

RESEARCH ARTICLE

Oviposition behaviour in *Drosophila melanogaster*: Genetic and behavioural decoupling between oviposition acceptance and preference for natural fruits

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Abstract

In phytophagous insects, oviposition behaviour is an important component of habitat selection and, given the multiplicity of genetic and environmental factors affecting its expression, is defined as a complex character resulting from the sum of interdependent traits. Here, we study two components of egg-laying behaviour: oviposition acceptance (OA) and oviposition preference (OP) in *Drosophila melanogaster* using three natural fruits as resources (grape, tomato and orange) by means of no-choice and two-choice experiments, respectively. This experimental design allowed us to show that the results obtained in two-choice assays (OP) cannot be accounted for by those resulting from no-choice assays (OA). Since the genomes of all lines used are completely sequenced, we perform a genome-wide association study to identify and characterize the genetic underpinnings of these oviposition behaviour traits. The analyses revealed different candidate genes affecting natural genetic variation of both OA and OP traits. Moreover, our results suggest behavioural and genetic decoupling between OA and OP and that egg-laying behaviour is plastic and context-dependent. Such independence in the genetic architectures of OA and OP variation may influence different aspects of oviposition behaviour, including plasticity, canalization, host shift and maintenance of genetic variability, which contributes to the adoption of adaptive strategies during habitat selection.

KEYWORDS

natural genetic variation, natural resources, oviposition acceptance, oviposition candidate genes, oviposition preference

1 | INTRODUCTION

Insects that utilize decaying plant tissues as feeding and rearing resources have the challenge of identifying and selecting feeding and breeding sites that warrant the development and survival of the progeny. This essential and difficult task requires the integration of traits involving behavioural, physiological and genetic aspects.

Oviposition behaviour is a complex character orchestrated by a conserved ensemble of microbehaviours that vary across species, like *Drosophila* (Bräcker et al., 2019; Cury et al., 2019; Markow, 2019; Mwingira et al., 2020; Yang et al., 2008). The first step of egg-laying

behaviour is the location of a suitable host, relying on the perception of relevant environmental clues (Bernays, 1998; Day, 2016; Gou et al., 2014; Joseph et al., 2009; Reisenman et al., 2009; Reisenman & Scott, 2019; Tait et al., 2020; Yang et al., 2008). During this step, chemosensory systems play a major role in the search of suitable hosts (Hansson & Stensmyr, 2011; Keesey et al., 2019). For those species in which the volatiles are the principal cues to locate resources (Bruce et al., 2005; Scheidler et al., 2015), the coding capacity of olfactory sensory systems is enormous because of the multidimensional nature of the olfactory code (Hallem & Carlson, 2006; Münch & Galizia, 2016, 2017). For instance, most

Drosophila species use decomposing resources as feeding and breeding sites, wherein a community of microorganisms, mainly yeasts, participate in the decay of plant tissues, producing volatile compounds that attract adult flies (Anagnostou et al., 2010; Barker & Starmer, 1999; Becher et al., 2012; Bellutti et al., 2018; Koerte et al., 2020). Also, it has been reported that volatiles in the frass and pheromones in the ejected sperm stimulate aggregation and oviposition (Billeter & Wolfner, 2018; Duménil et al., 2016; Ha & Smith, 2006; Keeseey et al., 2016). During the second step, females re-evaluate more closely the suitability of a substrate through gustatory organs to decide egg-laying (Karageorgi et al., 2017; Koerte et al., 2020). Although the organs associated with chemoreceptor systems are distributed all over the body of the fly, the taste sensory capacity directly related to the choice of the oviposition site is in the vaginal plate at the lower end of the abdomen (Agnihotri et al., 2016; Masek & Keene, 2016; Vosshall & Stocker, 2007) and in forelegs (Chen & Amrein, 2017). In *Drosophila*, adults can be highly mobile (Coyne et al., 1982; Wong et al., 2018), which makes it possible to face a wide variety of resources during their lives. Thus, fruit flies are excellent models to study variation and host-dependent effects in adaptive traits related to the ability to locate, oviposit and survive in alternative resources wherein phenotypically plastic responses may play a major role.

Phenotypic plasticity is defined as the ability of a genotype to produce different phenotypes in response to varying environments (Lavagnino et al., 2020; Schlichting & Pigliucci, 1998; Petino Zappala et al., 2018). Plasticity is expected to evolve for traits that present distinct phenotypic optima across frequently experienced environments (DeWitt & Scheiner, 2004; Flatt, 2005). Several studies have shown that the evaluation of phenotypic plasticity in adaptive traits, like oviposition behaviour, may be important to understand the underlying genetic changes that determine the population dynamics of cosmopolitan species, the ecological fate of genotypes and the expansion of species (Betti et al., 2014; Carreira et al., 2013; Fallis et al., 2014; Fanara & Hasson, 2001).

On the contrary, in species where larvae have limited mobility, larval nutrition depends on the mother's choice, that is, oviposition decisions (Reaume & Sokolowski, 2006). In this sense, it has been proposed (Craig & Itami, 2008; Gripenberg et al., 2010) that females lay eggs in resources that may benefit her offspring ('mother-knows-best' hypothesis). However, the evidence of supporting a positive relationship between preference and performance remains ambiguous (Clark et al., 2011; Koerte et al., 2020; Olazcuaga et al., 2019; Soto et al., 2011).

Egg-laying decisions can be analysed as the process through which females decide whether to invest in one action (egg-laying) or not by considering the costs associated with the available options (Karageorgi et al., 2017; Miller et al., 2011; Yang et al., 2008). If female oviposition decision is a consequence of interactions among diverse environmental variables, then the full picture should be analysed since in nature it is not a simple addition or combination of pieces of a puzzle (Anholt et al., 2020; Duménil et al., 2016; Miller et al., 2011). Furthermore, integrating a wide range of experimental

approaches (behavioural, ecological and genetics) is necessary to answer questions that remain elusive: are the genetic architectures of host acceptance and host preference independent? What is the relationship between the genetics of host use, the genetics of host shifts and the genetics of speciation? (Drès & Mallet, 2002; Forbes et al., 2017; Markow, 2019). Therefore, the characterization of behavioural and genetic associations between traits involved in egg-laying decisions is necessary to understand variation in oviposition behaviour and its implications in host plant use and speciation.

Oviposition acceptance (OA) and oviposition preference (OP) are two quantitative traits that are part of the process of host plant selection. Both traits denote different behavioural aspects of oviposition resource selection (Singer et al., 1988). The former is applied to situations in which an organism uses a putative resource in the absence of alternatives and is affected by motivation, the general willingness to feed or oviposit (Fanara & Hasson, 2001; Schoonhoven et al., 2005). In turn, oviposition preference (OP) refers to situations in which organisms consistently use or choose a host among many alternatives in dual- or multiple-choice assays (Soto et al., 2011, 2015).

Here, we study oviposition behaviour using lines of the *Drosophila* Genetic Reference Panel (DGRP, Huang et al., 2014; Mackay et al., 2012) to investigate the genetic variation and phenotypic plasticity of OA and OP when different natural resources are offered to the flies. We identify and characterize the genetic underpinnings of these two traits (OA and OP) involved in the decision to oviposit by means of a genome-wide association study (GWAS). Finally, our experimental design allowed us to evaluate behavioural independence between OA and OP by estimating an OP expected from the data of OA observed by line and resources analysed and compare with the OP observed. In this sense, our hypothesis proposes that OP can be only accounted for by differences in the absolute capacity of each substrate (OA), which induce to oviposition preference in our two-choice assays. The results revealed that both oviposition behaviours: OA and OP, are decoupled.

2 | MATERIALS AND METHODS

In this study, we used a set of 40 fully sequenced lines from the DGRP (Huang et al., 2014; Mackay et al., 2012). Briefly, these lines were started from isofemale lines collected in Raleigh (NC, USA) and inbred for 20 generations of full-sib mating. Lines were individually maintained under standard culture conditions (cornmeal-dextrose-agar medium, 25°C, 60%–75% relative humidity, 12:12-h light: dark cycle) and were never exposed to the resources prepared with the fruits used in the experiments described below.

2.1 | Oviposition behaviour assays

Oviposition behaviour was evaluated by means of two complementary approaches: no-choice assays that allowed the measurement

of oviposition acceptance (OA) and, 2-choice assays that allowed the study of oviposition preference (OP). In each assay, 20 couple (male and female) nonvirgin sexually mature flies (3–5-days-old) were released in an oviposition arena that consisted of a transparent plastic box (20 by 15 by 10 cm) containing four plates (2.5 cm in diameter by 1.5 cm height) distributed according to the specific oviposition assay. Each plate contained an oviposition medium prepared by blending pieces of a certain fruit (resource) using a blender. Ten milliliter of blended fruit plus a heated solution of 0.1 g of agar-agar in 100 ml of distilled water were poured into each plate. After cooling, plates were inoculated with 0.1 ml with the corresponding fermenting juice obtained from naturally rotting fruit (collected in the field). We evaluated OA for three different resources: tomato, grape and orange. OP was investigated using two resource combinations: grape/tomato and grape/orange. It is important to note that the four oviposition plates in the OP assay (two for each resource) were randomly distributed in the arena since the distribution of oviposition media can affect OP (Yang et al., 2008). Flies were released into the chambers and allowed to oviposit for 36 h under controlled conditions of temperature ($25^{\circ}\text{C} \pm 1^{\circ}\text{C}$), relative humidity (75%) and photoperiod (12-h light:12-h dark). All plates were removed after 36 h and photographed for egg counting using a digital camera attached to a binocular microscope. Five replicates (experimental unit [arena]) of each combination of line and oviposition experiment (three for OA: tomato, grape and orange; and two for OP grape/tomato and grape/orange) were run totalling 1000 assays: 600 for OA (40 lines \times 3 resources \times 5 replicates) and 400 for OP (40 lines \times 2 OP assays \times 5 replicates). We used the number of eggs deposited in each resource as variable to quantify OA. To quantify OP, we modelled a binomial distribution, where an egg laid in the grape resource was considered a success, while the alternative resource (grape or orange) was a failure. A value of OP equal to 0.5 indicates no preference, while higher and lower values than 0.5 suggest preference for grapes and the alternative resource (tomato or orange), respectively. We also estimated the following quantitative genetic parameters: genetic (σ^2_G), environmental (σ^2_E) and phenotypic (σ^2_P) variance. Under our experimental design, σ^2_G equals the sum of among line (σ^2_L) and line by resource interaction ($\sigma^2_{LR/T}$) components of variance; σ^2_E can be computed as the within line (error) component (σ^2_W) and σ^2_P as the sum of σ^2_G and σ^2_E . Based on these estimates, broad sense heritability (H^2) can be estimated as $H^2 = \sigma^2_G/\sigma^2_P$. We also computed coefficients of genetic ($CV_G = 100\sigma_G/\text{mean}$) and environmental ($CV_E = 100\sigma_E/\text{mean}$) variance.

Statistical analyses to evaluate OA and OP were performed in R v. 4.0.4 (R Core Team, 2021). Data from OA were fitted to a Poisson distribution with mixed effects using package 'lme4' (Bates et al., 2015). The resource offered for oviposition was included in the model as a fixed-effect variable and the line and line by resource interaction were included as random-effect variables. Data from OP analysis were modelled with package 'glmmTMB' (Brooks et al., 2017) as a binomial distribution, where every egg laid on grape was considered a 'success' and eggs laid on the alternative resource (orange or tomato) a 'failure'. The resource being contrasted with

grape was included as a fixed-effect variable and the line and line by resource interaction were included as random-effect variables. Since this model cannot differentiate the variance between lines in the grape versus tomato assays from that in the grape/orange assays, two separate models were fitted. Each model was fitted with a subset of the data containing only the grape/tomato assays or the grape/orange assays, respectively, and does not include a fixed-effect variable or the line by resource interaction. In order to avoid the effect of overdispersion present in both the OA and OP datasets and to allow for the estimation of residual variances, necessary for the estimation of variance partition coefficients (VPC) (Austin et al., 2018; Browne et al., 2005), an observation-level random effects (OLRE) variable was added to every model. All models were validated using package 'Dharma' (Hartig, 2022).

Components of variance were obtained from each model using package 'insight' (Lüdtke et al., 2019), and VPCs were estimated as each variance component divided by the sum of all components (line, line by resource and OLRE). Random-effect variable significance was determined by a likelihood ratio test of the full model against reduced models, which did not include the random-effect variable being tested. Fixed-effect variable significance was determined by a type II Wald test, using package 'car' (Fox & Weisberg, 2019).

2.2 | Genome-wide association studies

Genome-wide association studies (GWAS) were performed to identify and characterize possible candidate genes underlying both oviposition behaviour traits. We utilized the mean number of eggs laid for OA on each resource evaluated separately and the OP values for each combination of resources in OP assays. We utilize these phenotypic means and the 1 048 575 polymorphic markers of the 40 lines included in the study. The DGRP lines used in this study have their genome fully sequenced (Huang et al., 2014; Mackay et al., 2012). All the information about sequencing methodology and statistical analyses to execute the GWAS is publicly available in the site of the *Drosophila* Reference Genome Panel (DGRP Freeze 2.0: dgrp2.gnets.ncsu.edu). Before running GWAS, the mean number of eggs laid for OA and OP values was adjusted for the effects of *Wolbachia* sp. infection and of the five major chromosomal inversions (In(2L)t, In(2R)NS, In(3R)P, In(3R)K and In(3R)Mo) segregating in the DRGP. The adjusted phenotypic values were fitted using a linear mixed model $Y = \mu + M + G + \epsilon$, where μ is the population mean, M is the fixed effect of the marker, and G is a polygenic term with its covariance among lines determined by the genomic relationship matrix (Huang et al., 2014). Only the gene closest to each significant candidate variant (where the distance was less than 1 kb) was accounted as candidate for any given trait.

3 | RESULTS

We counted a total of 152 851 eggs experiment-wide, 85 560 in 600 OA arenas and 67 291 in 400 OP arenas, with an overall

average egg-laying per female of 7.1 and 8.4 in OA and OP assays, respectively.

The assessment of OA considering each resource separately showed that females laid more eggs in tomato (30240 total eggs laid) than in orange (27775 eggs) and grape (27 545 eggs). The lowest number of eggs laid was observed in line 786 when females were tested in tomato with an average of 27 eggs per arena, whereas the highest number of eggs laid (305.6 eggs per arena) was observed in line 380 in grape. Interestingly, none of the lines analysed were among the lines exhibiting the five highest or five lowest egg-laying records in all resources used in our OA assays.

The evaluation of the causal factors of the patterns observed for OA revealed that genetic factors accounted for 82.5% (line effect+line by resource interaction) of total phenotypic variation in OA (Table 1). As a matter of fact, fecundity differences among lines (genotype effect) explained 43.1% of total phenotypic variation for OA (Table 1), whereas the line by resource interaction accounted for 49.4% of OA variation. These results point out that the responses of lines, in terms of egg-laying, depended on the resource utilized as stimulus, suggesting that phenotypic plastic responses have genetic basis (Table 1, Figure 1). In this context, it is worth mentioning that the proportion of variance accounted for genetic differences among lines was high and similar across resources used as oviposition stimulus: 61.3%, 74.9% and 69.2% of total phenotypic variation in grape, tomato and orange, respectively.

We further analysed the causal factors underlying OA variation by estimating the cross-environment ($r_{G \times E_{(resources)}}$) genetic correlations ($r_{G \times E_{(resource\ i - resource\ j)}}$). All $r_{G \times E}$ s were significantly different from unity, 0.43 for the combination grape-tomato, 0.50 for grape-orange and 0.62 for tomato-orange. These results indicate that the genetic factors orchestrating fecundity variation among lines when flies were confronted to alternative oviposition substrates (tomato, grape and orange) are somehow independent. Thus, the lack of phenotypic plasticity for OA (nonsignificant resource effect in Table 1) is a consequence of the pattern of egg-laying by the lines that depended on the resource used as oviposition stimulus (Table 1, Figure 1).

The experiments aimed to investigate OP also showed great variation among lines (Figure 2). The average of OP in grape/tomato and grape/orange combinations was 0.65 (SD \pm 0.06) and 0.58 (SD \pm 0.09), respectively. The average OP was in both cases

significantly different (higher) from 0.5 ($t_{39} = 15.23$, $p < 0.001$ and $t_{39} = 5.18$, $p < 0.001$, for grape/tomato and grape/orange, respectively), indicating that females preferred to lay eggs in grape rather than on the alternative resources offered to flies: tomato or orange. However, it is important to mention that a few lines preferred orange over grape despite the general preference for grape, observed experiment-wide in two-choice assays (Figure 2). Even though differences among lines were not significant, the results of the ANOVA indicated a significant resource by line interaction that represents a 25.4% of the total phenotypic variance (Table 1). These results suggest that most of the genetic differences underlying OP variation depended on the combination of resources used as oviposition stimuli (Figure 2). Interestingly, when OPs were analysed separately for each resource combination, we detected that a 40.3% of total phenotypic variation in grape/orange assays can be attributed to genetic factors (line effect $\chi^2 = 35.24$, df 37, 197, $p = 2.92 \times 10^{-9}$) whereas in the case of grape/tomato assays the line effect was not significant (line effect $\chi^2 = 2.16$, df 37, 197, $p = 0.14$).

To gain further insights into the genetic architecture of OA and OP, we calculated quantitative genetic parameters (Table 2). The estimation of heritability (H^2) yielded similar values for OA_{grape} , OA_{tomato} , OA_{orange} and higher than the values obtained in both OP assays. The lowest heritability estimate obtained in this study was for OP in grape/tomato assay, likely due to the low value of genetic variance (σ^2_G) relative to environmental variance (σ^2_E). Estimations of evolvability (CV_G) showed higher values for OP as compared to OA indicating that OP exhibited more potential for evolutionary adaptation to the components of habitat choice investigated.

The results obtained in OA and OP assays suggest natural genetic variation underlying phenotypic variation for these adaptive traits. Thus, we may ask whether fecundity of each line in response to each specific fruit used as oviposition substrates in nonchoice experiments can account for the results obtained in two-choice experiments aimed to evaluate preference. To this end, we estimated an expected OP (OP_E) for each line based on OA results in the corresponding substrates using the formula: $OP_E = (OA_{grape} / (OA_{grape} + OA_{alternative\ resource\ [tomato\ or\ orange]}))$. Comparisons between OP_E with the corresponding observed OP (OP_O) for each line using correlation analyses revealed (Figure 3, Figure S1) that OP cannot be explained by the results obtained in OA assays neither for

TABLE 1 Results of the ANOVA examining differences among DGRP lines for oviposition acceptance (OA) for three resources: tomato, grape and orange, and for oviposition preference (OP) for the two-choice resources: grape/orange and grape/tomato. σ^2 , component of variance; df, degree of freedom.

	OA				OP			
	df	χ^2	p	σ^2 (%)	df	χ^2	p	σ^2 (%)
Line	35	249.78	2.2E-16	43.1	36	12.75	3.6E-4	0.3
Resource	2	0.18	0.91		1	14.06	1.8E-4	
Line \times Resource	114	157.42	2.2E-16	49.4	75	18.25	1.9E-5	25.4
Error	592			7.5	395			74.3

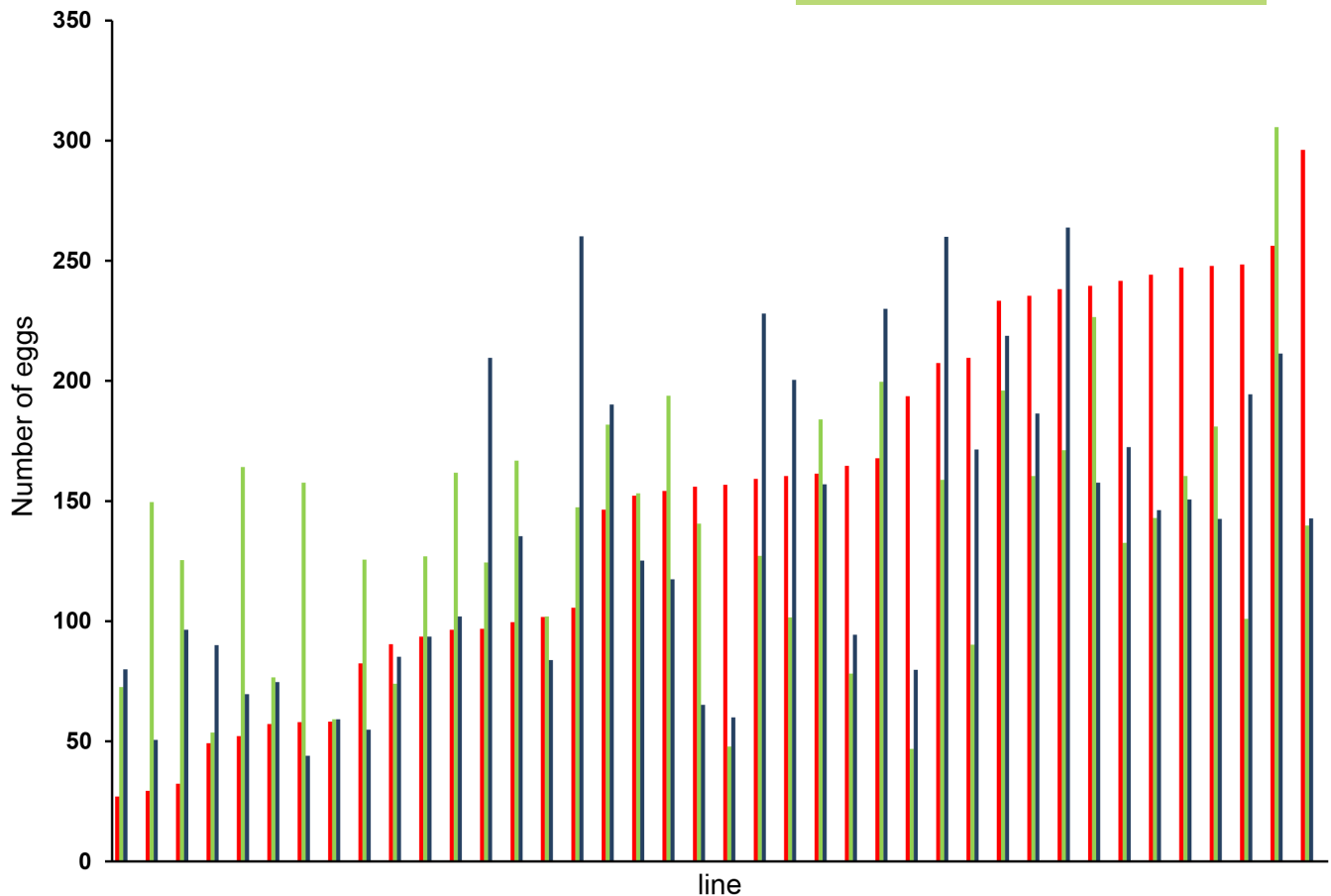


FIGURE 1 Variation in means in oviposition acceptance for three different resources: tomato (red), grape (green) and orange (blue) for DRGP lines. The DGRP lines are ordered according to higher egg-laying in tomato.

grape/tomato ($r = -0.12$, $p = 0.46$) nor grape/orange combinations ($r = -0.19$, $p = 0.25$), suggesting a behavioural decoupling between OA and OP. Moreover, the pattern of differences detected between OP_O and OP_E for each combination of resources evaluated (Figure 4) suggests that each line represents a particular combination of genetic factors underpinning acceptance and preference, pointing to plasticity in egg-laying behaviour. Thus, based on the apparent behavioural decoupling between OA and OP, we hypothesize that the genetic architectures of variation of these traits are relatively independent. To test this hypothesis and to identify the genetic factors involved in OA and OP variation, we conducted GWA studies in OA_{tomato} , OA_{grape} and OA_{orange} and for OP in both substrate combinations tested ($OP_{\text{grape/tomato}}$ and $OP_{\text{grape/orange}}$).

We identified experiment-wide a total of 184 SNPs (single-nucleotide polymorphisms) and INDELS (insertion or deletions <15bp), excluding duplicates (i.e. variants affecting more than one trait) that contribute to natural variation of the traits evaluated (Tables S1 and S2). Our results revealed that 106 out of 184 (57.6%) of the genetic polymorphisms identified were involved in OA variation (Table S1) as a result of 23, 32 and 52 genetic polymorphisms detected for OA_{grape} , OA_{tomato} and OA_{orange} , respectively. It is worth to mention that 1 SNP located in chromosome 2L at position 5669968, affected both OA_{tomato} and OA_{orange} . On the contrary,

78 genetic polymorphisms were detected in both OP assays (65 for $OP_{\text{grape/orange}}$ and 13 for $OP_{\text{grape/tomato}}$, Table S2). After filtering for variants in linkage disequilibrium (LD), considering a cut-off of 50bp (Mackay et al. 2012), the number of polymorphisms were 152 SNP and INDELS: 16, 27 and 43 in OA assays for grape, tomato and orange, respectively, and 13 and 53 in OP assays in grape/tomato and grape/orange combinations, respectively. On the contrary, GWAS analyses revealed that the sets of SNPs identified in OA and OP experiments were largely independent since none of the 184 variants affected pleiotropically OA and OP traits. We also evaluated whether the frequencies of site location classes relative to coding regions (down/upstream, intron, coding region and UTR) of candidate SNP/INDELS differ between OA and OP. These analyses indicated that the frequencies of site classes did not vary ($\chi^2_3 = 1.34$, $p = 0.35$) between oviposition behaviour traits.

A total of 95 genes are within <1 kb of candidate variants affecting oviposition traits assessed in this study (Table 3, Tables S1 and S2). Out of the 52 genes affecting OA, only three genes (5.8%) exhibited phenotypic plasticity. These are the cases of *Heat shock protein 60C* (*Hsp60C*) that affected acceptance for both tomato and orange, and *Bruno 3* (*Bru3*) and *Lysosomal α -mannosidase III* (*LManIII*) that were involved in acceptance of both grape and orange. After including plastic genes, the numbers of genes implicated in natural

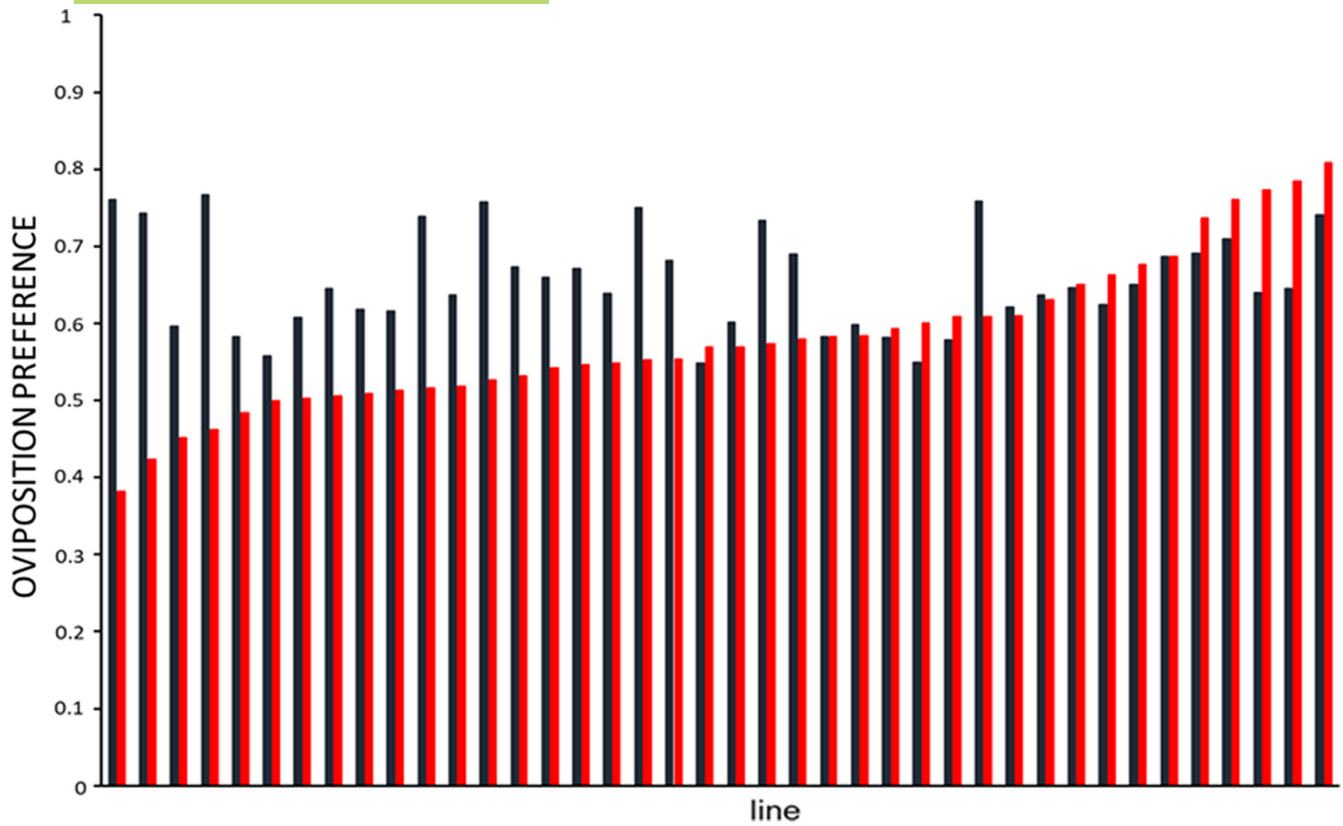


FIGURE 2 Variation in means in oviposition preference (OP) estimated as #eggs on grape/(#eggs on grape + #eggs on alternative resource) being the alternative resource orange or tomato. OP comparing grape/orange is indicated in blue, while OP evaluating grape/tomato is shown in red. An OP equal to 0.5 indicates an absence of OP.

	σ^2_G	σ^2_E	σ^2_P	h^2	CV_G	CV_E
OA	0.27	0.11	0.38	0.7	0.36	0.24
OA _{grape}	0.16	0.1	0.26	0.61	0.29	0.23
OA _{tomato}	0.4	0.13	0.53	0.75	0.42	0.24
OA _{orange}	0.24	0.11	0.34	0.69	0.35	0.23
OP _{grape-tomato}	0.03	0.3	0.33	0.08	24.7	83.53
OP _{grape-orange}	0.15	0.22	0.36	0.4	65.92	80.21

TABLE 2 Estimates of quantitative genetic parameters for oviposition acceptance (OA) for grape, tomato and orange; and for oviposition preference (OP) for grape-tomato and grape-orange.

Note: σ^2_G , σ^2_E and σ^2_P indicate the genetic, environmental and phenotypic variances, respectively. h^2 , CV_G and CV_E indicate broad sense heritability, coefficients of genetic variance and coefficients of environmental variance, respectively.

genetic variation affecting OA_{grape}, OA_{orange} and OA_{tomato}, respectively, were 11, 31 and 13, respectively. Contrarily, none of the 43 genes involved in OP (10 for OP_{grape/tomato} and 33 for OP_{grape/orange}) exhibited plasticity.

Finally, the results of phenotype–genotype association analyses performed in both oviposition behaviour traits reinforce our previous conclusion of a genetic decoupling between OA and OP. None of the 93 candidate genes identified affected pleiotropically OA and OP traits, indicating that these genes are trait-specific (Table 3). However, our results along with studies evaluating chemosensory-like and/or fecundity traits (Arya et al., 2015; Barish et al., 2018; Brown et al., 2013; Durham et al., 2014; Nakamura et al., 2002;

Sarkar & Lakhota, 2005, 2008; Toshima et al., 2014) showed that 39 candidate genes (41%) were pleiotropic (Table 3). Moreover, the proportions of pleiotropic candidate genes were not different (χ^2_1 : 1.28, $p > 0.05$) comparing pleiotropic candidate genes involved in OA (46.1%) and OP (34.9%).

4 | DISCUSSION

Establishing how environmental and genetic factors affect oviposition behaviour in insects is essential to understand the evolution of host use and host shifts. In the present study, we evaluated

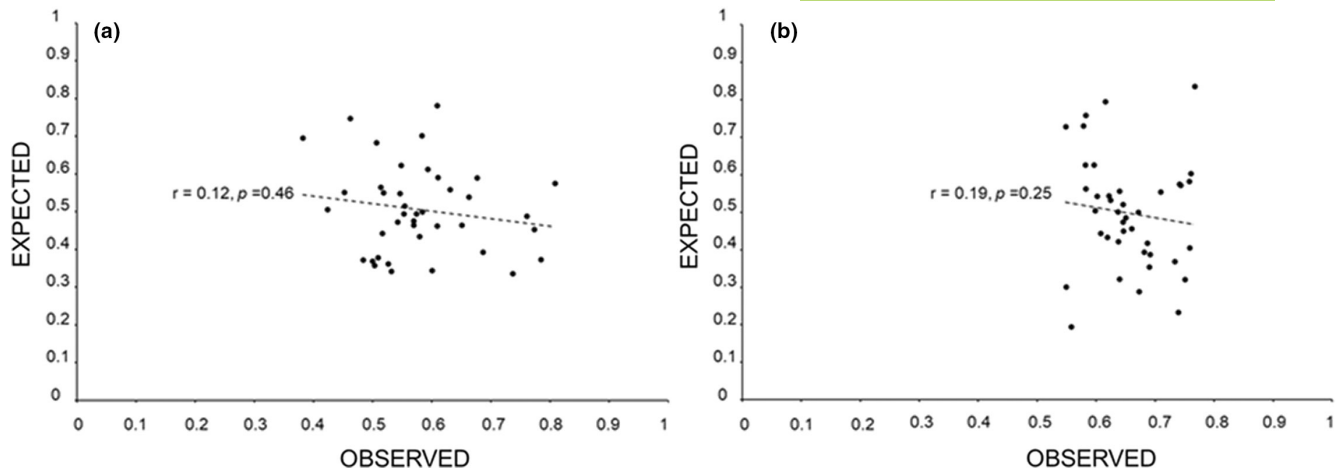


FIGURE 3 Scatter plots showing the comparison of oviposition preference difference between observed and expected values of each line for grape/orange (a) and grape/tomato (b). Expected values for each line were estimated from their corresponding oviposition acceptance (OA, no-choice essay) data as $OA_{\text{grape}} / (OA_{\text{grape}} + OA_{\text{alternative resource [tomato or orange]}})$. Coefficients of correlation (r) and the p -value for each oviposition preference are shown.

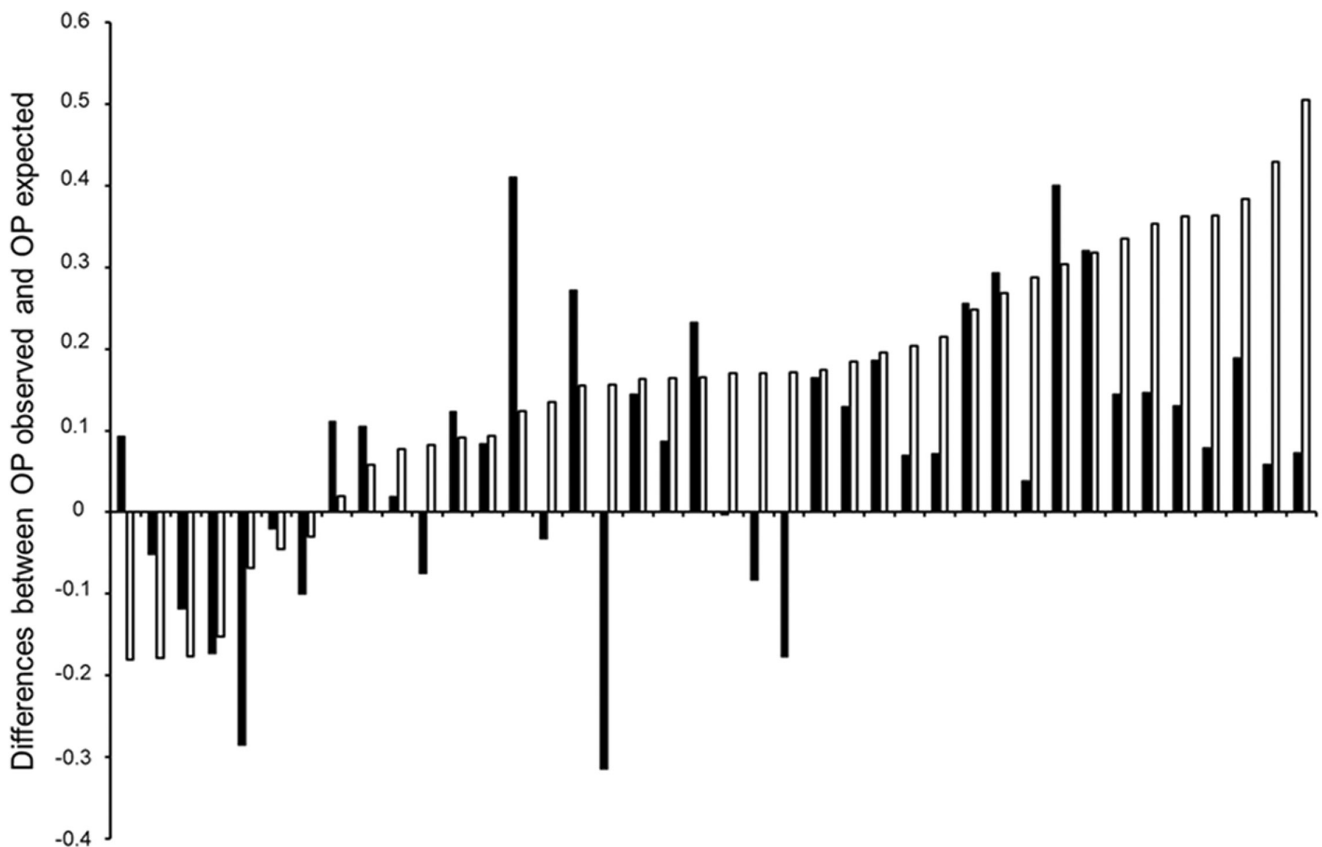


FIGURE 4 Differences in oviposition preference between observed (OP_O) and expected (OP_E) preferences among DGRP lines. For each line, expected values were estimated from their corresponding oviposition acceptance (OA) data as $OA_{\text{grape}} / (OA_{\text{grape}} + OA_{\text{alternative resource [tomato or orange]}})$. Positive values indicate a higher value of OP_O than OP_E . Differences in OP_O and OP_E are shown in rank order with respect to the value resulting from the calculation performed for the $OP_{\text{grape/tomato}}$ (open bars). The value obtained for differences between OP_O and OP_E for $OP_{\text{grape/orange}}$ is shown in filled bar.

oviposition acceptance (OA) and preference (OP), traits that are part of egg-laying behaviour, using different fruit as oviposition substrates in *D. melanogaster*. We also studied the genetic

architecture of these oviposition-site decision traits and their genetic basis. Our results revealed extensive variation among lines and across resources for OA and OP for different combinations

TABLE 3 Candidate genes for oviposition acceptance for grape, tomato and orange resources and for preference oviposition between grape-tomato and grape-orange. Candidate genes involved in more than one resource are indicated. We indicated those candidate genes affecting chemosensory traits (C) and/or fecundity (F) identified from our literature review.^a

Oviposition acceptance				Oviposition preference			
Resources	Genes	C	F	Resources	Genes	C	F
Grape	<i>CG15270</i>	X		Grape-Orange	<i>CG13280</i>		
Grape	<i>CG42747</i>			Grape-Orange	<i>CG31809</i>	X	
Grape	<i>CG4496</i>	X		Grape-Orange	<i>CG31810</i>	X	
Grape	<i>CG5565</i>			Grape-Orange	<i>CG33725</i>		
Grape	<i>Dref</i>			Grape-Orange	<i>AstC-R2</i>		
Grape	<i>Mnn1</i>			Grape-Orange	<i>Pp1α-96A</i>		
Grape	<i>NELF-B</i>			Grape-Orange	<i>CG43185</i>		
Grape	<i>rau</i>	X	X	Grape-Orange	<i>CG13272</i>		
Grape	<i>Spir</i>			Grape-Orange	<i>exp</i>		
Grape and Orange	<i>bru3</i>	X		Grape-Orange	<i>pncr003:2 L</i>	X	
Grape and Orange	<i>LManIII</i>			Grape-Orange	<i>Src64B</i>		
Orange	<i>baz</i>			Grape-Orange	<i>bves</i>	X	
Orange	<i>buGZ</i>			Grape-Orange	<i>Can</i>	X	
Orange	<i>Cad89D</i>	X		Grape-Orange	<i>CG10479</i>		
Orange	<i>cenG1A</i>	X		Grape-Orange	<i>CG10663</i>	X	
Orange	<i>CG13229</i>	X	X	Grape-Orange	<i>CG13284</i>		X
Orange	<i>CG1688</i>			Grape-Orange	<i>CG13622</i>		
Orange	<i>CG17571</i>			Grape-Orange	<i>CG17839</i>	X	
Orange	<i>CG34274</i>			Grape-Orange	<i>CG43120</i>	X	X
Orange	<i>CG6753</i>	X	X	Grape-Orange	<i>CR43858</i>		
Orange	<i>CG6793</i>	X		Grape-Orange	<i>CG10463</i>		
Orange	<i>CG7484</i>			Grape-Orange	<i>CG14764</i>		
Orange	<i>CR43836</i>	X		Grape-Orange	<i>CG16779</i>	X	
Orange	<i>Cyp6u1</i>	X		Grape-Orange	<i>CG9733</i>		
Orange	<i>Dgkε</i>	X		Grape-Orange	<i>Eaat1</i>		
Orange	<i>DIP-η</i>			Grape-Orange	<i>fbp</i>		
Orange	<i>dpr1</i>	X		Grape-Orange	<i>Lmpt</i>	X	
Orange	<i>dpr12</i>	X		Grape-Orange	<i>LManV</i>		
Orange	<i>Dscam2</i>	X		Grape-Orange	<i>Mhc</i>	X	X
Orange	<i>Hf</i>			Grape-Orange	<i>mwh</i>		
Orange	<i>kirre</i>	X	X	Grape-Orange	<i>PGRP-SC1b</i>	X	
Orange	<i>mRpS23</i>			Grape-Orange	<i>tst</i>		
Orange	<i>Msr-110</i>	X		Grape-Orange	<i>Ugt37E1</i>	X	X
Orange	<i>RanBPM</i>			Grape-Tomato	<i>bchs</i>		
Orange	<i>Rcd6</i>	X		Grape-Tomato	<i>Cyp311a1</i>		
Orange	<i>rdgA</i>	X	X	Grape-Tomato	<i>CG30015</i>	X	
Orange	<i>rols</i>	X		Grape-Tomato	<i>DIP-δ</i>		
Orange	<i>sick</i>	X	X	Grape-Tomato	<i>ics</i>		
Orange	<i>TTL3A</i>			Grape-Tomato	<i>jvl</i>		
Orange-Tomato	<i>Hsp60C</i>			Grape-Tomato	<i>Kcmf1</i>		
Tomato	<i>CadN2</i>	X		Grape-Tomato	<i>Nipped-A</i>		
Tomato	<i>CG2955</i>			Grape-Tomato	<i>Stam</i>		

TABLE 3 (Continued)

Oviposition acceptance				Oviposition preference			
Resources	Genes	C	F	Resources	Genes	C	F
Tomato	CG31817			Grape-Tomato	VGlut		
Tomato	CG4004						
Tomato	CG7384						
Tomato	dpr8	X					
Tomato	EcR	X					
Tomato	Eip63F-2						
Tomato	Fatp1						
Tomato	Treh						
Tomato	tRNA:CR30316						
Tomato	tRNA:K2:42Ad						

^aThe literature reviewed were Arya et al. (2015), Barish et al. (2018), Brown et al. (2013), Durham et al. (2014), Nakamura et al. (2002), Toshima et al. (2014), Sarkar and Lakhotia (2005, 2008).

of oviposition substrates. Furthermore, we detected that these traits, which constitute essential components of oviposition-site decision behaviour, are behaviourally and genetically decoupled. Moreover, our study shows that egg-laying behaviour is plastic and context-dependent since responses varied according to the ecological scenario faced by flies.

Several studies have shown that *Drosophila* females are highly selective about where to lay eggs and can withhold egg-laying when non acceptable substrates are available (Azanchi et al., 2013; Joseph et al., 2009; Schwartz et al., 2012; Yang et al., 2008, 2015). The three-oviposition media utilized in our study: grape, tomato and orange were accepted for egg-laying, as was also observed in other reports (Betti et al., 2014; Dweck et al., 2013; Jaenike, 1983; Soto et al., 2015). The overall ANOVA revealed that the three fruits were equally attractive for the flies, suggesting no differences among the resources that could modify the decision of egg-laying, after evaluation by females (Karageorgi et al., 2017; Miller et al., 2011; Yang et al., 2008, 2015). However, it is important to note that the significant line by resources interaction can be responsible for the absence of phenotypic plasticity since differences in fecundity varied across lines depending on the resources utilized. Moreover, our results showed that about two thirds of total OA variance can be accounted for by either variation among lines (Genotype effect) and by the significant line by resources interaction (genotype-by-environment interaction effect), indicating that fecundity differences among lines varied across egg-laying substrates. Further quantitative genetic analyses showed that the genotype-by-environment interaction can be mainly explained by the cross-environment genetic correlations (rGxE) between pairs of resources. These results point out that OA variation not only has a genetic basis but also, and more importantly, that the differential responses deployed by lines are dependent on the type of resource offered to females. Thus, even though the resources evaluated in this study exceed the threshold required for acceptance for egg-laying, the chemical cues coming from grape, tomato and orange seem to be different enough to trigger dissimilar

egg-laying outputs among lines. Therefore, it may be argued that the expression of the genetic factors that orchestrate OA variation is mostly host-specific.

Certainly, the results of our genome-wide association study give support to the hypothesis that genes affecting OA are host-specific since only one out of 107 SNPs, and three out of 52 candidate genes: *Heat shock protein 60C* (*Hsp60C*), *Bruno3* (*bru3*) and *Lysosomal α -mannosidase III* (*LManIII*) were associated with OA variation in different resources. Interestingly, the same SNP located at site 5669968 in *Hsp60C* gene, which was involved in OA_{grape} and OA_{orange} variation, has been shown to affect natural phenotypic variation of olfactory behaviour in the same panel of *Drosophila* lines (Arya et al., 2015). Specifically, the authors showed that SNP segregating at site 5669968 is involved in olfactory perception of 2-heptatone, a natural class of odorous ketones component of many fruits. Our study also revealed that 39 candidate genes identified in our study are pleiotropic since they were also shown to affect chemosensory and/or fecundity traits (Table 3). Remarkably, 46.2% of the candidate genes associated with oviposition acceptance are related to the perception of environmental cues emanating from potential hosts (Arya et al., 2015; Barish et al., 2018; Brown et al., 2013; Nakamura et al., 2002; Toshima et al., 2014), whereas six genes (11.5%) have critical roles in fecundity (Durham et al., 2014; Sarkar & Lakhotia, 2005, 2008).

The two-choice assays aimed to evaluate OP revealed that flies preferred to oviposit on grape rather than tomato or orange and that the degree of preference for grape varied across OP assays. Certainly, most lines showed a marked preference for grape in both fruit combinations; however, we detected a few lines in which females preferred orange over grape in the corresponding two-choice assay. The preference for orange over grape was observed in other studies (Betti et al., 2014; Dweck et al., 2013), indicating that *D. melanogaster* has the capacity to discriminate between these two resources. Moreover, Betti et al. (2014) showed that about 60% of total oviposition effort was on grape and

that a few lines preferred orange using the same assay in lines derived from three natural populations sampled in Argentina. In fact, if we calculate OP with the same OP estimator used in Betti et al. (2014), our results are strikingly coincident, since 60.5% of eggs were laid on grape. Altogether these results point to similar OPs in lines from populations inhabiting distant localities in Argentina and those of the DRGP collected in Raleigh (NC, USA), where fruits available as potential feeding and breeding sites are not strictly coincident.

Theoretical arguments and empirical data have shown that natural selection rapidly exhausts additive genetic variance for fitness traits as compared with morphological traits (Falconer & Mackay, 1996; Houle, 1992; Lavagnino et al., 2008; Mousseau & Roff, 1987). Therefore, populations are expected to harbour little additive genetic variance for fitness-related traits. However, previous studies (Betti et al., 2014; Miller et al., 2011; Soto et al., 2015,) along with this work, show that natural populations harbour genetic variation for OP. Furthermore, GWAS analyses allowed us to identify 43 candidate genes associated with OP variation, most of them (76.7%) in the OP_{grape/orange} assay. These analyses also showed that the sets of OP candidate genes were highly specific since there was no overlap between OP assays.

In a generalist insect species, variation in resource availability can be complex and dynamic and, therefore, decision-making circuits that regulate egg-laying behaviour could be affected by context-dependent variables (Azanchi et al., 2013; Betti et al., 2014; Dweck et al., 2013; Joseph et al., 2009; Miller et al., 2011; Schwartz et al., 2012; Yang et al., 2008, 2015). In this sense, most studies of egg-laying behaviour addressed the effect of the presence and concentration of sugars and alcohols, of media with different nutritional values among other aspects related to oviposition-site variation. Also, other studies demonstrated that females exhibited egg-laying preference for a particular fruit when they are given the opportunity to choose between natural resources (Betti et al., 2014; Dweck et al., 2013; Soto et al., 2011, 2015; and our results). In this sense, Yang et al. (2008) demonstrated that egg-laying behaviour is context-dependent. In effect, they showed that females laid more eggs in single-choice chambers (sucrose or lobeline) than in two-choice chambers (sucrose and lobeline), suggesting that females have the capacity to compare and assess available options.

Our study based on no-choice and single-choice experiments utilizing the same fruits as egg-laying substrates and the same set-up allowed us to compare two aspects of oviposition behaviour, acceptance and preference. In this context, we found that OP cannot be predicted on grounds of the results obtained in OA assays, suggesting that these behaviours are decoupled, as also indicated by correlation analyses. Moreover, such decoupling between acceptance and preference seems to be ubiquitous because the lack of correlation between OA and OP was observed in both oviposition-site preference assays: grape-tomato and grape-orange. Previous studies addressed the relationship between acceptance and preference using different strategies (Becher et al., 2012; Karageorgi et al., 2017; Olazcuaga

et al., 2019; Yang et al., 2008, 2015). For instance, Olazcuaga et al. (2019) studied variation in the ranking order of preference when 12 fruit resources were evaluated in choice experiments with respect to no-choice trials in the spotted wing fly *D. suzukii*. In turn, Karageorgi et al. (2017) demonstrated that the relative preference observed in single-choice assays resulted directly from the capacity of each substrate to elicit oviposition. Nevertheless, it should be noted that the study performed by Karageorgi et al. (2017) evaluated egg-laying on strawberry (genus *Fragaria*) in different stages of maturation whereas we evaluated three fruits as oviposition substrates. However, none of these studies provided statistical analyses that demonstrated lack of association between acceptance and preference for different fruit resources. Furthermore, our analyses also detected decoupling between acceptance and preference at the genetic level pointing to a complex genetic architecture of oviposition-site decision-making.

Our genetic analyses revealed that several genes participate in variation in oviposition behaviour and provide evidence that OA and OP have different underlying genetic architectures. In this sense, our results show that the sets of genes governing OA and OP are character-dependent, which agrees with the behavioural decoupling detected. Such independence of genetic architectures of acceptance and preference may influence different aspects of oviposition behaviour, including plasticity, canalization, plasticity of canalization, host shift and maintenance of genetic variation, which contributes to the adoption of different adaptive strategies in terms of habitat selection (Markow, 2019).

AUTHOR CONTRIBUTIONS

JJF, MILB and EH conceived the study. MILB collected the data. JJF, MILB and LG analysed the data. JJF and EH wrote the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The data associated with this manuscript can be found at Dryad: doi:10.5061/dryad.bnzs7h4dn.

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