

Oviposition Site Preference for Natural Breeding Sites in *Drosophila melanogaster* (Diptera: Drosophilidae) Populations from Argentina

Author(s): María I. L. Betti, Eduardo M. Soto and Esteban Hasson

Source: Annals of the Entomological Society of America, 107(5):944-953. 2014.

Published By: Entomological Society of America

URL: <http://www.bioone.org/doi/full/10.1603/AN14050>

BioOne (www.bioone.org) is a nonprofit, online aggregation of core research in the biological, ecological, and environmental sciences. BioOne provides a sustainable online platform for over 170 journals and books published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Web site, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/page/terms_of_use.

Usage of BioOne content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

Oviposition Site Preference for Natural Breeding Sites in *Drosophila melanogaster* (Diptera: Drosophilidae) Populations From Argentina

MARÍA I. L. BETTI, EDUARDO M. SOTO, AND ESTEBAN HASSON

Ann. Entomol. Soc. Am. 107(5): 944–953 (2014); DOI: <http://dx.doi.org/10.1603/AN14050>

ABSTRACT Maternal decisions, like the choice of a site for laying eggs, have important ecological and evolutionary implications. In the current study, we investigated variation both within and between populations in oviposition site preference (OSP) in a collection of isofemale lines derived from three *Drosophila melanogaster* Meigen natural populations of western Argentina. In the oviposition preference assay, we used two resources that fruit flies use as egg-laying sites in nature. Results revealed 1) the distribution of eggs across the two alternative resources offered to the flies deviated from random when flies were given the chance to choose between grape and orange, 2) OSP varied within and between populations, and 3) a substantial proportion of OSP variation has a genetic basis as suggested by the significant contribution of variation among lines to total trait variance. Our survey represents an initial step in understanding patterns of natural variation in oviposition preferences for natural resources in *D. melanogaster*.

KEY WORDS *Drosophila melanogaster* oviposition site preference, natural breeding resource, natural population, phenotypic variation, genetic variation

Habitat selection refers to all behavioral and ecological processes that result in an uneven use of the environment by a species. Habitat selection is not only considered an important determinant of community structure but also a key mechanism in the maintenance of genetic variation within and between populations when genetically different individuals differ in their capabilities to exploit alternative patches of a heterogeneous environment (Rosenzweig 1981; Jaenike 1986; Hedrick 1986, 1990; Fanara and Hasson 2001; Soto et al. 2011).

In phytophagous insects, differential exploitation of the environment occurs during the selection and utilization of host plants and involves a diverse array of complex behaviors. In *Drosophila*, olfaction plays a major role in long-range location of suitable sites for feeding, mating, oviposition, and breeding (Markow et al. 2009, Schwartz et al. 2012). In fact, volatile compounds produced by rotting fruit are used by *Drosophila melanogaster* Meigen females to locate suitable breeding sites (Azanchi et al. 2013). In addition, it is known that taste and olfaction are the primary sensory systems for interpreting nutritional values and potential toxicity of food in *D. melanogaster* (Hallem et al. 2006, Stensmyr et al. 2012, Masek and Keene 2013). Moreover, there is evidence that adaptation to a new host plant may be accompanied by

changes in the expression of genes involved in detoxification and metabolic pathways and gustatory and olfaction systems in *Drosophila sechellia* Tsacas & Baecli (Dworkin and Jones 2009).

The choice of a suitable host plant for egg laying involves decisions that are critical for a female's reproductive success, particularly in insect groups in which larvae are confined to the resources chosen by mothers. These decisions are influenced by factors, such as female habitat choice, egg-load, density dependence, and the availability of suitable oviposition sites (Van Randen and Roitberg 1996, Scheirs and De Bruyn 2002, Ellis 2008, Gripenberg et al. 2010).

The choice of an oviposition site is a complex reproductive trait and an important component of habitat selection (Fox 1993, Markow and O'Grady 2005). Oviposition site preference (OSP) can be defined as a measure of a female's tendency to lay eggs in a certain resource when given the choice (Singer 1986; Soto et al. 2011, 2012). OSP has received renewed attention as a neural model for decision-making behavior (Yang et al. 2008, Joseph et al. 2009) and also for the study of ecological factors in speciation (Jaenike and Holt 1991). Models of speciation based on the use of alternative hosts portray the evolution of OSP as a key process in incipient speciation (Jaenike 1987, Via 1990, Futuyma 1991, Hawthorne and Via 2001). Three main hypotheses have been proposed to account for the adaptive nature of OSP. The first is the "preference-performance" hypothesis, which postulates that females prefer to lay eggs on resources where progeny fitness is maximized (Levins and MacArthur 1969). Under this hypothesis, larvae should fare

¹ Instituto de Ecología, Genética y Evolución de Buenos Aires (IEGEB - CONICET), Buenos Aires, Argentina.

² Departamento de Ecología, Genética y Evolución (EGE), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina.

³ Corresponding author, e-mail: mariabetti@gmail.com.

better in preferred than nonpreferred hosts, a pattern that may manifest through a positive correlation between OSP and offspring performance (Jaenike 1978). The second is the “optimal foraging” hypothesis, which states that females prefer to lay eggs in resources that maximize their own fitness in terms of fecundity and longevity instead of maximizing larval fitness (Stephens and Krebs 1986, Gripenberg et al. 2010). Finally, the “free enemy space” hypothesis postulates that female choice of oviposition sites depends on the presence or absence of possible predators and parasitoids that could affect larval development and survival (Price et al. 1980, Silberbush and Blaustein 2011, Kacsoh et al. 2013). Available evidence suggests that the “preference-performance” hypothesis prevails in insects with sedentary offspring restricted to feed and develop in the resource chosen by their mothers, while the other two hypotheses prevail in insects with mobile offspring or offspring that are unable to predict the quality of the host plant (Scheirs and De Bruyn 2002, Videla et al. 2012). Nevertheless, “preference-performance” and “optimal foraging” hypotheses should not be seen as alternative processes, as females maximize fitness by optimizing adult and offspring performance.

The *melanogaster* subgroup (genus *Drosophila*, subgenus *Sophophora*) is an ensemble of nine closely related species that originated in sub-Saharan central Africa ≈ 5.1 million years ago (Lachaise et al. 1988). These fruit flies use decaying fruits or other plants tissues as sites for finding mates, oviposition, and adult and larval feeding (Lachaise and Silvain 2004, Anagnostou et al. 2010, Lebreton et al. 2012, Palanca et al. 2013). The *melanogaster* subgroup includes specialists, like *D. sechellia* and *Drosophila erecta* Tsacas & Lachaise, that use as breeding and feeding sites the rotting fruits of *Morinda citrifolia* L. and *Pandanus* sp., respectively, and generalist species, like *Drosophila yakuba* Burla and the cosmopolitan *Drosophila simulans* Sturtevant and *D. melanogaster*. The latter, in particular, is a model system in genetics and genomics (Mackay et al. 2012). However, studies of behavioral traits associated with the choice and use of natural breeding sites are relatively scarce (Soto et al. 2011).

Previous studies in *Drosophila* have shown that complex traits related to OSP, like odour-guided behavior and diet preference, are genetically variable (Mackay et al. 1996, Brown et al. 2013, Reddiex et al. 2013). Moreover, there is evidence that OSP in *D. melanogaster* has a genetic basis, although it is also influenced by environmental variables (Gripenberg et al. 2010, Miller et al. 2011). Nevertheless, most of the evidence is based on biological assays using isolated odorant compounds (Yang et al. 2008, Joseph et al. 2009, Miller et al. 2011, Abed-Vieillard et al. 2014) and yeasts (Becher et al. 2012, Lebreton et al. 2012, Palanca et al. 2013), but not natural substrates, which may have the advantage of testing the effect of the fruit substrate, the yeast, and the fruit–yeast interaction (Becher et al. 2012). However, a recent study on *D. melanogaster* by Dweck et al. (2013) tested a range of 15 fruits in six-way choice oviposition experiments.

The authors showed that orange is the preferred resource and that flies detect terpenes, characteristic of citrus fruits, via a single class of olfactory sensory neurons, expressing odorant receptor *Or19a*. In any case, the aforementioned studies reveal the quality of fruit flies as models for investigating the contribution of the plant substrate and the microbial community to habitat quality and habitat choice in insect–plant–yeast relationship.

The objective of the present article was to investigate patterns of variation in OSP in three natural populations of *D. melanogaster* sampled in Argentina. We performed dual choice assays employing seminatural media prepared with rotten fruits that female flies exploit as egg-laying resources in nature. The isofemale line technique (David et al. 2005), allowed us to estimate the relative contribution of genetic and environmental factors underlying phenotypic trait variation.

Materials and Methods

Fly Stocks. Flies were collected in three sampling localities in Argentina: Güemes (24° 38' S, 65° 03' W) in the northwest, Lavalle (32° 50' S, 68° 28' W) in West Central and Neuquén (38° 58' S, 68° 08' W) in the southwest (Fig. 1). Sampling sites were chosen, because they represent different environments (ecogeographical districts) that differ in the type of fruit crops available as potential feeding and breeding sites for the flies. Citrus orchards (especially oranges) offer the main potential resources for flies in Güemes; vineyards are abundant in Lavalle (though quince can also be found around the vineyards); and grape, apple, and pear provide suitable resources for the flies in Neuquén. The fruit crop information was obtained from reports by the official site of the Ministry of Agriculture of Argentina (<http://www.minagri.gov.ar>) and six-way choice oviposition experiments) and by personal notes taken at the sampling sites.

Sets of isofemale lines (lines from hereafter) were founded by placing individual gravid females in vials containing 5 ml agar-cornmeal-killed yeast medium (David et al. 2005). Lines were maintained at 25°C, 75% relative humidity (RH), and a photoperiod of 12:12 (L:D) h 20 generations until the experiments described below.

Oviposition Preferences Assay. OSP was measured by means of dual choice experiments. Briefly, twenty couples of mature flies (4–5 d old) of the same line were released in egg-collecting chambers (20 by 15 by 10 cm) containing six oviposition plates (2.5 cm in diameter \times 1.5 cm height; see Soto et al. 2011 for details). In each chamber, three plates contained a seminatural oviposition medium prepared with a mix of agar and smashed fermenting grape (*Vitis vinifera* L.) and the other three contained agar and smashed fermenting orange (*Citrus aurantium* L.). To prepare both types of media, pieces of fruit were smashed in a blender, and 10 ml of smashed fruit plus 0.1 g of agar-agar were poured into the plates. After cooling, plates were inoculated with 0.1 ml of fermenting juice



Fig. 1. Geographic location of the sampling localities studied in Argentina: Güemes, La Valle, and Neuquén.

obtained from naturally fermenting fruits. Plates were randomly distributed in each chamber, and the number of six plates was chosen to prevent saturation of the resources. Females were allowed to oviposit for 48 h under controlled conditions of temperature ($25 \pm 1^\circ\text{C}$), 75% RH, and a photoperiod of 12:12 (L:D) h. After 48 h, all plates were removed and photographed with a digital camera attached to a binocular microscope for egg counting. Ten lines of each population were tested, and 20 replicated egg-collecting chambers were run for each line.

Statistical Analysis. OSP was estimated as the number of eggs laid in grape divided by the sum of the number of eggs laid in grape and orange chambers.

Data were analyzed by means of a mixed two-way analysis of variance (ANOVA) according to the model:

$$Y = \mu + P + L(P) + \varepsilon,$$

where μ is the overall mean, P is the fixed population effect, L(P) stands for the random line effect nested in population, and ε is the error term.

Reduced ANOVAs were performed to investigate variation in each population according to the model:

$$Y = \mu + L + \varepsilon,$$

where L stands for the random Line factor, and μ and ε are as before defined.

We also assessed within- and between-population variation in fecundity using the number of eggs laid in each resource as fecundity estimates. We performed two and three way ANOVAs according to the models:

$$Y = \mu + L + R + L \times R + \varepsilon$$

and

$$Y = \mu + P + L(P) + R + P \times R + \varepsilon,$$

where μ is the population mean, P is the fixed Population effect, L(P) stands for the random Line effect nested in Population, R is the fixed Resource effect, P \times R the fixed interaction Population by Resource, L is the random Line effect, L \times R is the random Line by Resource interaction, and ε is the error term.

Finally, we performed a linear regression analysis of the mean number of eggs per population (averaged across lines) and the mean numbers of eggs laid in grape and orange as dependent variables on latitude to investigate the relationship between fecundity and latitude.

Before the statistical analysis, proportions were angularly transformed ($\arcsin \sqrt{x}$), and fecundity data were transformed using the formula $(x + \frac{3}{8})^{1/2}$ to satisfy ANOVA assumptions (Sokal and Rohlf 1995).

Estimation of Quantitative Genetics Parameters. Quantitative genetic parameters were estimated for OSP for each population. The contribution of among Line variation (σ^2_L) to total trait variance may be considered, under our experimental design, as an estimate of the genetic component (V_G) and variance among replicates (ε values) as an estimate of the environmental component (V_E) of total phenotypic trait variance (V_P), where $V_P = V_G + V_E$ (Lynch and Walsh 1998, Morgan and Mackay 2006, Goenaga et al. 2010). Thus, according to Falconer and Mackay (1996) broad sense heritability (H) can be calculated as:

$$H = V_G/V_P$$

We also estimated the coefficients of genetic and environmental variation using equations:

$$CV_G = [100 \cdot (V_G)^{1/2}] / \mu$$

$$CV_E = [100 \cdot (V_E)^{1/2}] / \mu,$$

where μ is the population mean of the proportion of eggs laid in grape, and V_G and V_E are the genetic and environmental variances. These estimates are based on the notion that trait means rather than variance values are more appropriate for standardizing genetic variance when the objective is to compare among traits and/or populations (Houle 1992, Lavagnino et al. 2008, Goenaga et al. 2010).

All statistical tests were performed using the General Linear model (GLM) and Variance component

(VARCOMP) implemented in the STATISTICA 6.0 software package (StatSoft 2001).

Results

Patterns of within and among populations variation in OSP are presented in Fig. 2A. The general ANOVA revealed that mean OSP did not vary significantly among populations ($F = 2.30$; $df = 2, 57$; $P = 0.12$), whereas differences among lines (within populations) were highly significant ($F = 20.14$; $df = 27, 571$; $P < 0.01$).

Partial ANOVAs showed that the line factor contributed significantly to total phenotypic variation in all populations (Güemes: $F = 7.35$; $df = 9, 189$; $P < 0.01$; Lavalle: $F = 7.69$; $df = 9, 193$; $P < 0.01$; Neuquén: $F = 55.69$; $df = 9, 190$, $P < 0.01$). Variance component analyses revealed that the contribution of among-line component to total trait variance varied widely among populations, from $\approx 25\%$ in Güemes and Lavalle to 73% in Neuquén. Under our experimental design, the significant among-line variance suggests that a large proportion of OSP variation has a genetic basis.

With the aim of characterizing and comparing variation across populations, lines were classified into three phenotypic classes according to OSP. Lines with mean values significantly > 0.5 were pooled into a class named G, and lines with mean OSP significantly lower than 0.5 into a class identified as O. Finally, lines with mean OSP values not significantly different from 0.5 were classified into a third class named I. Thus, the G and O classes include lines that preferred grape and orange, respectively, and I lines did not show any preference. These phenotypic classes were defined on the basis of the results of t -tests (Güemes: $t_a = 12.52$, $t_b = 6.78$, $t_c = 11.44$, $t_d = 19.42$, $t_e = 12.33$, $t_f = 9.7$, $t_g = 21.30$, $t_h = 9.21$, $t_i = 13.10$, $t_j = 10.26$; Lavalle: $t_k = 12.07$, $t_l = 19.49$, $t_m = 19.41$, $t_n = 11.99$, $t_o = 6.73$, $t_p = 6.49$, $t_q = 10.93$, $t_r = 1.32$, $t_s = 6.40$, $t_t = 6.11$; Neuquén: $t_u = 9.91$, $t_v = 16.65$, $t_w = 13.70$, $t_x = 10.01$, $t_y = 8.97$, $t_z = 2.81$, $t_1 = 6.69$, $t_2 = 3.49$, $t_3 = 0.93$, $t_4 = 0.27$, $df = 19$ in all comparisons; subindex stands for each of the 10 lines measured in each measured in each population). The relative proportions of the above-defined phenotypic classes varied substantially across populations (Fig. 2B). Consistent with the low level of variation in Güemes and Lavalle, only G lines were detected in the former, while 10% of the lines exhibited a significant preference for orange in the latter. In contrast, the greatest phenotypic diversity was observed in Neuquén, where we detected all phenotypic classes.

The comparative analysis of quantitative genetic parameters revealed similar patterns of variation in Güemes and Lavalle that were, in turn, consistently different from Neuquén. Broad sense heritability (H) was three times greater in the latter than in the other two populations (Table 1). In addition, estimates of genetic variance were about one-third of environmental variance in Lavalle and Güemes and exactly the opposite in Neuquén. The analysis of coefficients of genetic and environmental variation revealed a similar

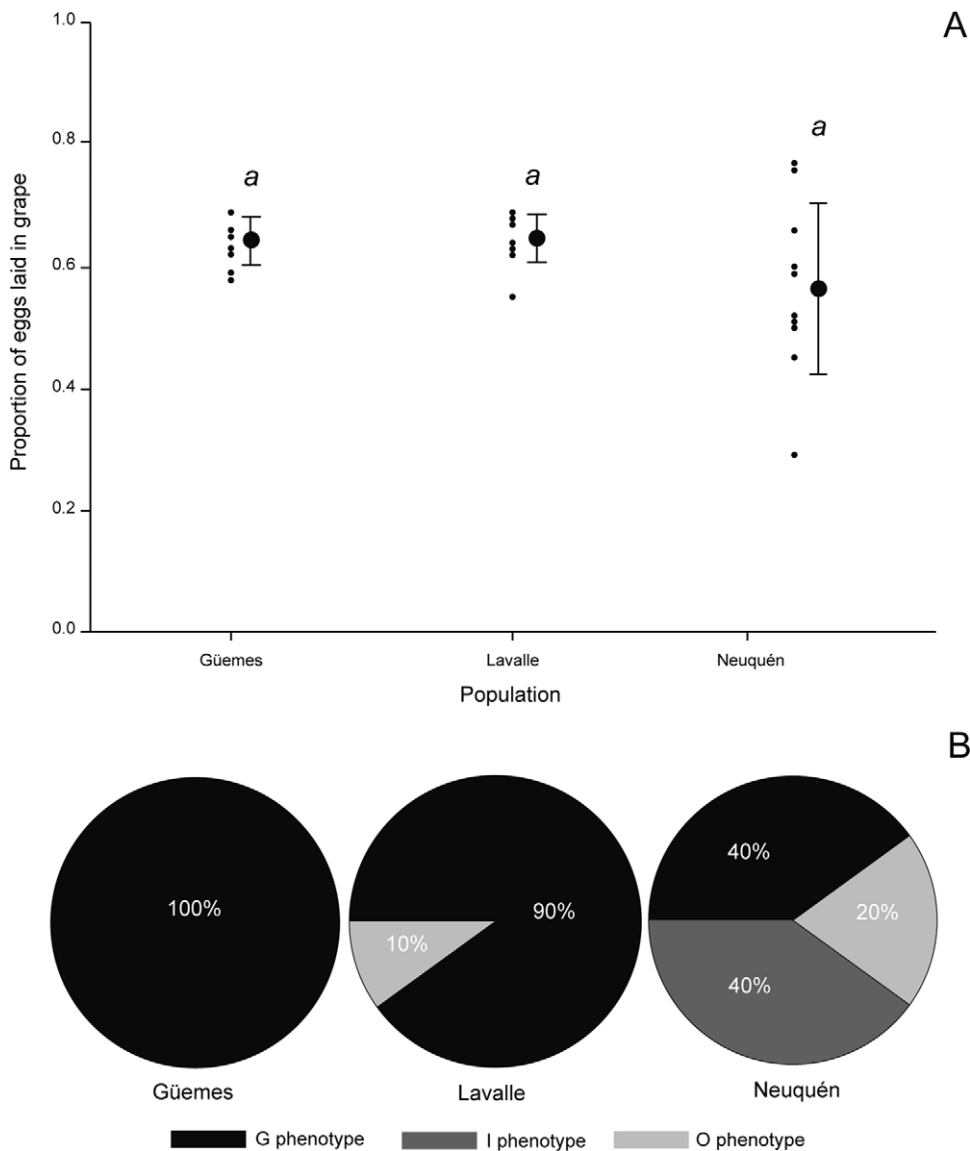


Fig. 2. OSP variation within and among populations. (A) Plots of the mean proportion of eggs laid in grape in the populations sampled, which are ordered from north to south. Each point represents the mean value of each isofemale line, and the overall population means are represented by circles with the corresponding standard deviation bars. *a* represents nonsignificant differences among populations: $F = 2.30$; $df = 2, 57$; $P = 0.12$. (B) Pie chart illustrating the relative frequencies of the G, O, and I phenotypic classes (see text for explanation) in each population.

pattern. The coefficient of environmental variation was greater than the coefficient of genetic variation in Güemes and Lavalle, and the reverse was true in Neuquén.

To further investigate the genetics of the trait, we measured OSP in the offspring of crosses between lines exhibiting the most contrasting phenotypes (G and O lines) among Neuquén lines. OSP was measured in F₁ females produced via both reciprocal crosses. To this end, 20 F₁ virgin females and 20 F₁ virgin males were released in the same type of oviposition chambers described above, and the number of eggs was

Table 1. Estimation of quantitative genetic parameters for OSP for each population

| Genetic quantitative | Güemes | Lavalle | Neuquén |
|----------------------|----------|---------|----------|
| V _G | 22.43 | 26.2 | 78.49 |
| V _E | 77.01 | 76.3 | 28.99 |
| V _P | 102.43 | 102.5 | 107.48 |
| H | 0.24 | 0.25 | 0.73 |
| CV _G | 666.52 | 796.83 | 1,577.85 |
| CV _E | 1,170.04 | 1,360.6 | 959.91 |

V_G corresponds to the genetic variance, V_E to the environmental variance, V_P to the phenotypic variance, H to the heritability, CV_G to the coefficient of genetic variation and CV_E to the coefficient of environmental variation. Arbitrary units.

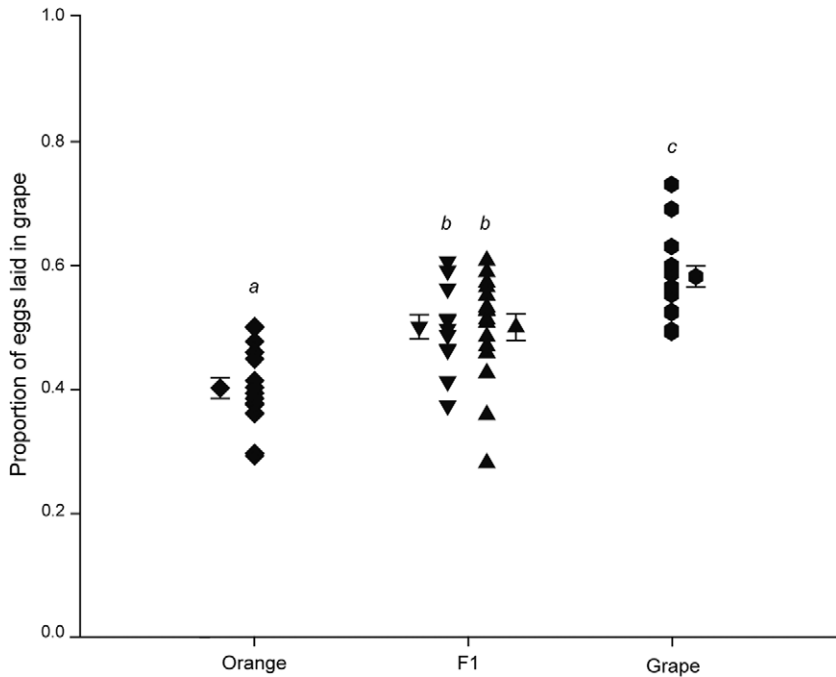


Fig. 3. Variability plot for the proportion of eggs laid in grape in crosses between *G* and *O* lines. Orange and Grape correspond to the parental lines and F_1 to the offspring obtained in reciprocal crosses. Each point represents the proportion of eggs laid in grape for each replicate. Means and SEs for the Orange (◆), Grape (●), and the F_1 s (the symbols ▲▼ stand for the two reciprocal crosses) are represented with SE bars. a, b, and c represent Tukey's a posteriori comparison of means, $P < 0.05$.

counted. OSP means in F_1 flies were intermediate to the means of the parental lines (Fig. 3). The ANOVA showed that differences among parental lines and F_1 s were significant ($F = 15.71$; $df = 3, 54$; $P < 0.001$). Tukey's a posteriori comparisons showed that parental lines maintained the differences registered in previous assays and that F_1 flies differed significantly from both parental lines but not between the two reciprocal crosses. These results suggest no maternal or paternal effects, and that genetic differences between strains are mainly additive.

We also measured variation in fecundity in the same set of lines. Two-way ANOVAs aimed to evaluate variation within populations revealed significant $L \times R$ in all populations (Güemes: $F = 5.67$; $df = 9, 189$; $P < 0.01$; Lavalle: $F = 3.75$; $df = 9, 179$; $P < 0.01$; Neuquén: $F = 24.63$; $df = 9, 180$; $P < 0.01$). The significant $L \times R$ implies that the number of eggs laid by females of each line were dependent on the fruit media. Thus, given our experimental design the $L \times R$ may be interpreted as a genotype by environment interaction or, in other words, as genetic variation in phenotypic plasticity. The number of eggs laid in each resource was examined by means of a three-way ANOVA, which revealed significant Population ($F = 6.9$; $df = 2, 540$; $P < 0.01$), Line (nested in Population; $F = 2.29$, $df = 28, 28$; $P < 0.01$), and Resource ($F = 86.71$; $df = 1, 540$; $P < 0.01$) effects. The Line (nested in Population) by Resource interaction was also significant ($F = 10.01$; $df = 28, 540$; $P < 0.01$).

Finally, we tested for clinal variation by linear regressing mean fecundity on latitude. The regression analysis revealed a negative and significant relationship between latitude and the total number of eggs ($b = -182.59$; $r^2 = 0.97$; $F = 57.25$; $df = 1, 3$; $P < 0.01$; Fig. 4A), the number of eggs laid in grape ($b = -196.40$; $r^2 = 0.95$; $F = 41.16$; $df = 1, 3$; $P < 0.02$) and orange ($b = -168.22$; $r^2 = 0.97$; $F = 57.94$; $df = 1, 3$; $P < 0.01$) (Fig. 4B). On average, flies laid significantly more eggs on grape than on orange, and overall fecundity decreased from northern to southern populations.

Discussion

Recently, it has been proposed that the expansion of the *melanogaster* subgroup began 10,000–20,000 yr ago in the final period of the ice age and that *D. melanogaster* first migrated from Africa to Europe and later to Asia (Laurent et al. 2011), accompanying the expansion of cultivars, probably banana (genus *Musa*), in the Natufian period (Bar-Yosef 1998). Thus, the association of *D. melanogaster* with human activities and the concomitant shift to domestic substrates has been a key factor in its recent evolutionary success. However, little is known about the diversity and type of breeding substrates used by *D. melanogaster* in nature. In fact, knowledge of breeding and feeding substrates is scarce and mainly based on the codistribution of species and potential host plants (Lachaise and Silvain

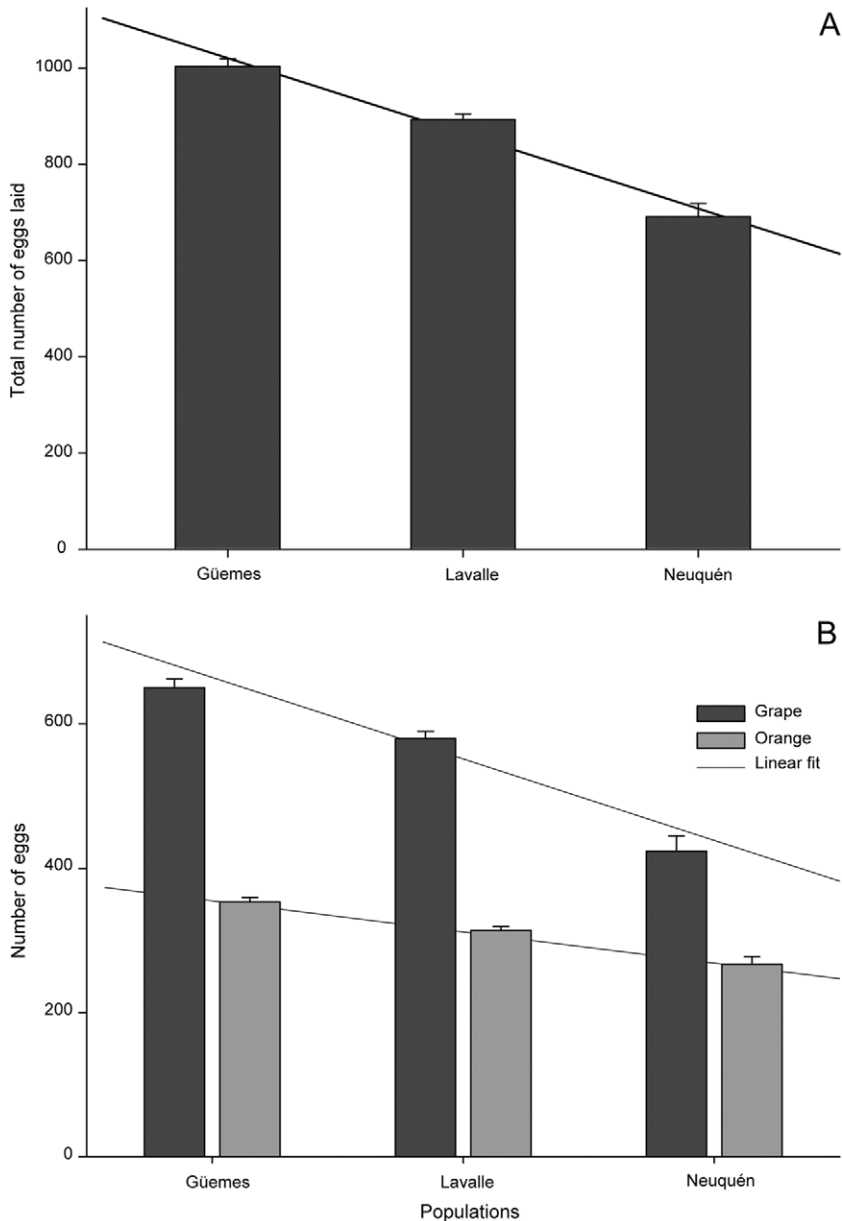


Fig. 4. (A) Bar charts for the mean numbers of eggs and (B) for the mean number of eggs laid in grape (black bars) and orange (gray bars). Lines in each graph represent the linear trend on the latitude in each case: (A) $b = -182.59$; $F_{1,3} = 57.25$; $P < 0.01$; (B) grape $b = -196.40$; $F_{1,3} = 41.16$; $P < 0.02$; and orange $b = -168.22$; $F_{1,3} = 57.94$; $P < 0.01$. Populations are ordered from north to south.

2004). In this context, our survey of OSP for natural substrates provides novel data on an ecological aspect scarcely addressed in this model species.

In our study, *D. melanogaster* females exhibited a general preference for grape; however, regardless of fecundity differences among populations and lines, we detected substantial within- and among-population variation in OSPs. Our results are clear in three main points: 1) the distribution of eggs across resources deviated from random expectations when flies were

given the chance to choose between grape and orange, 2) OSP varied within and between populations, and 3) substantial proportion of variation has a genetic basis, as suggested by the significant contribution of variation among lines to total trait variance.

Although the general ANOVA showed that among-population variation did not explain a substantial proportion of OSP variance, patterns of within-population variation differed sharply among the populations sampled. Moreover, the relative contribution of the line

component of variation to total phenotypic variance varied across sampling localities. Bearing in mind that isofemale lines analyzed in this article derived from different environments and that grape or orange are absent in some of the locations sampled, the observed patterns may have different explanations depending upon the population. For example, in Güemes, all lines were of the same *G* phenotypic class. Considering that this locality is in a geographic region characterized by the presence of citric fruit orchards and that agricultural activity based on wine industry is absent, the marked preference for grape can be linked to cryptic genetic variation (Le Rouzic and Carlborg 2008). Cryptic genetic variation refers to all genetic components of the genetic architecture of a complex trait that have the potential to express and become target of natural selection if the environmental or genetic scenario is modified. Thus, the exclusive presence of *G* lines may be a reflection of the prevailing preference in the flies that colonized the area and the observed pattern may be considered as the expression of cryptic genetic variation under the conditions of our assays. Although, at least two phenotypic classes were detected in the other two localities studied, both showed a general preference for grape, which incidentally is an abundant resource (though not all year round) in the geographic areas surveyed. These results are at variance with a recent report showing that when *D. melanogaster* females are given the choice of multiple fruits, they exhibit an innate preference for *Citrus* spp. (except lemon) as oviposition sites and that the presence of limonene, a terpene compound characteristic of citric fruits, is necessary for the increased rate of oviposition seen toward citric fruits (Dweck et al. 2013). Based on these results, the authors argue that the citrus preference may be an ancestral trait in *D. melanogaster* that presumably represents an adaptation toward African fruits similar to citrus. However, there are two aspects of the Dweck et al. (2013) study that may explain the differences between their study and our present survey. First, Dweck et al. (2013) considered only the effect of several fruits, not taking into account the fact that the microbial composition of the decaying fruits as oviposition and rearing substrate is a critical factor, whereas in our study, we used naturally decaying fruits. Second, and equally important is the fact that their conclusions on oviposition preference are based on the assessment of only one fly stock (Canton-S), while ours is based on the assessment of several isofemale lines sampled from three localities representing different environments.

Based on the assessment of several isofemale lines per population, our study also allowed the estimation of quantitative genetic parameters (David et al. 2005). Under our experimental design, variation among lines and among replicates can be considered as estimates of the contribution of genetic (V_G) and environmental (V_E) variance to total phenotypic variance ($V_P = V_G + V_E$), respectively. Broad sense heritability (H) and coefficients of genetic variation (CV_G) also exhibited extensive variation among populations. Because H provides an estimate of total genetic variation

that includes additive, dominant, and epistatic effects, we performed crosses between *G* and *O* lines exhibiting the most extreme phenotypes to further investigate the genetic basis of OSP variation. The analysis of OSP in the F_1 progeny of these crosses revealed that the phenotype of the F_1 was intermediate between the parental strains, suggesting that genetic differences between *G* and *O* lines are mainly additive. Previous investigations of the genetics of OSP compared egg-laying preference between yeast and yeast-free media (Miller et al. 2011). The authors reported broad sense heritability close to 50% and largely dominant effects in the F_1 . However, the dominant effect got diluted in the next generations, reaching values compatible with additivity.

Our survey also unveiled a trend of increasing OSP variation in the southernmost sampling locality, which contrasts with the negative cline in fecundity. Although these results must be taken with caution because the number of sampled populations was relatively low, the trends observed suggest that flies of the southernmost sampling locality (Neuquén) produced lower numbers of eggs and were less choosy when they were offered alternative oviposition sites. Neuquén is the most extreme population because it faces the most stringent climatic conditions with a strong seasonal availability of substrates (information obtained from <http://www.smn.gov.ar> and <http://www.minagri.gov.ar>). Surveys of quantitative trait variation in the same set of natural populations revealed differences in the patterns of within- and among-population variation for adult and larval olfactory behavior (Lavagnino et al. 2008), thermal-stress tolerance (Fallis et al. 2011), positive latitudinal clines for developmental time and viability (Folguera et al. 2008, Mensch et al. 2010) and a negative latitudinal cline for starvation resistance (Goenaga et al. 2010). Overall, these findings are in line with the idea that specific population processes drive the evolutionary trajectories of each trait and suggest the adaptive nature of the OSP cline.

What are the possible ecological and physiological mechanisms underlying OSP variation? In *Drosophila*, fermenting fruits serve as adult food, mating, and egg-laying sites. Thus, to understand the mechanisms driving insect-fruit-yeast relationship, it is necessary to establish the relative contribution of the plant substrate and the yeasts to habitat quality and habitat choice by the flies. Our results of host plant oviposition preference can be interpreted in the light of two recent reports, investigating the significance of baker's yeast (*Saccharomyces cerevisiae* Meyen ex E. C. Hansen) and fruit (grape) as individual components involved in fly attraction, oviposition, and larval development (Becher et al. 2012), and assessing variance in fly attraction among yeast isolates (Palanca et al. 2013). In the former, the authors concluded that yeast is not only required but is also sufficient to account for fly attraction, oviposition, and larval development. In turn, Palanca et al. (2013) found that yeast strains isolated from fruits were more attractive than strains isolated from nonfruit sources. Though, we did not characterize the microbial community in the fruit me-

dia used in the OSP assay, our results indicate that alternative fruits prompted different egg-laying responses among genotypes.

We can offer two alternative explanations for the observed variation in egg-laying response. On one hand, OSP variation may be due to differential attraction mediated by differences in the mix of volatile compounds produced by different microbial communities associated with grape and orange media. However, the production of different bouquets of volatile compounds may be the result of the interaction between the same microbial community and different fruit substrates. However, future investigations in this respect should focus on the dissection of the microbial components associated with decaying grape and orange and their individual roles in attraction and oviposition behavior.

In conclusion, our study reveals substantial genetic variation for OSP in natural populations. These results are important because genetic variation is an essential condition for the action of natural selection. However, further work that identifies genes involved in oviposition behavior is necessary to understand the physiological mechanisms and the genetic architecture underlying this complex trait and its potential role in speciation.

Acknowledgments

We thank the three anonymous reviewers for insightful comments and suggestions. This work was supported by grants of Universidad de Buenos Aires, Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). M.I.L.B. is recipient of a PhD scholarship awarded by CONICET. E.M.S. and E.H. are members of CONICET.

References Cited

- Abed-Vieillard, D., J. Cortot, C. Everaerts, and J. F. Ferveur. 2014. Choice alters *Drosophila* oviposition site preference on menthol. *Biol. Open* 3: 22–28.
- Anagnostou, C., E. A. LeGrand, and M. Rohlf. 2010. Friendly food for fitter flies?—Influence of dietary microbial species on food choice and parasitoid resistance in *Drosophila*. *Oikos* 119: 533–541.
- Azanchi, R., K. R. Kaun, and U. Heberlein. 2013. Competing dopamine neurons drive oviposition choice for ethanol in *Drosophila*. *Proc. Natl. Acad. Sci. USA* 8: 3–8.
- Bar-Yosef, O. 1998. The natufian culture in the levant, threshold to the origins of agriculture. *Evol. Anthropol.* 6: 159–177.
- Becher, P. G., G. Flick, E. Rozpędowska, A. Schmidt, A. Hagman, S. Lebreton, M. C. Larsson, B. S. Hansson, J. Piškur, P. Witzgall, et al. 2012. Yeast, not fruit volatiles mediate *Drosophila melanogaster* attraction, oviposition and development. *Funct. Ecol.* 26: 822–828.
- Brown, E. B., J. E. Layne, C. Zhu, A. G. Jegga, and S. M. Rollmann. 2013. Genome-wide association mapping of natural variation in odour-guided behavior in *Drosophila*. *Genes Brain Behav.* 12: 503–515.
- David, J. R., P. Gibert, H. Legout, G. Pétavy, P. Capy, and B. Moreteau. 2005. Isofemale lines in *Drosophila*: an empirical approach to quantitative trait analysis in natural populations. *Heredity* 94: 3–12.
- Dweck, H. K., S. A. Ebrahim, S. Kromann, D. Bown, Y. Hillbur, S. Sachse, B. S. Hansson, and M. C. Stensmyr. 2013. Olfactory preference for egg laying on *Citrus* substrates in *Drosophila*. *Curr. Biol.* 23: 2472–2480.
- Dworkin, I., and C. D. Jones. 2009. Genetic changes accompanying the evolution of host specialization in *Drosophila sechellia*. *Genetics* 181: 721–736.
- Ellis, A. M. 2008. Incorporating density dependence into the oviposition preference-offspring performance hypothesis. *J. Anim. Ecol.* 77: 247–256.
- Fanara, J. J., and E. R. Hasson. 2001. Oviposition acceptance and fecundity scheduled in the cactophilic sibling species *Drosophila buzzatii* and *D. koepferae* on their natural hosts. *Evolution* 55: 2615–2619.
- Falconer, D. S., and T.F.C. Mackay. 1996. Introduction to quantitative genetics. 4th ed. Longman, Harlow, United Kingdom.
- Fallis, L. C., J. J. Fanara, and T. J. Morgan. 2011. Genetic variation in heat-stress tolerance among South American *Drosophila* populations. *Genetica* 139: 1331–1337.
- Folguera, G., S. Ceballos, L. Spezzi, J. J. Fanara, and E. Hasson. 2008. Clinical variation in developmental time and viability, and the response to thermal treatments in two species of *Drosophila*. *Biol. J. Linn. Soc.* 95: 233–245.
- Fox, C. W. 1993. A quantitative analysis of oviposition preference and larval performance on two hosts in the bruchid beetle, *Callosobruchus maculatus*. *Evolution* 47: 166–175.
- Futuyma, D. 1991. Evolution of host specificity in herbivorous insects: genetic, ecological, and phylogenetic aspects. In P. Price, T. Lewinsohn, G. Fernandes, and W. Benson (eds.), *Plant-animal interactions: evolutionary ecology in tropical and temperate regions*. Wiley, New York, NY.
- Goenaga, J., J. J. Fanara, and E. Hasson. 2010. A quantitative genetic study of starvation resistance at different geographic scales in natural populations of *Drosophila melanogaster*. *Genet. Res.* 92: 253–259.
- Gripenberg, S., P. J. Mayhew, M. Parnell, and T. Roslin. 2010. A meta-analysis of preference-performance relationships in phytophagous insects. *Ecol. Lett.* 13: 383–393.
- Halle, E., A. Dahanukar, J. R. Carlson. 2006. Insect odor and taste receptors. *Annu. Rev. Entomol.* 51: 113–135.
- Hawthorne, D. J., and S. Via. 2001. Genetic linkage of ecological specialization and reproductive isolation in pea aphids. *Nature* 412: 904–907.
- Hedrick, P. W. 1986. Genetic polymorphism in heterogeneous environments: a decade later. *Annu. Rev. Ecol. Syst.* 17: 535–566.
- Hedrick, P. W. 1990. Genotypic-specific habitat selection: a new model and its application. *Heredity* 65: 145–149.
- Houle, D. 1992. Comparing evolvability and variability of quantitative traits. *Genetics* 130: 195–204.
- Jaenike, J. 1978. Optimal oviposition behavior in phytophagous insects. *Theor. Popul. Biol.* 14: 350–356.
- Jaenike, J. 1986. Genetic complexity of host-selection behavior in *Drosophila*. *Proc. Natl. Acad. Sci. USA* 83: 2148–2151.
- Jaenike, J. 1987. Genetics of oviposition-site preference in *Drosophila tripunctata*. *Heredity* 59: 363–369.
- Jaenike, J., and R. D. Holt. 1991. Genetic variation for habitat preference: evidence and explanations. *Am. Nat.* 131: 67–97.
- Joseph, R. M., A. V. Devineni, I.F.G. King, and U. Heberlein. 2009. Oviposition preference for and positional avoidance of acetic acid provide a model for competing behavioral drives in *Drosophila*. *Proc. Natl. Acad. Sci. USA* 106: 11352–11357.

- Kacsoh, B. Z., Z. R. Lynch, N. T. Mortimer, and T. A. Schlenke. 2013. Fruit flies medicate offspring after seeing parasites. *Science* 339: 947–950.
- Lachaise, D., M. L. Cariou, J. R. David, F. Lemeunier, L. Tsacas, and M. Ashburner. 1988. Historical biogeography of the *Drosophila melanogaster* species subgroup. *Evol. Biol.* 22: 159–225.
- Lachaise, D., and J. P. Silvain. 2004. How two Afrotropical endemics made two cosmopolitan human commensals: the *Drosophila melanogaster* - *D. simulans* palaeogeographic riddle. *Genetica* 120: 17–39.
- Laurent, S.J.Y., A. Werzner, L. Excoffier, and W. Stephan. 2011. Approximate Bayesian analysis of *Drosophila melanogaster* polymorphism data reveals a recent colonization of Southeast Asia. *Mol. Biol. Evol.* 28: 2041–2051.
- Lavagnino, N. J., R. R. Anholt, and J. J. Fanara. 2008. Variation in genetic architecture of olfactory behaviour among wild-derived populations of *Drosophila melanogaster*. *J. Evol. Biol.* 21: 988–996.
- Le Rouzic, A., and O. Carlborg. 2008. Evolutionary potential of hidden genetic variation. *Trends Ecol. Evol.* 23: 33–37.
- Lebreton, S., P. G. Becher, B. S. Hansson, and P. Witzgall. 2012. Attraction of *Drosophila melanogaster* males to food-related and fly odours. *J. Insect. Physiol.* 58: 125–129.
- Levins, R., and R. MacArthur. 1969. An hypothesis to explain the incidence of monophagy. *Ecology* 50: 910–911.
- Lynch, M., and B. Walsh. 1998. *Genetics and analysis of quantitative traits*. Sinauer Associates, Sunderland, MA.
- Mackay, T. F., J. B. Hackett, R. F. Lyman, M. L. Wayne, and R. R. Anholt. 1996. Quantitative genetic variation of odor-guided behavior in a natural population of *Drosophila melanogaster*. *Genetics* 144: 727–735.
- Mackay, T. F., S. Richards, E. A. Stone, A. Barbadilla, J. F. Ayroles, D. Zhu, S. Casillas, Y. Han, M. M. Magwire, J. M. Cridland, et al. 2012. The *Drosophila melanogaster* genetic reference panel. *Nature* 482: 173–178.
- Markow, T. A., and P. M. O'Grady. 2005. *Drosophila: a guide to species identification and use*. Academic, London, United Kingdom.
- Markow, T., S. Beall, and L. M. Matzkin. 2009. Egg size, embryonic development time and ovoviviparity in *Drosophila* species. *J. Evol. Biol.* 22: 430–434.
- Masek, P., and A. C. Keene. 2013. *Drosophila* fatty acid taste signals through the PLC pathway in sugar-sensing neurons. *PLoS Genet.* 9: e1003710.
- Mensch, J., V. Carreira, N. Lavagnino, J. Goenaga, G. Folguera, E. Hasson, and J. J. Fanara. 2010. Stage-specific effects of candidate heterochronic genes on variation in developmental time along an altitudinal cline of *Drosophila melanogaster*. *PLoS One* 5: e11229.
- Miller, P. M., J. B. Saltz, V. A. Cochrane, C. M. Marcinkowski, R. Mobin, and T. L. Turner. 2011. Natural variation in decision-making behavior in *Drosophila melanogaster*. *PLoS One* 6: e16436.
- Morgan, T. J., and T.F.C. Mackay. 2006. Quantitative trait loci for thermotolerance phenotypes in *Drosophila melanogaster*. *Heredity* 96: 232–242.
- Palanca, L., A. C. Gaskett, C. S. Günther, R. D. Newcomb, and M. R. Goddard. 2013. Quantifying variation in the ability of yeasts to attract *Drosophila melanogaster*. *PLoS One* 8: e75332.
- Price, P. W., C. E. Bouton, P. Gross, B. A. McPherson, J. N. Thompson, and A. E. Weis. 1980. Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annu. Rev. Ecol. Syst.* 11: 41–65.
- Reddiex, A. J., T. P. Gosden, R. Bonduriansky, and S. F. Chenoweth. 2013. Sex-specific fitness consequences of nutrient intake and the evolvability of diet preferences. *Am. Nat.* 182: 91–102.
- Rosenzweig, M. L. 1981. A theory of habitat selection. *Ecology* 62: 327–335.
- Scheirs, J., and L. De Bruyn. 2002. Integrating optimal foraging and optimal oviposition theory in plant-insect research. *Oikos* 96: 187–191.
- Schwartz, N. U., L. Zhong, A. Bellemer, and W. D. Tracey. 2012. Egg laying decisions in *Drosophila* are consistent with foraging costs of larval progeny. *PLoS One* 7: e37910.
- Silberbush, A., and L. Blaustein. 2011. Mosquito females quantify risk of predation to their progeny when selecting an oviposition site. *Funct. Ecol.* 25: 1091–1095.
- Singer, M. C. 1986. The definition and measurement of oviposition preference in plant feeding insects. In J. Miller and T. A. Miller (eds.), *Insects-plant relations*. Springer-Verlag, New York, NY.
- Sokal, R. R., and F. J. Rohlf. 1995. *Biometry*. Freeman, New York, NY.
- Soto, E. M., I. M. Soto, M. D. Cortese, and E. Hasson. 2011. Oviposition site preferences and performance in natural resources in the human commensals *Drosophila melanogaster* and *D. simulans*. *Fly* 5: 102–109.
- Soto, E. M., J. Goenaga, J. P. Hurtado, and E. Hasson. 2012. Oviposition and performance in natural hosts in cactophilic *Drosophila*. *Evol. Ecol.* 4: 975–990.
- StatSoft Inc. 2001. *STATISTICA* (data analysis software system) 6.0 Edition. StatSoft Inc., Tulsa, OK. (www.statsoft.com).
- Stensmyr, M. C., H. K. Dweck, A. Farhan, I. Ibba, A. Strutz, L. Mukunda, J. Linz, V. Grabe, K. Steck, S. Lavista-Llanos, et al. 2012. A conserved dedicated olfactory circuit for detecting harmful microbes in *Drosophila*. *Cell*, 151 1345–1357.
- Stephens, D. W., and J. R. Krebs. 1986. *Foraging Theory*. Princeton University Press, Princeton, NJ.
- Van Randen, E. J., and B. D. Roitberg. 1996. The effect of egg load on superparasitism by the snowberry fly. *Entomol. Exp. Appl.* 79: 241–245.
- Via, S. 1990. Ecological genetics in herbivorous insects: the experimental study of evolution in natural and agricultural systems. *Annu. Rev. Entomol.* 35: 421–446.
- Videla, M., G. R. Valladares, and A. Salvo. 2012. Choosing between good and better: optimal oviposition drives host plant selection when parents and offspring agree on best resources. *Oecologia* 169: 743–751.
- Yang, C. H., P. Belawat, E. Hafen, L. Y. Jan, and Y. N. Jan. 2008. *Drosophila* egg-laying site selection as a system to study simple decision-making processes. *Science* 319: 1679–1683.

Received 29 March 2014; accepted 15 July 2014.