A geometric analysis of the macronutrient needs of Drosophila suzukii larvae

Simon Fellous, Anne Xuéreb

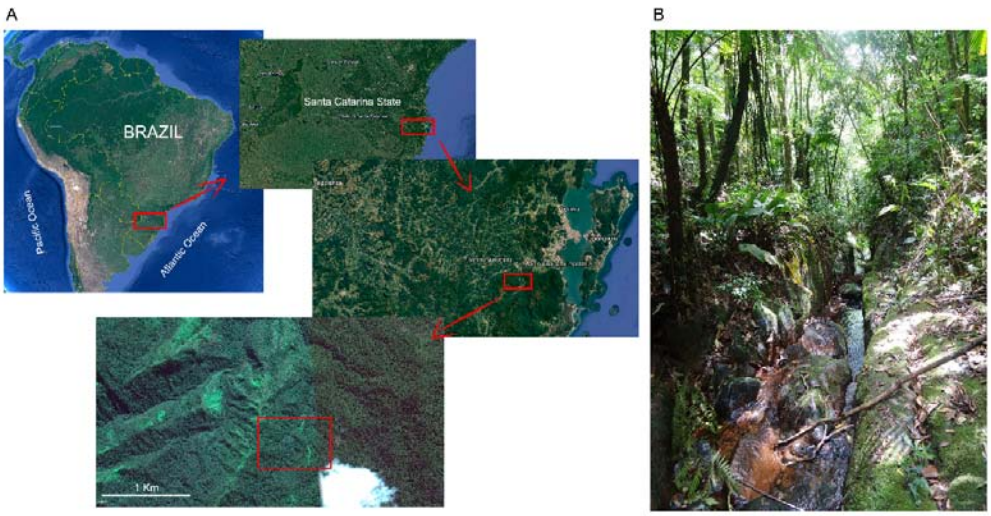

Figure 1. Characterization of the study site. A) Map of the study site in Brazil, showing Santa Catarina State. Map of Santa Catarina State, highlighting the collection area at Santo Amaro da Imperatriz, with satellite images of the region limited by the red rectangles (Reference: http://maps.google). B) Typical collecting site.

Next, polytene chromosome preparations were obtained from third instar larvae and photographed for analysis of heterozygous chromosomal inversions. Three chromosomal inversion polymorphisms were detected, all located on the right arm of chromosome 2.

These results reveal that there is a well-established inversion chromosomal polymorphism, which allows characterizing these continental populations and contrasting them with other conservation units, both continental and insular. This indicates the need for stabilizing effective conservation policies in this park.

References: Anderson, A., A. Hoffman, S. Mckechnie, P. Umina, and A. Weeks 2005, Molecular Ecology 14: 851–858; Bizzo, L., T. Vanderlinde, B. Wildemann, and D.C. De Toni 2012, Dros. Inf. Serv. 95: 121-122; Cordeiro, J., D.C. De Toni, G.S. Silva, and V.L.S. Valente 2014, Genetica 142: 461-472; Dobzhansky, Th., 1970. Columbia University Press, New York; Ishiy, S.T. *et al*., 2009. Fundação do Meio Ambiente – FATMA 80; Kirkpatrick, M., and N. Barton 2006, Genetics 173: 419–434; Krimbas, C., and J. Powell 1992, C.R.C. Press, Boca Raton, FL, 2-52; Rohde, C., and V.L.S. Valente 1996, Braz. J. Genetics 19: 27-32; Roque, F., S. Oliveira, and R. Tidon 2011, Dros. Inf. Serv. 94: 140-141.

A geometric analysis of the macronutrient needs of *Drosophila suzukii* larvae.

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Abstract

The nutritional needs of animals largely depend on their ecology and habitat. Phenotypes and general performance often depend on the synergistic influence of multiple nutrients. These effects are currently studied within the geometric framework of nutrition. Contrary to its close relative *Drosophila melanogaster*, the invasive Spotted-Wing *Drosophila*, *Drosophila suzukii*, attacks fresh, undamaged fruit devoid of microbial growth. Different oviposition habits suggest different nutritional needs by the two species. We investigated the combined influence of carbohydrate and protein concentrations on the larval performance of a *D. suzukii* population. Proportions of individuals that survived until the adult stage were maximal at intermediate protein and low sugar concentrations. Larval development was shortest under high protein diets. Observations on this population are congruent with what is known of *D. suzukii* larval ecology, as ripening, undamaged fruit is generally poor in sugars and proteins in comparison to ripe, yeast-colonized fruit. We discuss the limitations

of fly nutrition experiments based on laboratory food, such as ours, where the natural dynamics of microbial growth is neglected.

Introduction

Animals need to acquire a variety of resources to develop and reproduce. The relative proportions of nutrients, as well as their absolute amounts, influence phenotype and ultimately fitness (Raubenheimer *et al.*, 2009). Importantly, numerous nutrients have synergistic effects: the effect of given resource on animal phenotype has different effect when another resource is abundant or scarce (Jacobs *et al.*, 2009). This observation has led to the formulation of the geometric framework of nutrition (*i.e.*, nutritional geometrics), whereby several types of nutrients are jointly studied in a multi-dimensional space (Raubenheimer *et al.*, 2009). This framework has, for example, allowed to disentangle the effect of caloric restriction and protein intake on longevity in *Drosophila melanogaster*, the ubiquitous fly encountered in kitchens and laboratories world-wide (Lee *et al.*, 2008).

The spotted-wing *Drosophila*, *Drosophila suzukii*, our focal organism, is a particular species among Drosophilid flies as it is able to oviposit in undamaged fruit whose skin it pierces with the aid of a large, serrated ovipositor (Atallah *et al.*, 2014). Current understanding of *Drosophila suzukii* ecology indicates larvae develop in conditions that differ from those of species such as *D. melanogaster*. Larvae of *D. suzukii* and *D. melanogaster* would hence have distinct but partly overlapping nutritional niches. First, both species can be found in ripe fruit, if the skin barrier has been broken, but *D. suzukii* also oviposits in unripe fruit (*e.g.*, Swoboda-Bhattarai and Burrack, 2015) that do not contain high concentrations of sugars yet (Prasanna *et al.*, 2007) and are free from microbial growth. Second, unlike *D. suzukii*, *D. melanogaster* often oviposits on decaying fruit that can be very ripe (*i.e.*, with high sugars levels) and, most importantly, largely colonized by bacteria and yeast (Becher *et al.*, 2012; Rombaut *et al.*, 2017). Accordingly, the behaviors of female flies that seek oviposition sites are different in the two species. *D. melanogaster* responds to yeast volatiles (Oakeshott *et al.*, 1989; Becher *et al.*, 2012), whereas *D. suzukii* responds to fruit volatiles (Karageorgi *et al.*, 2017; Swoboda-Bhattarai *et al.*, 2017). Female attraction to yeast relates to the beneficial effects it has on larval nutrition: in several *Drosophila* species yeast is the main source of proteins of larvae (Starmer and Fogleman, 1986; Anagnostou *et al.*, 2010; Becher *et al.*, 2012). In *D. suzukii*, yeasts do associate with adults (Hamby *et al.*, 2012), but their role in larval ecology is poorly known. It is nonetheless reasonable to assume *D. suzukii* mothers inoculate larval medium by depositing yeast cells during oviposition, a form of pseudo-vertical transmission. Based on female behavior, we hypothesized that *D. suzukii* larvae would have access to lower yeast concentrations than those of *D. melanogaster*, because *D. melanogaster* eggs are deposited in fruit where yeast has already had time to develop and reach high densities, while yeast concentration would be lagging behind when *D. suzukii* eggs hatch. This hypothesis translates into the prediction that the performance of *Drosophila suzukii* larvae would be best in nutritive media with low or intermediate protein concentrations, not benefiting from high protein concentrations. Similarly, as ripening fruit is usually poorer in sugars than ripe fruit, we expected *D. suzukii* larvae to perform poorly on - or at least not benefit from - high sugar concentrations.

To investigate the nutritional needs of *Drosophila suzukii* larvae, we used the nutritional geometric framework (Raubenheimer *et al.*, 2009). As for most other *Drosophila* studies within this framework, we chose to vary carbohydrate and protein availability. Proteins availability was manipulated by varying the proportion of dead yeast in the medium recipe. Carbohydrates were manipulated by varying the amount of saccharose (*i.e.*, short-chained carbohydrates, sugar) in medium recipe. Yeast cells also contain long-chained carbohydrates; therefore, yeast amounts also affected carbohydrate concentrations, which was taken into account in medium composition calculations. Larval performance was assessed by recording the proportion of larvae that reached the adult stage (*i.e.*, survival to emergence) and time to adult emergence (*i.e.*, developmental rate).

Material and Methods

Biological material

Drosophila suzukii is native from Asia and has invaded Europe, North and South America in the last 10 years (Adrion *et al.,* 2014, Fraimout *et al.,* 2017). Females are known to oviposit on fresh, undamaged fruits (Lee *et al.,* 2015). Eggs hatch within 24 h; time until adult emergence ranges from 10 days to 4 weeks, depending on conditions.

We used a *D. suzukii* population founded with *ca*. 30 females captured in Southern France, near Montpellier, a year earlier (*ca*. 20 generations). As population size was kept small - on average less than 30 reproducing females - we expect drift to have occurred and some genetic diversity to have been lost. Besides, microsatellite analyses have revealed low genetic diversity in natural populations around Montpellier (Fraimout *et al.,* 2017). Despite a lack of genetic variation, it is possible the population had adapted (in evolutionary terms) to laboratory conditions, an unfortunate caveat that our study shares with the other studies on *Drosophila* nutritional ecology we compare our results to (Rodrigues *et al.,* 2015).

Table 1. Recipe and macronutrient composition of the 25 nutritional media used in the experiment. In addition to water, yeast and sugar (*i.e.*, saccharose), recipe contained 3 g of Agar and 1.5 g of Nipagin (diluted in alcohol that evaporates during medium preparation). Note the diet used to maintain our *D. suzukii* population was close to treatment number 9.

Treatment number	Yeast mass (in g)	Sugar mass (in g)	Water in mL	Concentration in proteins	Concentration in carbohydrates	Proportion of carbohydrates brought by yeast input
1	37	0	300	0.053	0.015	1.000
2	37	5.2	300	0.052	0.030	0.499
3	37	10.4	300	0.052	0.044	0.332
4	37	20.7	300	0.050	0.071	0.200
5	37	41.4	300	0.047	0.122	0.111
6	18.5	2.6	300	0.028	0.016	0.499
7	18.5	7.8	300	0.027	0.031	0.249
8	18.5	18.1	300	0.027	0.061	0.125
9	18.5	38.9	300	0.025	0.115	0.062
10	18.5	80.3	300	0.022	0.206	0.031
11	9.3	3.9	300	0.014	0.016	0.250
12	9.3	9.1	300	0.014	0.032	0.125
13	9.3	19.4	300	0.014	0.062	0.063
14	9.3	40.1	300	0.013	0.117	0.031
15	9.3	81.6	300	0.012	0.210	0.016
16	4.6	4.5	300	0.007	0.016	0.125
17	4.6	9.7	300	0.007	0.032	0.062
18	4.6	20.1	300	0.007	0.063	0.031
19	4.6	40.8	300	0.006	0.118	0.016
20	4.6	82.2	300	0.006	0.212	0.008
21	2.3	4.9	300	0.004	0.017	0.062
22	2.3	10	300	0.004	0.033	0.031
23	2.3	20.4	300	0.003	0.063	0.016
24	2.3	41.1	300	0.003	0.119	0.008
25	2.3	82.6	300	0.003	0.213	0.004

Experimental methods

Larvae were reared in vials containing one of 25 different nutritional treatments (Table 1). We varied the concentration in proteins and carbohydrates by manipulating the amount of sugar (*i.e.*, saccharose) and dead bakers' yeast, *Saccharomyces cerevisiae*, in the medium recipe. This recipe was based on standard fly food initially designed for *Drosophila melanogaster*. Our choice of treatments was guided by the idea that laboratory foods are richer in nutrients than those encountered by flies in the wild. By consequent, we mostly explored concentrations of nutrients lower than that of standard medium. The macronutrient composition of the medium on which our *D. suzukii* population was maintained before the experiment was similar to treatment number 9 (Table 1), but also contained fresh banana. Banana was excluded from experiment's recipes in order to allow comparison of our results with those from other studies.

We used known composition in proteins and carbohydrates in yeast provided by our supplier to convert yeast quantities into protein and carbohydrates concentrations (1 g yeast contained 0.49 g of proteins and 0.14 g of digestible, long-chained carbohydrates). Note that we further distinguish total carbohydrates from the fraction of carbohydrates provided by yeast input (see statistical methods and results). Media also contained agar for consistency and nipagin to prevent the development of mold (Table 1). It should be noted that with this type of dietary manipulation, water content in the medium decreases as macronutrient concentration increases. The effect of water availability is, therefore, confounded with that of high macronutrient availability.

An experimental unit consisted of a 39 mm diameter drosophila vials with 15 mL of medium. Each received 40 *D. suzukii* eggs that we manually transferred from oviposition plates (*i.e.*, petri dishes with a medium made of grape juice and agar, exposed to females for *ca.*12 h). Note that manipulating eggs, rather than allowing females to naturally oviposit on experimental media, was mandatory in order to control for larval density and ensure all eggs came from females exposed to similar conditions. Unfortunately numerous eggs were killed in the process as *D. suzukii* eggs are notoriously more fragile than those of *D. melanogaster*, explaining the moderate rates of survival to adulthood reported below. Each treatment consisted of 3 replicates spread in 3 blocks each initiated 24 h apart. To assess fly development, experimental units were checked daily for newly emerged flies, which were removed from the vials and sexed. The adults collected and frozen during this experiment were unfortunately lost, preventing their phenotyping. The experimental room was maintained at 23°C under a 14 L : 10 D photoregime.

Statistical methods

We used standard linear models to analyse the response of survival and age at adult emergence to macronutrients concentrations. Protein and carbohydrate concentrations were square-root transformed, the proportion of carbohydrates contributed by yeast was log transformed. Initial models contained the term describing the proportion of carbohydrates from yeast, the terms describing nutrient contents and their squared value (*i.e.*, factor^2) so as to permit non-linear relationships between factors and responses, as well as 2nd order interactions between nutrient composition terms. Basic model hence had the form:

$$\begin{aligned}\text{Trait} = {} & \sqrt{\text{Proportion.proteins}} + (\sqrt{\text{Proportion.proteins}})^2 + \sqrt{\text{Proportion.carbohydrates}} + (\sqrt{\text{Proportion.carbohydrates}})^2 \\ & + \sqrt{\text{Proportion.carbohydrates}} * \sqrt{\text{Proportion.proteins}} * + (\sqrt{\text{Proportion.carbohydrates}})^2 * (\sqrt{\text{Proportion.proteins}})^2 \\ & + \sqrt{\text{Proportion.carbohydrates}} * (\sqrt{\text{Proportion.proteins}})^2 + \sqrt{\text{Proportion.proteins}} * (\sqrt{\text{Proportion.carbohydrates}})^2 \\ & + \text{Log}(\text{proprotion.carbohydrates.from.yeast}) + \varepsilon.\end{aligned}$$

For the analysis of development time we also included a factor describing sex-ratio, the proportion of females among the adults that emerged. Using a backward selection procedure we removed non-significant terms starting from highest order interactions. Data distribution complied with the main assumptions of the linear model, namely independence, homoscedasticity and normality of the residuals. All analyses were carried out with the statistical software JMP 12.1.

Data presented in Figures 1 and 2 are heat-maps fitted over values predicted by the final models for each of the 25 nutritional treatments. Predicted values are presented, rather than raw data, so as to facilitate comparison with other nutritional geometric studies in *Drosophila* that used the same method.

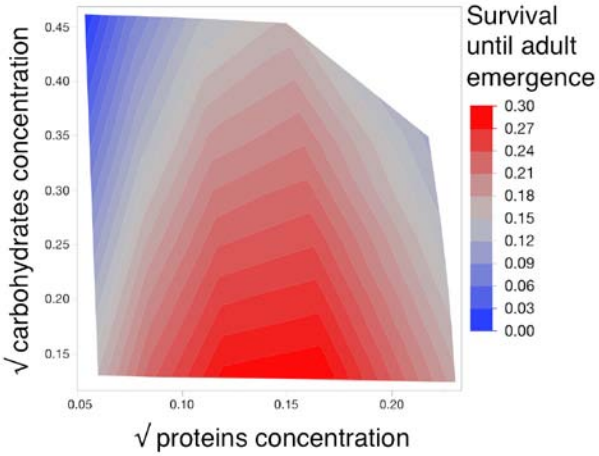

Figure 1. Combined effects of the concentrations of proteins and carbohydrates on the proportion of eggs that developed until the adult stage. Protein and carbohydrate concentrations were square-root-transformed. There were 25 treatments, each replicated 3 times.

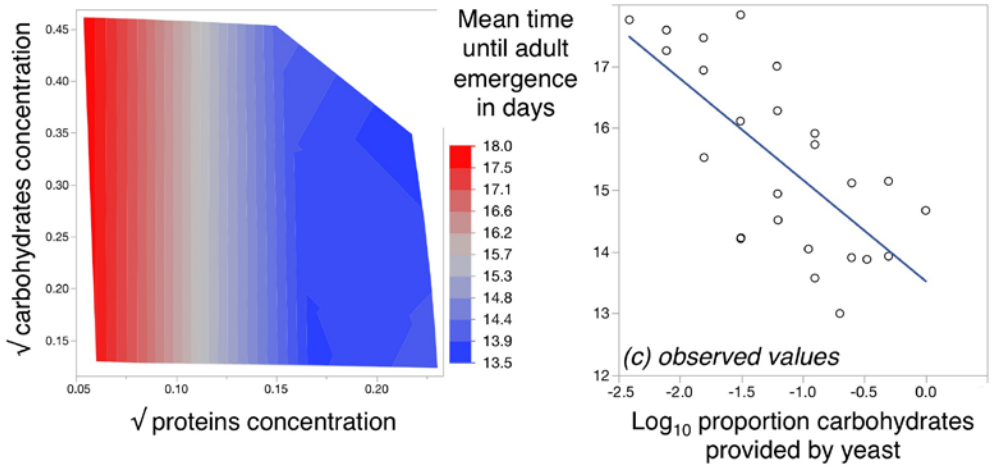

Figure 2. Combined effects of the concentrations of proteins and carbohydrates on the time until adult emergence. Protein and carbohydrate concentrations were square-root-transformed; proportion of carbohydrates from yeast was log-transformed. There were 25 treatments, each replicated 3 times.

Table 2. Statistical analyses of (a) the proportion of eggs that developed into adults, (b) the average time between oviposition and adult emergence. Proteins and carbohydrates concentrations were square-root-transformed; proportion of carbohydrates from yeast was log-transformed. We used standard linear models and only present final models, without non-significant terms.

Trait	Factors	D.F.	F	*p* value
(a) proportion of eggs that developed into adults	Carbohydrates concentration	1	17.7	< 0.0001
	Proteins concentration	1	10.7	0.002
	Proteins concentration ^{2}	1	23.4	< 0.0001
	Error	71		
(b) Time until adult emergence	Proteins concentration	1	132	< 0.0001
	Proteins concentration ^{2}	1	17.9	< 0.0001
	Proportion of carbohydrates from yeast	1	5.62	0.021
	Carbohydrates concentration	1	1.33	0.25
	Error	64		

Results

Survival to adulthood

The proportion of eggs that developed until adulthood decreased as total carbohydrate concentration increased (Figure 1, Table 2a). Survival peaked at intermediate protein concentrations showing this macronutrient has a non-monotonous effect on this trait. There was no significant effect of the proportion of carbohydrates from yeast on survival. Overall, the proportion of eggs that produced adults never exceeded 31%, which is partly due to egg mortality during their manual transfer to nutritive media.

Time until adult emergence

The average time until adult emergence decreased when the concentration of proteins in the diet increased (Figure 2a, Table 2b). In contrast, neither the concentration of carbohydrates, nor sex-ratio had a significant effect time until adult emergence. As the proportion of carbohydrates brought by yeast increased, there was a marginally significant acceleration of development (Table 2b, Figure 2b).

Relationship between survival and development speed

Treatments that produced many survivors also tended to favor fast development (correlation r = -0.37, P = 0.066) (Figure 3 dashed line). It appeared that treatments with the greatest protein concentrations had opposite effects on survival and developmental rate. Excluding from the analysis the 3 diets with more than 10% proteins and 10% carbohydrates (squares in Figure 3) greatly improved the correlation between the two traits (correlation r = -0.67, P = 0.001) (Figure 3 solid line). Comparison of Figures 1 and 2 indeed show they mostly differ in the top right part of the panels (*i.e.*, high proteins and carbohydrate concentrations), where survival is poor but development fast.

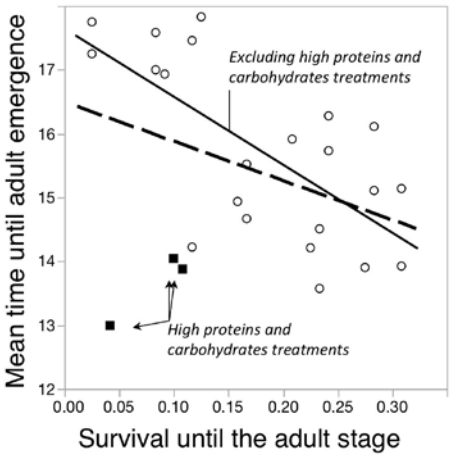

Figure 3. Relationship between survival until the adult stage and time until adult emergence. Each point (circle and square) corresponds to a diet treatment. Squares indicate treatments with protein and carbohydrate concentrations above 10%. Full line indicates the relationship between the two traits including all treatments; dashed lines indicate relationship excluding the 3 treatments with protein and carbohydrate concentrations above 10%. Correlations are there to highlight how treatments with high protein and carbohydrate concentrations depart from the general inverse relationship between survival and developmental rate.

Discussion

The study of larval response to variation in carbohydrate and protein concentrations in the nutritive medium revealed different effects on survival until the adult stage and time until emergence. Survival was maximized with an intermediate protein concentration and decreased as carbohydrates increased in concentration (Figure 1). However, there was no visible effect of carbohydrates on time until adult emergence, whereas greater protein concentrations led to faster development (Figure 2).

Congruence between field ecology and laboratory reaction norms

Drosophila suzukii is famous for having a different ecology from that of most Drosophilid species, and, in particular, from *Drosophila melanogaster* (Cini *et al.*, 2014). A notable feature is its ability to lay eggs in healthy, unripe fruit (Atallah *et al.*, 2014) and, therefore, exploit resources not yet colonized by bacteria and yeast, at least before egg deposition. As yeasts are the main source of dietary proteins of *Drosophila* larvae,

we expected *D. suzukii* to maintain good performance even in media with low proteins (i.e., yeast) concentrations (see detailed reasoning in the introduction). This prediction is met by our results on survival, which is maximized at intermediate protein concentration (Figure 1). Another prediction was that *D. suzukii* larvae would not perform well in high sugar conditions as ripening fruit, where *D. suzukii* females would lay eggs, contain less sugar than ripe fruit. Again, this prediction was matched by our survival data: high sugar content had a detrimental effect on the proportion of eggs that developed as adults. These results may be compared to those of a recent study on *D. suzukii* nutrition (Silva-Soares *et al.,* 2017) that also used the geometric framework and similar macronutrients concentrations in larval diets. In both experiments, larval survival was optimal at intermediate protein concentration. However, in the study by Silva-Soares *et al.* (2017) carbohydrate concentration had no significant effect on survival, while higher concentrations were detrimental in our study (Figure 1). Speed of development responded similarly proteins concentration in the two studies; even though there was a marginal difference when concentrations were maximal as reduced performance is only noticeable in the previous study (note quadratic terms are significant in both experiments). Comparison with similar experiments on *D. melanogaster* may reveal whether the patterns we and Silva-Soares *et al.* (2017) observed are specific to *D. suzukii*. It appears that both survival and development speed in the two species respond differently to protein and carbohydrate availability. In a recent study that used methods similar to ours (Rodrigues *et al.,* 2015), survival of *D. melanogaster* larvae until the adult stage improved with protein concentration and showed only limited decrease at high protein concentration (see Figure 1A in Rodrigues *et al.,* 2015). Effects on developmental rate were also contrasted between our observations and the *D. melanogaster* study. We observed a monotonic acceleration of development with increasing protein concentrations, whereas in *D. melanogaster* this trait was optimal at intermediate protein concentrations (see Figure 1B in Rodrigues *et al.,* 2015). It, therefore, appears that the congruence between our results and the available knowledge on *Drosophila* ecology is best for the rate of survival: for this trait *D. suzukii* performed best in media poorer in proteins than did *D. melanogaster*. The congruence between predictions and observations is less for speed of development, which is not as important to fitness than survival and is, therefore, under weaker selective pressure. It is important to note that the study by Rodrigues *et al.* (2015) contained treatments with greater concentrations of proteins than ours. Their range of conditions should, therefore, have permitted the detection of reduced larval performance at high protein concentrations. We can, therefore, conclude that, at least for the studied populations, nutritional landscapes of *D. suzukii* and *D. melanogaster* are likely different. *D. suzukii* and that survival reaction norms match what is known of the oviposition habits of gravid females.

Treatments that combined high protein and high carbohydrate availability were detrimental for survival but not for developmental speed (Figure 3). In all other treatments, greater survival associated with faster development. The surprising pattern at high protein concentration could be interpreted as an alternative developmental strategy triggered by diet composition (Pigliucci, 2005). It is frequent to observe effects of the proportion of yeast in the diet of *Drosophila* larvae on their phenotype (Anagnostou *et al.,* 2010), or on that of the adults they produce (Fellous and Lazzaro 2010). The adaptive value of this phenotypic plasticity may even be discussed (Gould and Lewontin, 1979). Sometimes, different developmental trajectories produce different combinations of traits that nonetheless have similar fitness (Schmidt *et al.,* 2012). But in our case, it is unlikely that faster development, at the cost of greater mortality (black squares in Figure 3), reflects adaptive plasticity. A slightly faster development (here 1-2 days) probably cannot offset the cost of reduced survival (here ± 50%). However, our results suggest that larval food composition may affect important parameters of fly demography. It is thus possible that population structure (*i.e.*, the relative proportion of individuals from different age classes) may be affected by nutritional composition of the available resources (de Roos and Persson, 2013). The growth of populations feeding and developing on fruits with distinct macronutrient composition may then be limited by constrains exerted at different stages of the life-cycle (Nicholson, 1957), with consequences at the community level (Miller and Rudolf, 2011).

Challenges with laboratory studies of insect nutrition

Our approach has a caveat that is common to most studies of nutrition in *Drosophila* flies; namely, we did not re-create the microbial environment in which flies normally develop. We did manipulate dead yeast concentration, but in nature yeasts are alive and their concentration responds to larval feeding (Stamps *et al.,*

2012). It is clear that adults *D. suzukii* and *D. melanogaster* have different relationships with fruits and microbes: if both species can be attracted to yeast volatiles at least for adult feeding (Becher *et al.*, 2012) only *D. suzukii* responds to fruit volatiles (Keesey *et al.*, 2015, Swoboda-Bhattarai *et al.*, 2017). However, our understanding of fly-microbe relationships in the natural environment is for the moment shallow. Some studies on adult feeding ecology have shed light on the trade-off between feeding and oviposition (Lihoreau *et al.*, 2016; Plantamp *et al.*, 2017), or the reliance of adults on yeast for nutrient acquisition (Becher *et al.*, 2012; Yamada *et al.*, 2015). However, larval ecology remains poorly known. Other unidentified environmental factors - beyond macronutrient concentration - probably influence the development of *Drosophila* larvae. For instance, it is unknown whether the feeding apparatus of *D. suzukii* is different from that of other *Drosophila* species; one could indeed hypothesize their greater ability to chew through firm, unripe fruit flesh. The difference between the nutritional environments used in lab assays and that encountered by flies in nature may explain some hard-to-interpret results: for example, why do *D. melanogaster* females sometimes prefer to lay their eggs in environments that do not seem optimal for larval development (Rodrigues *et al.*, 2015). In addition to effects of yeast symbionts, bacterial symbionts involved in processing and assimilating nutrients greatly differ between lab and wild conditions (Chandler *et al.*, 2011; Chandler *et al.*, 2014), increasing the mismatch between lab conditions and the environment to which flies are adapted. In the case of *D. suzukii*, which oviposits on ripening fruit still attached to the host plant, ripeness, and, therefore, sugar concentrations may be dynamic and vary during larval life. Along these lines, we believe yeast growth during fruit infestation (Stamps *et al.*, 2012), and the subsequent variations of proteins concentrations, is one of the most important phenomena that is not taken into account in the geometric framework of nutrition.

Relationships between laboratory and field phenomena are further blurred by the genetic adaptation that occurs during domestication (Hoffmann *et al.*, 2001; Fragata *et al.*, 2014), as may have been the case in our study. This is, however, a problem met by other *Drosophila* studies to which we compare our results. Similarly, most studies only test a single population or strain per species, even though intra-specific genetic variation is very common for plant-exploitation traits in herbivores (*e.g.*, Jaenike, 1985; Fellous *et al.*, 2014) and interactions with symbiotic microbes *(e.g.*, Fellous *et al.*, 2012).

An unexpected result was the response of developmental rate to the proportion of carbohydrates that came from yeast input (Figure 2b). Carbohydrates are generally separated into short- and long-chained carbohydrates that correspond to readily accessible sugars and storage molecules such as starch, respectively. In our experiment, yeast provided long-chained carbohydrates in complement to the saccharose (a type of sugar) of our medium recipe. We cannot explain the effect of the proportion of carbohydrates provided by yeast, but relate it to the stoichiometry of nutrients involved in physiological processes (Jacobs *et al.*, 2009). As such, it is in favor of the geometric framework of nutrition (Raubenheimer *et al.*, 2009). Along these lines, variations of unidentified nutrients - and the discrepancy between lab environments and natural microbial communities - may explain why in another recent study *D. suzukii* larvae developed more slowly in yeast-rich medium than on real fruits (Jaramillo *et al.*, 2014), while in our experiment yeast enrichment accelerated development.

The nutritional framework of nutrition is a powerful tool to study insect physiology. However, the caveats discussed above - such as the dynamic nature of the microbial symbionts that provide nutrients and the existence of unidentified environmental factors - show artificial diets may not be adequate to understand the ecology of *Drosophila* flies. We believe this objective will be better met with alternative methods using real fruit and naturally occurring communities of microbes. In the wild, *D. suzukii* is frequently found associated with the yeast *Hanseniaspora uvarum* (Hamby *et al.*, 2012), which prompts for an in-depth study of the nature of this symbiosis.

Conclusions

We studied the performance of *D. suzukii* larvae across a range of nutritional conditions following the geometric framework of nutrition (Raubenheimer *et al.*, 2009). Our main results - that survival peaked at intermediate protein concentration in the larval diet and that carbohydrates are detrimental - are congruent with the behavior of *D. suzukii* females that are known to oviposit in sugar-poor fruit, not yet colonized by yeast. However, in retrospect, we identified a number of factors that may limit the significance of this type of

experiment for *in-natura* processes. Overcoming these challenges will be necessary to better understand the nutritional ecology of *Drosophila* flies.

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Literature cited: Adrion, J.R., A. Kousathanas, M. Pascual, H.J. Burrack, N.M. Haddad, A.O. Bergland, H. Machado, T.B. Sackton, T.A. Schlenke, M. Watada, D. Wegmann, and N.D. Singh 2014, Molecular Biology and Evolution; Anagnostou, C., M. Dorsch, and M. Rohlfs 2010, Entomologia Experimentalis et Applicata 136: 1-11; Atallah, J., L. Teixeira, R. Salazar, G. Zaragoza, and A. Kopp 2014, Proceedings of the Royal Society of London B: Biological Sciences 281: 20132840; Becher, P.G., G. Flick, E. Rozpędowska, A. Schmidt, A. Hagman, S. Lebreton, M.C. Larsson, B.S. Hansson, J. Piškur, and P. Witzgall 2012, Functional Ecology 26: 822-828; Chandler, J.A., P.M. James, G. Jospin, and J.M. Lang 2014, PeerJ 2: e474; Chandler, J.A., J. Morgan Lang, S. Bhatnagar, J.A. Eisen, and A. Kopp 2011, PLoS Genet 7: e1002272; Cini, A., G. Anfora, L.A. Escudero-Colomar, A. Grassi, U. Santosuosso, G. Seljak, and A. Papini 2014, Journal of Pest Science: 1-8; de Roos, A.M., and L. Persson 2013, *Population and Community Ecology of Ontogenetic Development*. Princeton University Press, Princeton; Fellous, S., G. Angot, M. Orsucci, A. Migeon, P. Auger, I. Olivieri, and M. Navajas 2014, Journal of Evolutionary Biology 27: 911-919; Fellous, S., A.B. Duncan, E. Quillery, P.F. Vale, and O. Kaltz 2012, Ecology Letters 15: 186-192; Fellous, S., and B.P. Lazzaro 2010, Molecular Ecology 19: 1462-1468; Fragata, I., P. Simões, M. Lopes-Cunha, M. Lima, B. Kellen, M. Bárbaro, J. Santos, M.R. Rose, M. Santos, and M. Matos 2014, PLoS ONE 9: e96227; Fraimout, A., V. Debat, S. Fellous, R. Hufbauer, J. Foucaud, P. Pudlo, J.-M. Marin, D.K. Price, J. Cattel, X. Chen, M. Deprá, P.F. Duyck, C. Guedot, G. Loeb, M. Kenis, M.T. Kimura, I. Martinez, M. Pascual, M.P. Richmond, P. Shearer, N. Singh, K. Tamura, A. Xuéreb, J. Zhang, A. Loiseau, and A. Estoup 2017, Molecular Biology and Evolution. 34: 980-996; Gould, S.J., and R.C. Lewontin 1979, Proceedings of the Royal Society of London Series B-Biological Sciences 205: 581-598; Hamby, K.A., A. Hernandez, K. Boundy-Mills, and F.G. Zalom 2012, Applied and environmental microbiology 78: 4869-4873; Hoffmann, A.A., R. Hallas, C. Sinclair, and L. Partridge 2001, 55: 436-438; Jacobs, D.R., M.D. Gross, and L.C. Tapsell 2009, The American Journal of Clinical Nutrition 89: 1543S-1548S; Jaenike, J., 1985, Evolution: 362-369; Jaramillo, S.L., E. Mehlferber, and P.J. Moore 2014, Physiological Entomology 40: 2-9; Karageorgi, M., L. Bräcker, S. Lebreton, C. Minervino, M. Cavey, K. Siju, I. Grunwald Kadow, N. Gompel, and B. Prud'homme 2017, Current Biology 27: 847-853; Keesey, I., M. Knaden, and B. Hansson 2015, Journal of Chemical Ecology 41: 121-128; Lee, J., D. Dalton, K. Swoboda-Bhattarai, D. Bruck, H. Burrack, B. Strik, J.M. Woltz, and V. Walton 2015, Journal of Pest Science: 1-10; Lee, K.P., S.J. Simpson, F.J. Clissold, R. Brooks, J.W.O. Ballard, P.W. Taylor, N. Soran, and D. Raubenheimer 2008, Proceedings of the National Academy of Sciences 105: 2498-2503; Lihoreau, M., L.-A. Poissonnier, G. Isabel, and A. Dussutour 2016, Journal of Experimental Biology; Miller, T.E., and V.H. Rudolf 2011, Trends in Ecology & Evolution 26: 457-466; Nicholson, A.J., 1957, The self-adjustment of populations to change. Pages 153-173, In: Cold Spring Harbor Symposia on Quantitative Biology. Cold Spring Harbor Laboratory Press; Oakeshott, J.G., D.C. Vacek, and P.R. Anderson 1989, Oecologia 78: 533-541; Pigliucci, M., 2005 Trends in Ecology & Evolution 20: 481-486; Plantamp, C., V. Estragnat, S. Fellous, E. Desouhant, and P. Gibert 2017, Basic and Applied Ecology; Prasanna, V., T.N. Prabha, and R.N. Tharanathan 2007, Critical Reviews in Food Science and Nutrition 47: 1-19; Raubenheimer, D., S.J. Simpson, and D. Mayntz 2009, Functional Ecology 23: 4-16; Rodrigues, M.A., N.E. Martins, L.F. Balancé, L.N. Broom, A.J.S. Dias, A.S.D. Fernandes, F. Rodrigues, É. Sucena, and C.K. Mirth 2015, Journal of Insect Physiology 81: 69-80; Rombaut, A., R. Guilhot, A. Xuéreb, L. Benoit, M.-P. Chapuis, P. Gibert, and S. Fellous 2017, Royal Society Open Science; Schmidt, B.R., W. Hödl, and M. Schaub 2012, Ecology 93: 657-667; Silva-Soares, N.F., A. Nogueira-Alves, P. Beldade, and C.K. Mirth 2017, BMC Ecology 17: 21; Stamps, J.A., L.H. Yang, V.M. Morales, and K.L. Boundy-Mills 2012, PLoS ONE 7: e42238; Starmer, W.T., and J.C. Fogleman 1986, Journal of Chemical Ecology 12: 1037-1055; Swoboda-Bhattarai, K., and H.

Burrack 2015, Pages 419-430, In: XI International Rubus and Ribes Symposium 1133; Swoboda-Bhattarai, K.A., D.R. McPhie, and H.J. Burrack 2017, Journal of Economic Entomology; Yamada, R., S.A. Deshpande, K.D. Bruce, E.M. Mak, and W.J. William 2015, Cell reports 10: 865-872.

Developmental homeostasis reflected in symmetry of cell death in the *Bar* eye of *Drosophila*.

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One of our recent interests is the identification of genetic modifiers of cell death in compound eye facets using the sequenced strains of *Drosophila* developed by Mackay and her colleagues (Mackay *et al*., 2012; DGRP strains available from the Bloomington *Drosophila* Stock Center; for earlier pilot data see Thompson *et al*., 2015). But these experiments also provide an opportunity to explore a somewhat unrelated phenomenon: the degree of symmetry in the extent of developmentally-patterned cell death. Using *Drosophila* eyes carrying the mutation *Bar*, deviations from symmetry are a potential measure of developmental homeostasis – the compensations required to generate a symmetrically bilateral body plan. Cell death in *Bar* eyes is clearly variable, with phenotypes in our experiments ranging from less than 50 to over 300 facets per eye. Our hypothesis is that, in spite of this tendency to vary, there is developmental coordination within an individual that tends toward symmetrical expression in facet number.

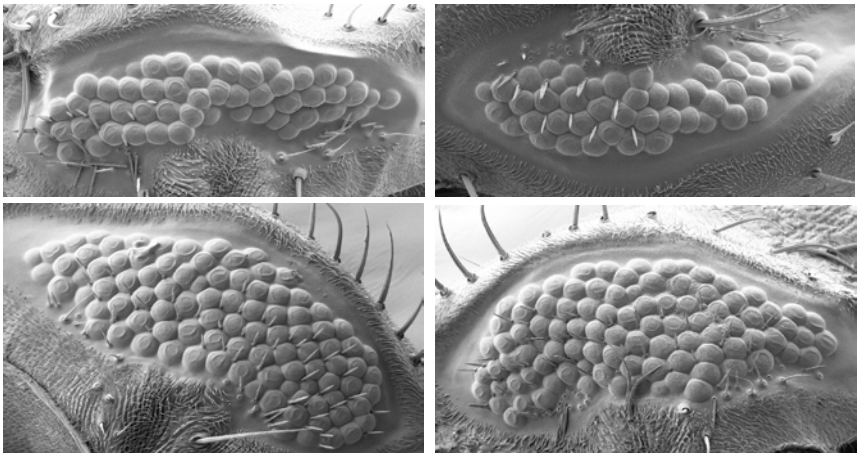

Figure 1. Representative pairs of *Bar* eyes from an F_1 male from #25745 (top row) and a male from # 25185 (lower row).

We have chosen two DGRP strains that yield quite different numbers of facets when heterozygous in males carrying the sex-linked mutation *Bar*. The average facet number from strain #25745 is 71 ± 13 (n = 80), while for #25185 it is 169 ± 44 (n = 62). We are able to get such precise facet numbers by using the Zeiss NEON 40EsB scanning electron microscope to image individual eyes (Figure 1). Inbred females carrying the balancer *Basc* with the dominant sex-linked *Bar* eye mutation were mated to males from each of two of the DGRP lines that had shown quite different F_1 facet numbers in earlier crosses. F_1 males carry the *Bar* mutation and are heterozygous for eye facet number modifiers from a sequenced DGRP line. Individual heads were removed with a razor blade and bisected between the eyes. Pairs of eyes were mounted on SEM plugs, air dried for several days, and then coated with gold-palladium in a Hummer 6 sputter coater. High resolution images were taken of each eye at an average magnification of 350×. Facet numbers were then counted in duplicate by several participants. This is clearly excessive replication, but, for this step in our project, it allowed each participant to become directly involved