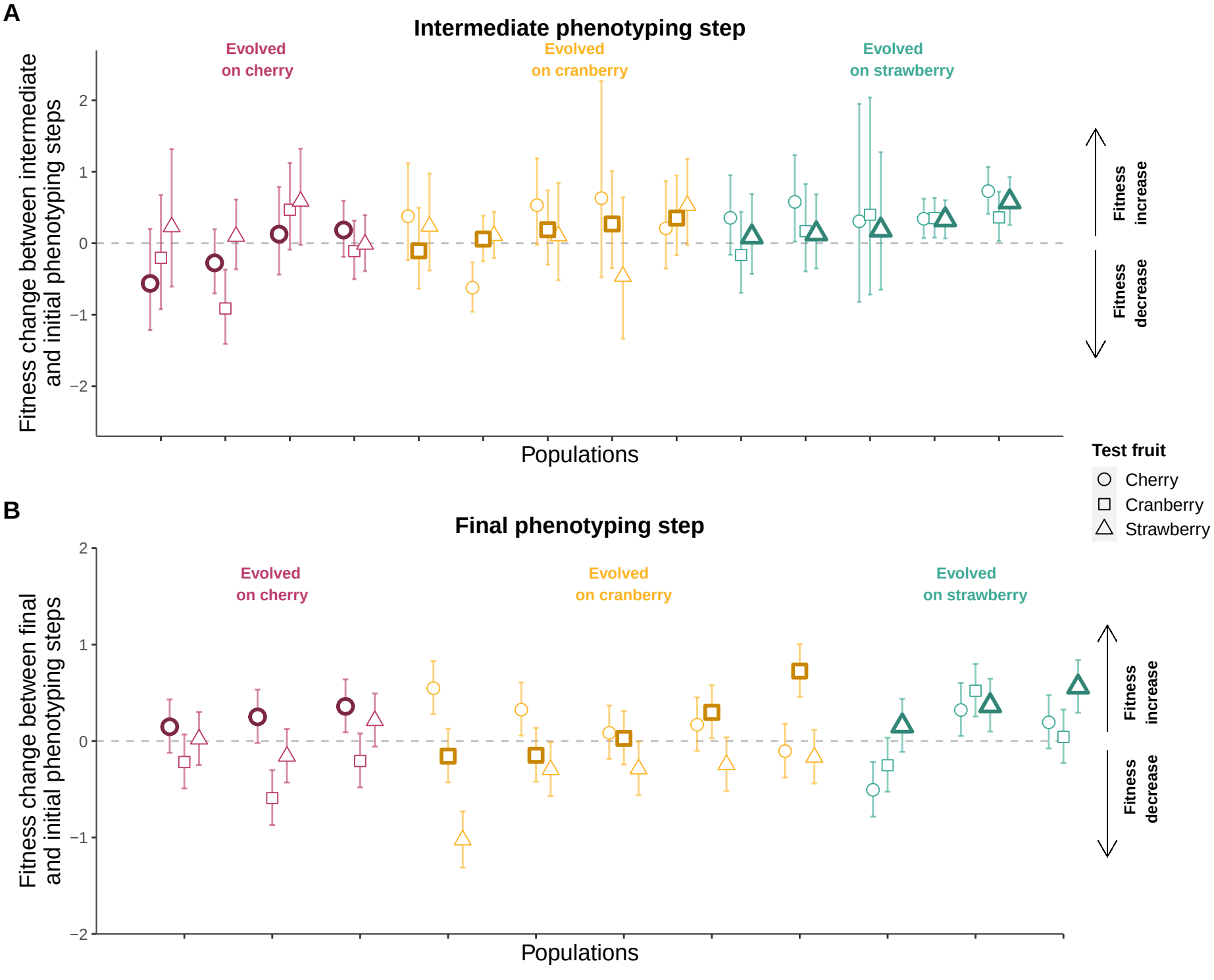
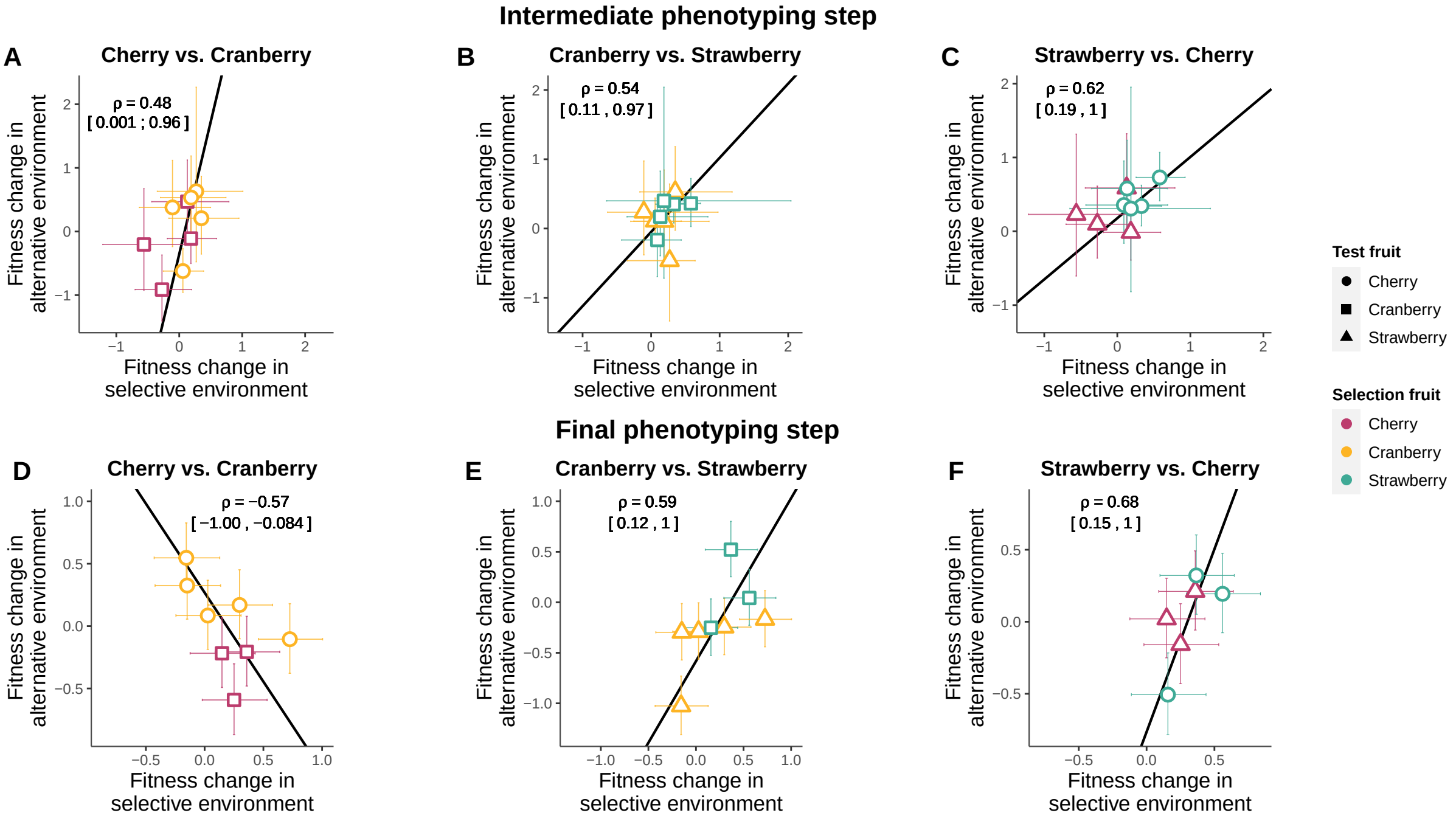
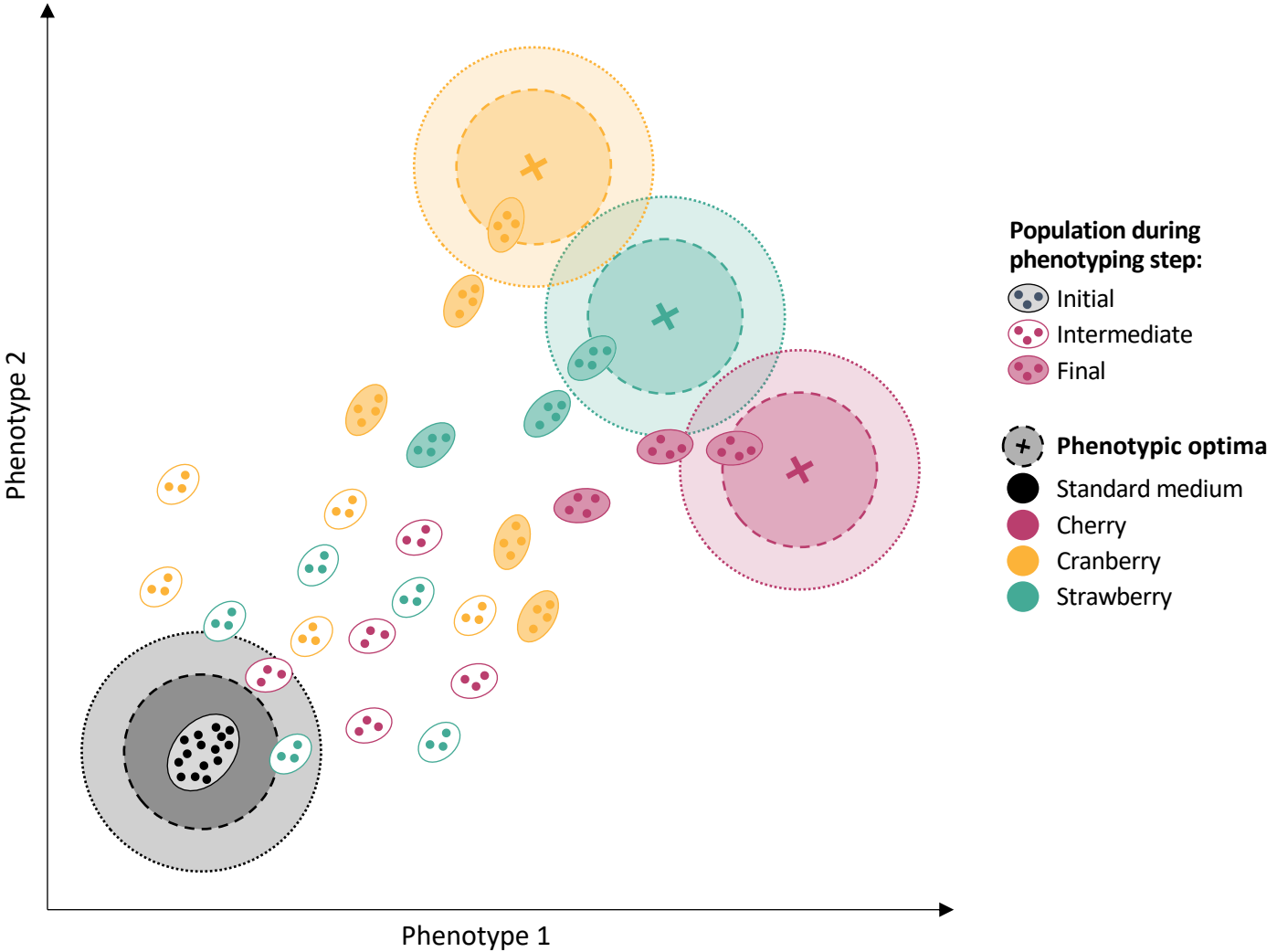


Fig
Grape
Blackcurrant
Rose hips
Tomato
Cherry
Cranberry
Strawberry
G0
G1 G2 G3 G4 G5 G6 G7 G8 G9 G10 G11 G12 G13 G14 G15 G16 G17 G18 G19 G20 G21 G22 G23 G24 G25 G26 G27 G28 G29
Initial phenotyping step
PHASE 1
Intermediate phenotyping step
PHASE 2
PHASE 3
Final phenotyping step


A
Cherry
***
**
**
Fitness
2
0
−2
B
Cranberry
***
**
**
Fitness
2
0
−2
C
Strawberry
***
**
**
Fitness
2
0
−2
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
Generation


A
Intermediate phenotyping step
Evolved on cherry
Evolved on cranberry
Evolved on strawberry
Fitness change between intermediate and initial phenotyping steps
2
1
0
−1
−2
Fitness increase
Fitness decrease
Populations
Test fruit
Cherry
Cranberry
Strawberry
B
Final phenotyping step
Evolved on cherry
Evolved on cranberry
Evolved on strawberry
Fitness change between final and initial phenotyping steps
2
1
0
−1
−2
Fitness increase
Fitness decrease
Populations


Intermediate phenotyping step
A
Cherry vs. Cranberry
ρ = 0.48
[0.001 ; 0.96]
B
Cranberry vs. Strawberry
ρ = 0.54
[0.11 , 0.97]
C
Strawberry vs. Cherry
ρ = 0.62
[0.19 , 1]
Final phenotyping step
D
Cherry vs. Cranberry
ρ = −0.57
[−1.00 , −0.084]
E
Cranberry vs. Strawberry
ρ = 0.59
[0.12 , 1]
F
Strawberry vs. Cherry
ρ = 0.68
[0.15 , 1]
Fitness change in alternative environment
Fitness change in selective environment
Test fruit
Cherry
Cranberry
Strawberry
Selection fruit
Cherry
Cranberry
Strawberry


Phenotype 2
Phenotype 1
Population during phenotyping step:
Initial
Intermediate
Final
Phenotypic optima
Standard medium
Cherry
Cranberry
Strawberry