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Corresponding Author: Dr. Christen Kerry Mirth, Ph.D.

Corresponding Author's Institution: Instituto Gulbenkian de Ciência

First Author: Cristiane Matavelli

Order of Authors: Cristiane Matavelli; Maria João A Carvalho; Nelson E Martins; Christen Kerry Mirth, Ph.D.

Abstract: Species coexist using the same nutritional resource by partitioning it either in space or time, but few studies explore how species-specific nutritional requirements allow partitioning. Zaprionus indianus and Drosophila simulans co-exist in figs by invading the fruit at different stages; Z. indianus colonizes ripe figs, whereas D. simulans oviposits in decaying fruit. Larvae feed on yeast growing on the fruit, which serves as their primary protein source. Because yeast populations increase as fruit decays, we find that ripe fruit has lower protein content than rotting fruit. Therefore, we hypothesized that Z. indianus and D. simulans larvae differ in their dietary requirements for protein. We used nutritional geometry to assess the effects of protein and carbohydrate concentration in the larval diet on life history characters in both species. Survival, development time, and ovariole number respond differently to the composition of the larval diet, with Z. indianus generally performing better across a wider range of protein concentrations. Correspondingly, we found that Z. indianus females preferred to lay eggs on low protein foods, while D. simulans females chose higher protein foods for oviposition when competing with Z. indianus. We propose the different nutritional requirements and oviposition preference of these two species allows them to temporally partition their habitat.

INSTITUTO GULBENKIAN DE CIÊNCIA

September2nd, 2015

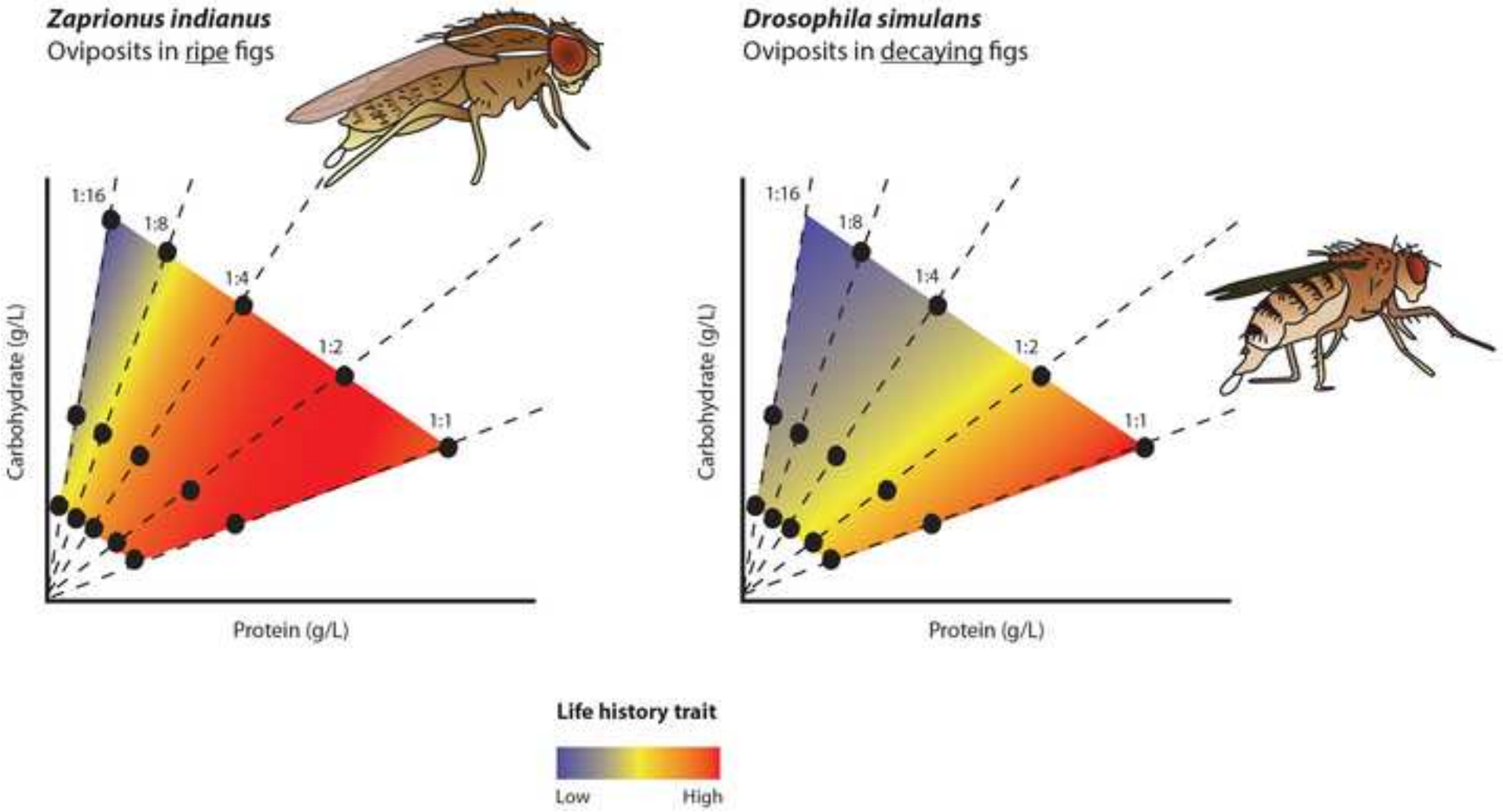
Dear Dr. Spencer Behmer,

Please find enclosed our revised manuscript "***Differences in larval nutritional requirements and female oviposition preference reflect the order of fruit colonization of Zaprionus indianus and Drosophila simulans***", for consideration as a research paper in Journal of Insect Physiology.We have addressed your two remaining comments:1) adding the species name and order of colonization to the graphical abstract, and 2) discussing your paper, Behmer and Joern, 2008, in the first introductory paragraph to build up our argument about how insects can coexist by differentially utilizing the same resource. We hope you now find this manuscript suitable for publication.

Sincerely,

Christen Mirth and coauthors

Graphical Abstract (for review)



***Highlights (for review)**

1. *Zaprionus indianus* and *Drosophila simulans* colonize figs at different stages of decay.
2. We compared the effects of protein and carbohydrate in the larval diets on life history traits.
3. *Z. indianus* performed better across a broader range of protein concentrations than *D. simulans.*
4. *Z. indianus* oviposit on low protein diets, while *D. simulans* make oviposition choices to avoid competition.
5. Nutritional requirements and oviposition choice reflect colonization time.

Differences in larval nutritional requirements and female oviposition preference reflect the order of fruit colonization of *Zaprionusindianus* and *Drosophila simulans*

CristianeMatavelli[#,a,b], Maria João A. Carvalho[#,b], NelsonE. Martins[b]and Christen K. Mirth*[b]

[#]joint first authorship

* corresponding author:Christen K. Mirth (christen@igc.gulbenkian.pt)

[a]Programa de Pós Graduação em Zoologia, Departamento de Zoologia, Universidade Estadual Paulista, UNESP, 13506-900, Rio Claro, SP, Brasil.

[b]Instituto Gulbenkian de Ciência, Rua da Quinta Grande, 6, 2780-156 Oeiras, Portugal

short title: resourcepartitioning in nutrient space

ABSTRACT

Species coexist using the same nutritional resource by partitioning iteither in space or time, but few studies explore how species-specific nutritional requirements allow partitioning. *Zaprionusindianus* and *Drosophilasimulans*co-exist in figsby invadingthe fruit at different stages; *Z. indianus*colonizesripe figs, whereas *D. simulans*oviposits in decaying fruit.Larvae feed on yeast growing on the fruit, which servesas their primary protein source. Because yeast populations increase as fruit decays,we find that ripe fruit has lower protein content than rotting fruit.Therefore, we hypothesized that *Z. indianus*and *D. simulans*larvae differ in theirdietary requirements for protein.We used nutritional geometry to assess the effects ofprotein and carbohydrate concentration in the larval diet on life history characters in both species. Survival, development time,and ovariole numberrespond differently to the composition of the larval diet, with*Z. indianus*generally performing better across a wider range of protein concentrations. Correspondingly, we found that*Z. indianus*females preferred to lay eggs on low protein foods,while*D. simulans*femaleschose higherprotein foods for oviposition when competing with *Z. indianus*.We proposethedifferent nutritional requirements and oviposition preference of these two speciesallows them to temporally partition theirhabitat.

KEYWORDS

Larval diet; Life-history traits;Macronutrient requirements;Nutritional geometry;Oviposition preference; Stage of ripeness/decay; Temporal partitioning

INTRODUCTION

Species that use the same ecological niche are faced with the problem of interspecific competition,whichaffects their fitness and population structure. Priority effects studies between two fungal-breeding Drosophilid species, *Drosophila phalerata* and *Drosophilasubobscura*, show thatthe species that arrives late at a patch has decreased survival, decreased body size (wing length), and increased mean developmental time (Shorrocks & Bingley 1994), thus lowering their fitness.Exploiting specific nutritional niches decreases competition amongclosely-related generalist species, allowing their coexistence.For example, species of grasshoppers within the genus *Melanoplus*coexist using the same food resources by actively selecting different protein and carbohydrate amounts from their environment (Behmer & Joern 2008). Another way species can avoid competition is by partitioning their resource,either spatially or temporally. Homogeneous niches can be partitioned in spacethroughlow interspecific and high intraspecific aggregation(Shorrocks 1975; Atkinson & Shorrocks 1981, 1984).Alternatively, in more heterogeneous environments different speciescan specialize in feeding and breeding on particular structures within the resource.Species from the Hirtodrosophila and immigrans groups that breed on mushrooms differ in where they prefer to lay their eggs, either on the stipe, lamella or pileus(Kimura 1980). Finally, species can exploit a resource at different times(Nunney 1990).The succession of changes that take place in decaying organic matter such as dung, carrion, fruit, fungi, and dead wood generate a range of temporally distributed niches for the animals that exploit these substrates for feeding and breeding sites(Kimura 1980; Lachaise *et al.* 1982; Nunney 1990; Morais *et al.* 1995).

Drosophilid fruit flies feed on species-specific ranges of decaying mushrooms, fruit, flowers and other plant parts.They are major vectors of yeasts, which provide a source of essential nutrients to these flies (Starmer & Fogleman 1986). Yeasts alsoshow a species-specific pattern of succession in their colonization of the decaying fruits(Morais *et al.* 1995), wood(Gonzalez *et al.* 1989), and logs of *Pseudotsugamenziesii*(Crawford *et al.* 1990). In amapa fruits, more than 19 different yeast species were identified in succession over the course of 14 days after the fall of the fruit(Morais *et al.* 1995).Thus, yeast succession in fruits provides a patchy environment for Drosophilidsand other insects sharing this ephemeral substrate (Morais *et al.* 1995). Importantly, yeast successionallows not only for spatial partitioning, as there may be several yeasts growing simultaneously in different patches, but also temporal partitioning, which sustains a consequent succession of insects.

The succession of yeasts and other microorganisms change the characteristics of the decomposing matter, leading to its change in toxin load, pH, taste, and nutrient composition over time. For example, carbohydrate composition changes as fruit ripens, the stage where it achieves the maximum sweetness. Starch hydrolyses during ripening to produce sugars in various fruits (Pech & Latche 1972; Pesis *et al.* 1978). In addition, the protein source for flies mostly comes from the yeast that colonizes the fruit and not from the fruit itself. The maturation process of fruits, from ripening to rotting, leads to changes in the density and diversity of yeast growing in the aging fruit (Morais *et al.* 1995), resulting in changes in the ratio of protein to carbohydrate (P:C) depending on the stage of fruit decay (Tournas & Katsoudas 2005). Thus, both the

carbohydrate composition and the protein content of fruit changes with time, providing temporal diversity in macronutrient composition of the resource.

A succession of Drosophilidsemerges from rotting fruit such as oranges (Nunney 1990), amapa fruits (Morais *et al.* 1995), and figs (Lachaise *et al.* 1982). Amongst these examples, the order of colonization of *Zaprionusindianus* and *Drosophila simulans*provides an interesting opportunity to understand how species might adapt their nutritional requirements to partition a resource at different stages of maturation. *Z. indianus* and *D. simulans* are the two most abundant Drosophilid species in fig monocultures of the Valinhos region, São Paulo (Pires & Bélo 2005). They coexist in fig monocultures as they show temporal partitioning of this breeding site. *Z. indianus* females are attracted to the figs for oviposition before the ripening phase, laying their eggs near the ostiole and inside of the immature, pre-ripened fig, therebycolonizing it with yeasts (Lachaise *et al.* 1982; Stein *et al.* 2003). As *Z. indianus* invades the fig before harvest, it renders it unusable for commercial purposes.In contrast, *D. simulans*females only colonize figs at an advanced stage of ripening, when the fruit ison its way to rotting (Lachaise *et al.* 1982; Stein *et al.* 2003). Since larvae have limited mobility compared to adults, their food sources are largely determined by their mother's choice of oviposition site (Shorrocks 1975), making oviposition site choice crucial for the survival of the eggs and larvae. Due to differences in when the females oviposit in fruit, we would expect that the developing larvae are adapted to different macronutrient environments.

Understanding how species adapt to nutritional niches within a dynamic environment involves considering a multitude of factors, which rapidly can become intractable. One way of coping with this complexity is to parse down changes in the

nutritional environment to two nutritional parametersvaried across a broad range of values, an approach termed nutritional geometry (Raubenheimer & Simpson 1997; Simpson & Raubenheimer 1999). This approach allows us to decrease the nutritional complexity of foods down to manageable sizes, while introducing sufficient complexity to allow the exploration of interactions between macronutrients(Raubenheimer & Simpson 1997; Simpson & Raubenheimer 1999). Nutritional geometry has been used to explore the response of life history traits and behavioural strategies to the macronutrients in a broad range of animals(Kohler *et al.* 2012; Simpson *et al.* 2015,Rothman, 2014 #2003).

Previous studies in *D. melanogaster* show that the protein content of the larval diet regulates their growth (Bakker 1959; Tu & Tatar 2003), their development time (Beadle *et al.* 1938), their body and organ sizes (Tu & Tatar 2003), and the development of their reproductive organs(Güler *et al.* 2014). Protein consumption, not carbohydrate consumption, regulates body and tissue growth in larvae(Britton & Edgar 1998; Colombani *et al.* 2003). However, larvae show the shortest development times in diets containing a mix of protein and carbohydrates (Rodrigues *et al.* 2015). Thus, in *D. melanogaster* both the protein and the carbohydrate compositions of the larval diet appear to playimportant roles in shaping life history characters.

To understand how *Z. indianus* and *D. simulans* utilize different larval macronutrient environments, we used nutritional geometry to explore the effects of the macronutrient composition of the larval diet on three life history traits. Our results show significant differences in the responses of life history traits to the larval diets between*Z. indianus*and *D. simulans*. Additionally, adult females of the two species also show differences in the preferred macronutrient balance foroviposition. Overall,our

results indicate that differences in the nutritional requirements of larvae and oviposition preference of the females allow resourcepartitioning between species.

MATERIAL AND METHODS

Fly stocks and stock maintenance conditions

Z. indianus was a generous gift from Dr. Jean David (CNRS, Gif-Sur-Yvette, France). *D. simulans* was obtained from the *Drosophila* Species Stock Center (#14021-0251.187). Both during stock maintenance and experiments, flies were maintained at 25^{o}C, in a 12h light: 12h dark regime, 60-70% humidity. Adults were kept on standard food used in the laboratory, which included 45 g/L molasses, 75 g/L sugar, 70 g/L corn flour, 20 g/L yeast extract, 10 g/L agar and 0.25 % of nipagen.

Protein and sugar quantification in decaying figs

To assess the protein and sugar content of figs from ripening to decay, we placed 10 plastic cups each containing a single freshly-harvested fig inside a population cage (11x20.5x27 cm), with three replicate population cages. Figs were inoculated with yeast by introducing 50 males (25 *Z. indianus* and 25 *D. simulans* males) and, for the first two days of the experiment, a petri dish (5.5 cm-diameter) filled with standardfoodand yeast paste (Baker's yeast). One fig per cage was collected and frozen on days 2, 5, 8, 11, 14, 16, 19, 21, 23, and 26.

We blended each of the figs collected, and distributed2 ml of the blended fig intoone of threeeppendorfs. Samples were lysed with metal beads using a QiagenTissueLyzer for 10 minutes at maximum speed. Samples were centrifuged for 10 minutes at 6189 g. Supernatant was collected and used for protein quantification using

a Pierce BCA protein assay kit (Thermo Scientific #23227) and for glucose/sucrosequantification using a Glucose and Sucrose Colorimetric/FluorimetricAssay Kit (Sigma #MAK013).

Nutritional geometry and life history traits

We used the geometric framework for nutrition, raising larvae of each species in fifteen different diets that differed in their caloric, protein, and carbohydrate content. We produced each of these diets by combining yeast (LesaffreSAF-Instant Red #15909, 31105, 31150) and sucrose(Sidul, Santa Iria de Azóia, Portugal)solutions of different concentrations (45, 90, and 180 mg/ml, each containing 0.5% agar) to produce one of five P:C ratios (1:1; 1:2; 1:4; 1:8, and 1:16)(Lee *et al.* 2008)and one of three caloric concentrations (0.18, 0.36, and 0.72 kcal/ml).To prevent bacterial and fungal growth, we autoclaved the diets and added 0.25 % nipagen and 0.6% (v/v) propionic acid to the cooled mixtures before pouring them into bottles. Within this nutritional framework, we assessed the response of threelife history traits, survival from embryo to pupae, developmental time, and ovariole number.

Embryosfrom each species were collected overnight in each diet. Thirty 24h-old larvae were transferred to bottles with the same diet as the respective oviposition plate. Three replicates were made per dietary regimen, all from the same oviposition period. To assess survival and development time, we checked each bottle daily and recorded the number of animalspupariating. Once the adults emerged, we allowed them to feed for 3-5 days on standard diet supplemented with yeast. They were then kept at -20°C until the time of dissection. Ovaries from the adult females were extracted in phosphate buffered saline to assess the number of ovarioles per female, a measure of reproductive potential.

Oviposition Assays

To assessoviposition preference, we reared animals from egg to eclosion at densities of thirty eggs per vial on 0.72 kcal/ml food with P:C ratio of 1:1. We then designed a three-choice assay plate by adhering the caps from nine 0.5 µl microcentrifuge tubes to a 60 mm diameter petri dishas in (Rodrigues *et al.* 2015). Twelve females and five males were then placed into 200 ml plastic cups and the assay plate was fitted over the end of the cup. Each assay plate offered a choice of three different foods that contained the same caloric values (0.72 kcal/ml), but differed in P:C ratio (1:16, 1:4 and 1:1). These foods were dyed red, green, or blue(4.5ml of Globo food dye/100 ml of food) to distinguish between them, and we controlled for colour preference by alternating the colour of each food and conducting the assays in the dark. Flies were left in the oviposition chambers for 15 hours after which the chambers were frozen and the number of eggs laid in each food counted. We further assessed oviposition choice in competition by placing ten females and five males of each species together in the assay chambers. We could distinguish between the eggs of *Z. indianus*and *D. simulans*by the number of dorsalappendages;while *Z. indianus*eggs havefour (Sturtevant 1920), *D. simulans*eggshave only two dorsal appendages(Hutchinson 1978).

Statistical Analysis

Protein content, sugar content, and the protein to sugar ratio in the figs increased exponentially over time. To characterize the macronutrient composition of the fig over time, we log transformed the macronutient data and fit it with linear mixed effects models, including replicate as the random effect.

We estimated the response of life-history traits tothe larval dietfollowing the methods outlined in(Lee *et al.* 2008). For survival probabilities, we fit a generalized linear model assuming a quasibinomial distribution, to account for the overdispersion of the data, with a logit link function. For development timeand ovariole number, we fit the data with linear mixed effects models, including replicates as a random effect. Our models include the effects of both the linear and quadratic components of carbohydrate and protein, and their cross product, on the dependent variables. Toassess differences in the life-history trait responses both within and between species, we first standardized the dependent variables to a mean of zero with unit standard deviations, and then used partial F tests to compare the response surfaces generated from the models outlined above.

Finally, we tested for significant differences in the proportion of eggs laid in each P:C ratio for each species by fitting the data with a generalized linear model, using a quasibinomial distribution to account for the overdispersion of the data. We then compared the proportion of eggs laid in each P:C ratio against a null distribution of $\mu=0.33$ (no choice between all three food types) and, in the oviposition site competition experiment, compared the least squared means for each P:C ratio for each species. We adjusted the*p*-values for tests involving multiple comparisons using Bonferroni correction. All datasets and scripts are publically available from Dryad (reference to be provided).

RESULTS

The macronutrient composition of figs changes with decomposition

We assessed the change in protein content, sugar content, and protein to sugar ratio in figs over 27 days after yeast inoculation (Supplementary Figure 1). We found that the log protein concentration increased with time (χ^2=4.61, p value=0.032, R^2=0.24), while the log sugar content decreased with time (χ^2=75.30, p value<0.001, R^2=0.70). As a result, the log protein to sugar ratio increased with time after yeast inoculation (χ^2=113.44, p value<0.001, R^2=0.76).

Zaprionusindianus: the effects of larval nutrition on life history traits

Z. indianus lays its eggs in figs as they ripen. At this stage, figs have highersugar content, lower protein content, and lowerP:C ratios than they do at later stages. We predicted the response of life history traits to the larval diet would reflect the nutritional content of the figs at this stage. We first analyzedthe response of all four life-history traitsin*Z. indianus* towards the range of protein and carbohydrate concentrationsof our nutrientspace. We also compared the nutritional response curves of the different traits to identify potential trade-offs between traits.

SURVIVAL:The proportion of animals surviving from larva to pupa across the larval diets in *Z. indianus* ranged between 0.43 to 0.97,and correlated positively with the linear component and negatively with the quadratic component of protein (Figure 1A, Table 1). Neither the carbohydrate composition of the diet, nor the cross product between protein and carbohydrate significantly correlated with survival (Table 1). This resulted in a relatively flat response surface with maximum survival proportions at

intermediate protein concentrations, and with survival decreasing as protein concentration either decreasedor increasedaway from these values (Figure 1A).

DEVELOPMENTAL TIME: The developmental time of *Z. indianus* varied between 6-14 days depending on the diet(Figure 1B). The full model explained 76% of the variance observed in this trait. Development time correlated negatively with the linear component of protein and positively with the quadratic component of protein (Table 1). This resulted in the shortest development times across a range of intermediate to high protein concentrations.

OVARIOLE NUMBER: In *Z. indianus,*ovariole number ranged between 25-35 ovarioles across the larval diets (Figure 1C). The full model explained 31% of the observed variance (Table 1). Ovariole number increasedwith the linear component of protein and decreasedwith the quadratic component of protein. Thus, ovariole number wasmaximized when the larvae wereraised in diets with intermediate proteincontent, with ovariole number decreasing as protein decreased or increased away from these intermediate values (Figure 1C, Table 1).

COMPARISON BETWEEN RESPONSE SURFACES: We tested for significant differences in the shape of the responsesurfaces between *Z. indianus* life history traits by comparing the standardized parameter values for each trait using partial F tests (Table 2). To compare development time to the remaining traits, we inverted these values. The shape of the response for survival differed significantly to the other two traits. In addition, the responses of ovariole number and developmental time to the protein and carbohydrate composition of the larval diet differed significantly. From these

comparisons, we identified three groups of response surfaces: one for survival, a second for ovariole number, and a third for developmental time.

Drosophila simulans:the effects of larval nutrition on life history traits

D. simulans invades the fig monocultures when the figs are beginning to decay. At this stage, the fig contains lowersugar content, higherprotein content and higher protein to sugarratios than in the ripe fruit. Thus, we would expect that in *D. simulans* the responses of life history traits would show maximum values in diets with higher protein content and higher P:C ratios. To explore this possibility, we subjected larvae to the same fifteen diets as for *Z. indianus* and measured survival from larva to pupa, development time, and ovariole number.

SURVIVAL:The proportion of animals surviving from larva to pupa ranged from 0.27 to 1 in *D. simulans*. Although the survival appeared to increase with increasing protein (Figure 2A), neither the carbohydrate content, the protein content, nor the their cross product correlated significantly with the proportion of animals surviving (Table 3).

DEVELOPMENTAL TIME:Across the nutrient space explored,*D. simulans* took between 4-12 days to develop from egg to pupae (Figure3B). A linear mixed-effects model showed that 56% of the observed variance in development time was due to the protein and the carbohydrate content of the larval diet (Table 3). Developmental time correlated positively with the linearcomponent of carbohydrate and the quadratic component of protein, and negatively with the linear component of protein and with the cross product between protein and carbohydrate. The shortest developmental time occurred in diets with the lowest carbohydrates and intermediate to high protein (Figure2B).

OVARIOLE NUMBER:Within the nutrient space examined, larval diet resulted in females with 24-33 ovarioles (Figure 2C). Similar to survival, although ovariole number appeared to increase with increasing protein, none of the variables showed significant correlation with this trait(Table 3).

COMPARISON BETWEEN RESPONSE SURFACES:We next compared the shapes of the response surfaces between traits by applying partial F tests on the scaled parameter values. Because development time showed the opposite relationship with protein and carbohydrate, decreasing with increasing protein, we compared survival and ovariole number to the inverse values for development time (Table 4). The shape of the response surfaces for ovariole number and development time differed significantly (Table 4). Taken together, we can identify two types of response surfaces in *D. simulans*: one for survival and developmental time, a second for survival, andovariole number.

Life history traits in *Zaprionusindianus*and *Drosophila simulans*differed in theirresponse to the macronutrient composition of the larval diet

Next, we assessed whether*Z. indianus* and *D. simulans*showed significant differencesin the shapes of their response surfaces for each of the life history traits examined. We compared the response surfaces of the standardized values for each of the life history traits between the two species using partial F tests. The response surfaces for all traitstested, survival, developmental time, and ovariole number,significantly differed between the two species (Table 5).

*Zaprionusindianus*and *Drosophila simulans*adult females show different ovipositionsite preferences

The nutritional response surfaces for *Z. indianus* larvae indicate these larvae show highest survival and highest ovariole number at intermediate protein contents, and show fastest development timeacross a broad range of dietary protein, from intermediate to high protein concentrations. *D. simulans* larvae show fastest development times in high protein diets. Therefore,we asked if the oviposition preference of adult females correlated with our observed differences in response surfaces between the two species. We hypothesized *D. simulans*females would prefer the foods with higherP:Cratiosfor oviposition, whereas the *Z. indianus* would prefer the lower P:C ratios in accordance with its preference for ripening rather than rotting fruit.

We first tested the oviposition preference of each species on its own by offering them a choice between three different diets of the same caloric value, 0.72 Kcal/ml,but differing in P:C ratios, 1:1, 1:4 and 1:16. To distinguish between the three food-types, we dyed each ratio blue, red or green, alternating colours to control for colour preference.We did not observe any significant colour preference for either species (Supplementary Table S1).

*Z. indianus*laida significantlylower proportion of their eggs in the1:1 food, but did not show a significant preference for either 1:4 or 1:16 foods (Figure 3A, Table 6).In contrast,*D. simulans* showed no significant preference for any of the three foodsoffered (Figure 3B, Table 6). Thus, when tested alone, the*Z. indianus* show a preference for low and intermediate P:C ratiosfor oviposition, whereas *D. simulans*does not exhibit a preferred P:C ratio.

Because*Z. indianus* and*D. simulans*co-inhabit fig monocultures, we next assessed whether competition for oviposition sites between the two species would change theiroviposition preference. In competition,*Z. indianus*females behaved similarly to when assayed on their own, laying a significantly lower proportion of its eggs in the 1:1 food with no preference between the 1:4 and 1:16 foods(Figure 3C, Table 6).In contrast, *D. simulans*females laid significantly more of their eggs in the 1:1 food, and significantly fewer of their eggs in the 1:4 food (Figure 3C, Table 6). The proportion of eggs laid in the 1:16 food was indistinguishable between either the 1:1 or 1:4 foods in this species. Finally, *Z. indianus* females laid significantly higher proportions of their eggs in the 1:4 food than *D. simulans,* while *D. simulans* females laid significantly more of their eggs in the 1:1 food (Figure 3C, Supplementary Table S2). Thus,it appears that *Z. indianus*makes oviposition choices that correlate with their response to larval nutrition andtheir timing of fig exploitation, whereas *D. simulans*chooses oviposition sites to avoid competition.

DISCUSSION

Ecologists have long observedthat diversification of ecological niches together with a differentiation of the resource-habitat preferences between species results in increased biodiversity within that habitat. By partitioning their resources, species avoid competitionand are able to co-exist as they no longer rely onthe same limited resources(Hutchinson 1978). Although they colonize thesame substrate, *Z. indianus* and *D. simulans* species coexist by occupying different temporal patches in fig monocultures(Lachaise *et al.* 1982; Matavelli 2014).While *Z. indianus* invades the figs in their ripening stage,*D. simulans*arrives later, laying their eggs on the fruit only as it

begins to rot (Lachaise *et al.* 1982; Matavelli 2014). We used this difference in the order of colonization to explore how the known temporal partitioning of thisnutritional resourcecorrelateswith larval macronutrient requirements and changes in macronutrient preference for oviposition.

As expected from the literature, we confirmed an increase in the total protein content and in the ratio of protein to sugar as the fig decays and yeasts and other microorganisms grown on it. We hypothesized that macronutrient requirements of *Z. indianus* and *D. simulans* larvae would also parallel the change in protein and carbohydrate content of the fruit.

Consistent with their colonization of ripe fruits containing lower densities of yeasts, our results show that *Z. indianus* survives, develops faster, and shows the highest reproductive potential, measured byovariole number, in food with intermediate protein content.Carbohydrate levels did not bear significant impact on the response of any of the traits.

In contrast, only developmental time showed a significant response to the macronutrient concentration of the food in *D. simulans*.In this case, both carbohydrate and protein content ofthe larval diet affected developmental time, with the shortest development times in the foods with the highest protein contents. The lack of significant correlation between macronutrients in the diet and survival and ovariole number may be because*D. simulans* breeds in a highly complex environment and can adjust its development to a wide range of protein and carbohydrate compositions, because survival and ovariole number respond to other nutrients not present in our larval diets, or, perhaps,due to a lack of statistical power.

In Drosophilids, the yeast composition of the substrate is an important cue for attraction and oviposition(Dobzhansky 1956; Barker & Starmer 1999; Becher *et al.* 2012). We observed oviposition preferences differed between the two species. When given the choice between three diets with different P:C ratios, *Z. indianus*females avoided laying their eggs in the diet with highestP:C ratios, and laid their eggs in equal proportions in either of the other two choicesoffered. In contrast, *D. simulans* adult females showed no preference when assayed on their own, but when competing for oviposition sites with *Z. indianus*chose to lay their eggs in the food with the highest P:C ratio. Thus, it seems as though *Z. indianus* has a strong preference for macronutrient content of the food, whereas *D. simulans*females make choices to avoid *Z. indianus*. Given that previous studies have shown that larval residuals from *Z. indianus* alter viability and development time in *D. simulans*larvae, but not vice versa (Galego & Carareto 2005), *D. simulans*females may occupy their characteristic temporal nutritional resource to avoid competition with *Z. indianus* rather than out of dietary preference.

While yeasts are the major source of protein for Drosophilids, the flies, in turn, are the major inoculators of yeasts in the fruits and the primary vectorsfor yeast dispersal (Begon 1982; Buser *et al.* 2014). Furthermore, different species of Drosophilids prefer to consume different species of yeasts (Dobzhansky 1956; Phaff *et al.* 1956; Fogleman *et al.* 1981; Morais *et al.* 1995; Barker & Starmer 1999). Our results show that both*Z. indianus* and *D. simulans*show oviposition preferences based on the amount of yeast of a single species, *Saccharomyces cerevisiae*. However, *Z. indianus*is known to inoculate figs with the yeast species*Candida tropicalis*(Gomes *et al.* 2003).Assessing the effects of macronutrient on life history traits and on oviposition preference by

coupling the amount of yeast with speciesof yeast that are part of the succession in decaying fruitwould be an interesting avenue of future research.

To generate our nutrient space and to test oviposition preference, we useddietary media composed of only sucrose and yeast. However, decaying fruits are far more complex, containing several types of carbohydrates in addition to sucrose(Widdowson & McCance 1935), as well as many other macro- and micronutrients. All these components may change in space and time from ripening to rotting. Therefore, life history traits are likely to respond to complex interactions between these other nutritional elements. Additionally, other characteristics of the fruits also change through time, such as their texture and volatile compounds. As discussed in Rodriques*et* al(Rodrigues *et al.* 2015), these and other factors may account for female oviposition preferences. Further dedicated experiments including field research and the use of a holidic medium, such as that developed by Piper *et* al (Piper *et al.* 2014), would further refine our understanding of the effects of larval dietary composition on life history traits and how the balance of macronutrients affect oviposition choice.

CONCLUSIONS

Zaprionusindianus and *Drosophila simulans* coexist in fig monocultures inBrazil and other regions of the world by invading the fruit at different stages of its maturation. We have shown the nutritional composition of the fig is dynamic and changes continuously throughout the decaying process. We also show that these two species differ in their response to the macronutrient composition of the larval diet for all life history traits examined. Differences in their species-specific oviposition preference paired withdifferences in their developmental response to the macronutrients in the

diet, allow these two species toexplore temporal patches with different nutritional characteristics. This temporal partitioningpresents a solution for coexistence, since it prevents interspecific competition and increases intraspecific aggregation.

AUTHORS' CONTRIBUTIONS

CM and CKM conceived the study and designed all experiments. CM conducted the nutritional geometry and oviposition experiments. MJAC collected the ovariolenumber data. NEM and CKM completed the statistical analysis. MJAC and CKM wrote the manuscript and all authors gave final approval for publication.

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DATA ACCESSIBILITY

All data and scripts are available on Dryad (doi to be provided).

FIGURE LEGENDS

FIGURE 1. Larval diet affects the development of life history traits in *Zaprionusindianus*. (A-C) shows the fitted response surfaces of the effects of 15 different diets varying in protein, carbohydrate and caloric contents for (A) proportion of larvae surviving from egg to pupae, (B) development time from egg to pupae, and (C) ovariole number.

FIGURE 2. Larval diet affects the development of life history traits in *Drosophila simulans*. (A-C) shows the fitted response surfaces of the effects of 15 different diets varying in protein, carbohydrate and caloric contents for (A) proportion of larvae surviving from egg to pupae, (B) development time from egg to pupae, and (C) ovariole number.

FIGURE 3. *Zaprionusindianus* and *Drosophila simulans* show different oviposition site preferences across different protein to carbohydrate ratios (P:C). Females were offered diets with three protein to carbohydrate (P:C) ratios for oviposition. The oviposition site preference was estimated by the percentage of eggs laid in each P:C ratio. (A) shows the proportion of eggs laid in each P:C ratio for *Z. indianus* females. (B) showsthe proportion of eggs laid in each P:C ratio for *D. simulans* females. (C) Oviposition preference for *Z. indianus*and *D. simulans*when competing for egg laying sites in the same chamber. The letters (black for *Z. indianus*, grey italics for *D. simulans*) indicate significant differences in the proportion of eggs laid between P:C ratios within a species, with significant differences marked by different letters, as determined by generalized linear models assuming a quasibinomial distribution of the proportions and a no-choice value of μ=0.33 (dashed line in all panels). The asterisks in (C) indicate the P:C ratios where the species differed significantly in preference. We replicated each assay 12-22 times.

SUPPLEMENTARY FIGURE 1. Figs change their macronutrient composition with stage of decay. The plots show the log transformations of protein (A) and sugar (sucrose and glucose)

amounts in ug per ul (B) and protein to sugar ratio (C) over the course of 27 days in rotting figs. Panel (D) shows photos of the figs collected 2, 8, and 19 days after the start of the experiment. Black lines indicate the regression estimates from linear models and the grey shaded areas represent 95% confidence intervals.

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Table(s)

Life History Trait		C	P	C^2	P^2	C x P	R^2
Survival	β	0.0081	**0.074**	-0.000056	**-0.00081**	-0.00023	-
	t value	0.70	**3.38**	-1.05	**-4.15*****	-1.26	
Development Time	β	0.018	**-0.29**	0.000047	**0.0030**	-0.00055	0.76
	t-value	0.92	**-8.35*****	0.50	**8.95*****	-1.78	
Ovariole Number	β	-0.12	**0.52**	0.00047	**-0.0046**	-0.00048	0.31
	t-value	-1.97	**4.76*****	1.59	**-4.23*****	-0.49	

Table 1: Effects of carbohydrate (C), protein (P) and their squares and products in the larval diet on three life history characters: survival from egg to pharate adults, development time, and ovariole number in *Zaprionus indianus*. For development time and ovariole number, the models were linear mixed-effects models fit by maximum likelihood. Survival data was analysed with a generalized linear model, assuming a quasibinomial distribution of survival probabilities and a logit link. Significant coefficients are in bold: *p<0.05, **p<0.01, ***p<0.001.

Life History Trait A	Life History Trait B	Degrees of Freedom	L Ratio	p-value
Survival	**Ovariole Number**	**5**	**15.72**	**0.023***
Survival	**Inverse Development Time**	**5**	**50.28**	**<0.0001*****
Ovariole Number	**Inverse Development Time**	**5**	**18.32**	**0.010****

Table 2: Comparisons between the response surfaces of the four three history traits in *Zaprionus indianus*. Using partial F tests, we compared the response surfaces generated from linear mixed effects models on the scaled parameter values, using replicates as our random effect. For development time, we inverted the data for comparison. Response surfaces that show significant differences are highlighted in bold. The p values were adjusted using the Holm method. *p<0.05, **p<0.01, ***p<0.001

Life History Trait		C	P	C^2	P^2	C x P	R^2
Survival	β	-0.029	-0.0032	0.000091	0.0014	0.000186	-
	t-value	-1.50	-0.060	0.98	1.083	0.45	
Development Time	β	**0.048**	**-0.093**	-0.000002	**0.0014**	**-0.0012**	0.56
	t-value	**4.13*****	**-4.63*****	-0.042	**6.84*****	**-6.43*****	
Ovariole Number	β	-0.056	0.17	0.00014	-0.0016	0.0013	0.15
	t-value	-1.02	1.95	0.52	-1.86	1.59	

Table 3: Effects of carbohydrate (C), protein (P) and their squares and products in the larval diet on three life history characters: survival from egg to pharate adults, development time, and ovariole number in *Drosophila simulans*. For development time and ovariole number, the models were linear mixed-effects models fit by maximum likelihood. Survival data was analysed with a generalized linear model, assuming a quasibinomial distribution of survival probabilities and a logit link. Significant coefficients are in bold: *p<0.05, **p<0.01, ***p<0.001.

Life History Trait A	Life History Trait B	Degrees of Freedom	L Ratio	P value
Survival	Ovariole Number	5	9.64	0.176
Survival	Inverse Development Time	5	7.91	0.176
Ovariole Number	**Inverse Development Time**	**5**	**41.29**	**<0.0001*** **

Table 4: Comparisons between the response surfaces of the three life history traits in *Drosophila simulans*. Using partial F tests, we compared the response surfaces generated from linear mixed effects models on the scaled parameter values, with replicates as the random effect. For development time, we inverted the data for comparison. Response surfaces that show significant differences are highlighted in bold. The p values were adjusted using the Holm method. *p<0.05, **p<0.01, ***p<0.001

Life History Trait	Degrees of Freedom	L Ratio	p-value
Survival	**5**	**43.34**	**<0.0001*** **
Development Time	**5**	**212.09**	**<0.0001*** **
Ovariole Number	**5**	**54.61**	**<0.0001*** **

Table 5: Differences in the response surfaces between *Zaprionus indianus* and *Drosophila simulans* for each trait. Using partial F tests, we compared the response surfaces generated from linear mixed effects models on the scaled parameter values. Response surfaces that show significant differences are highlighted in bold: *p<0.05, **p<0.01, ***p<0.001 **.**

Oviposition Choice *Zaprionus indianus*							
Not Competing				Competing			
Food P:C	lsmean	st error	group	Food P:C	lsmean	st error	group
1:1	-1.86	0.351	1	1:1	-1.82	0.287	1
1:4	-0.34	0.243	2	1:4	-0.13	0.200	2
1:16	-0.21	0.241	2	1:16	-0.44	0.204	2
	Oviposition Choice *Drosophila simulans*						
Not Competing				Competing			
Food P:C	lsmean	st error	group	Food P:C	lsmean	st error	group
1:1	-0.42	0.188	1	1:1	-0.43	0.119	1
1:4	-0.70	0.196	1	1:4	-0.87	0.128	2
1:16	-0.99	0.208	1	1:16	-0.79	0.125	12

Table 6: *Z. indianus* and *D. simulans* females show significant preferences for P:C ratios for oviposition. We fit the data with generalized linear models using a quasibinomial distribution to account for the overdispersion of the data. Our models showed significant differences in the proportion of eggs laid in each P:C ratio for both *Z. indianus* (not competing: χ^2=20.60, df=2, p-value=<0.0001, competing: χ^2=34.85, df=2, p-value==<0.0001) and *D. simulans* (not competing: χ^2=4.28, df=2, p-value=0.12, competing: χ^2=6.25, df=2, p-value=0.043). The table above shows the least squared means (lsmean), standard errors (st error), and groups for each food type, with significant differences denoted by different numbers in the group column (adjusting p-values using the Bonferroni method for a significance level of 0.05).

Figure 1

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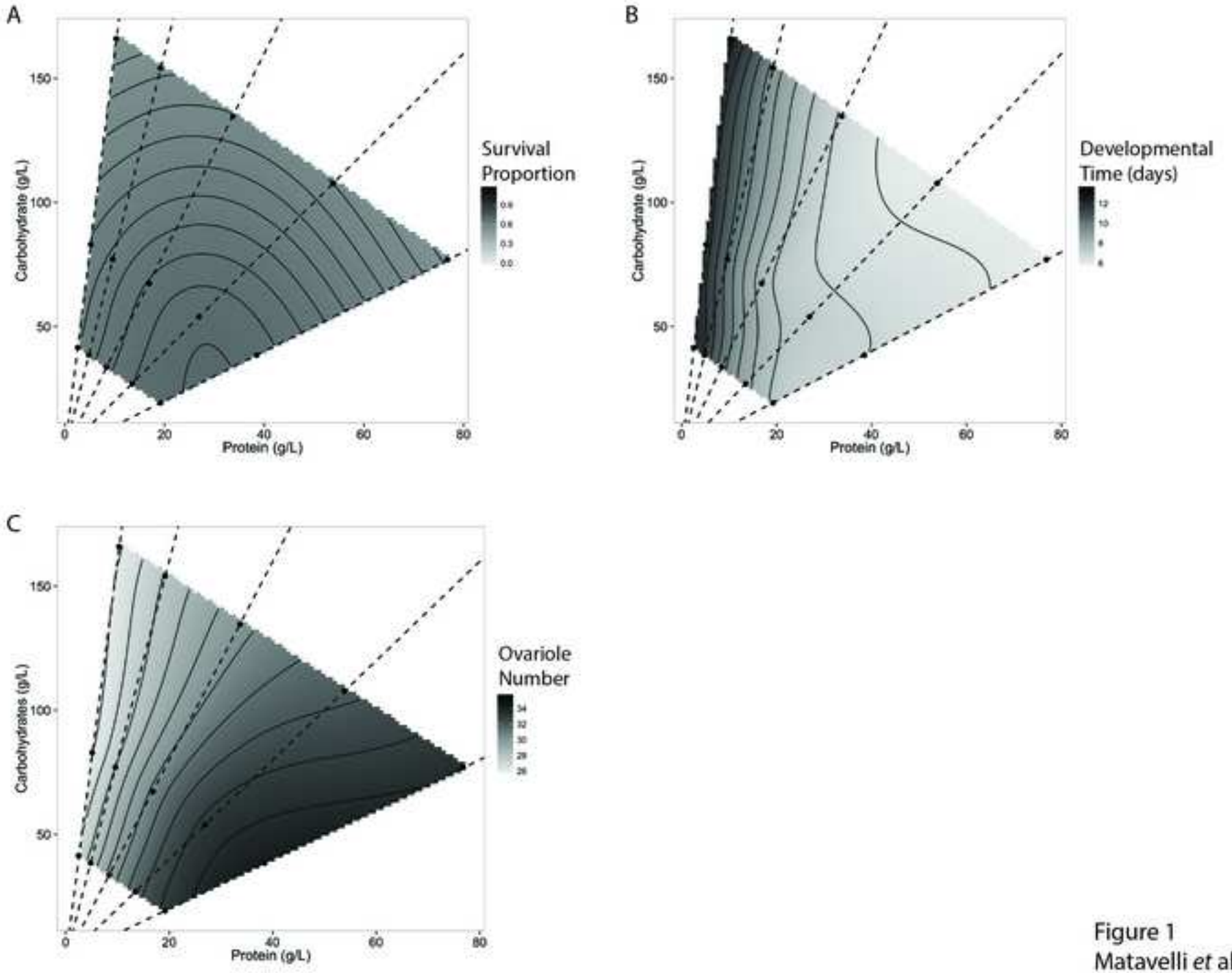

Figure 1
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Figure 2

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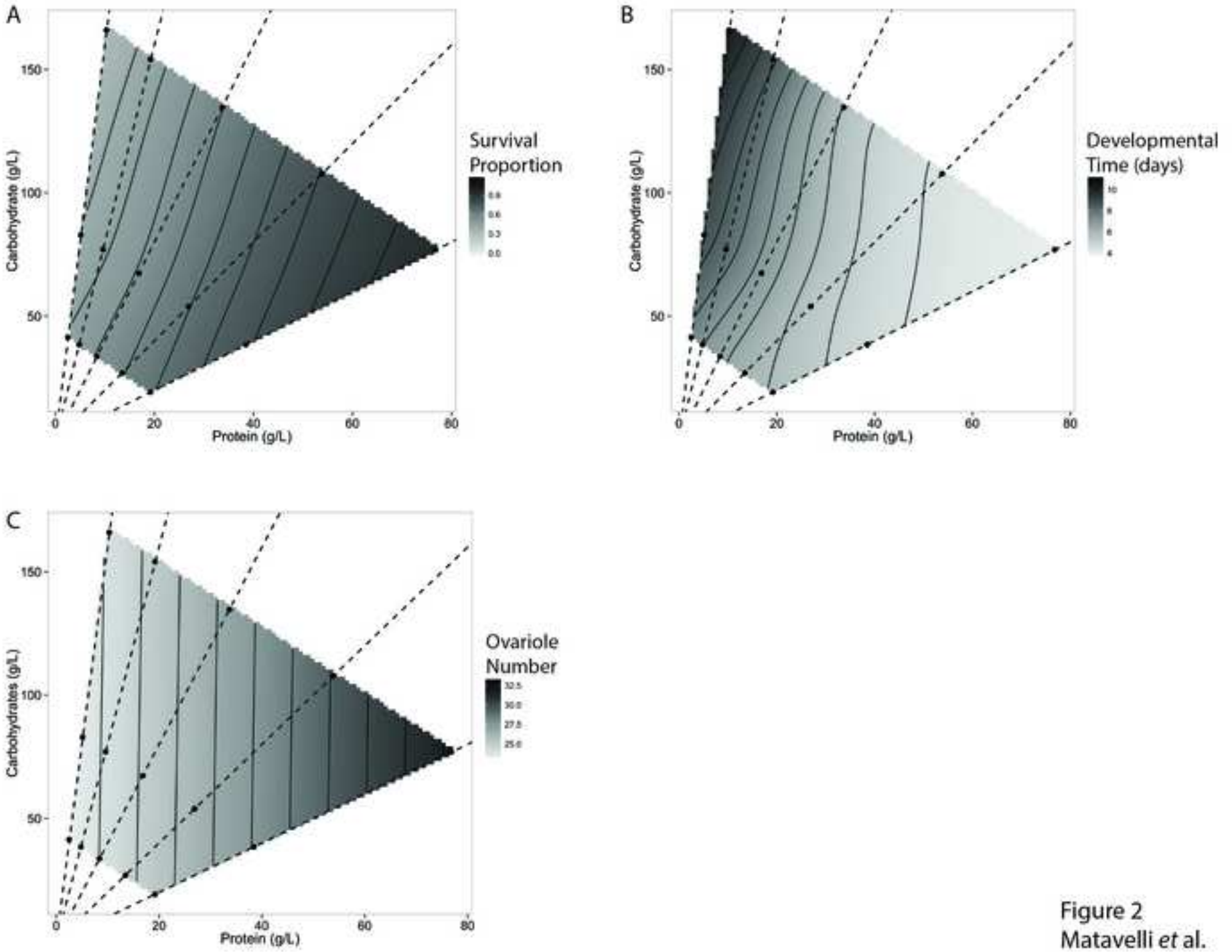

Figure 2
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Figure 3

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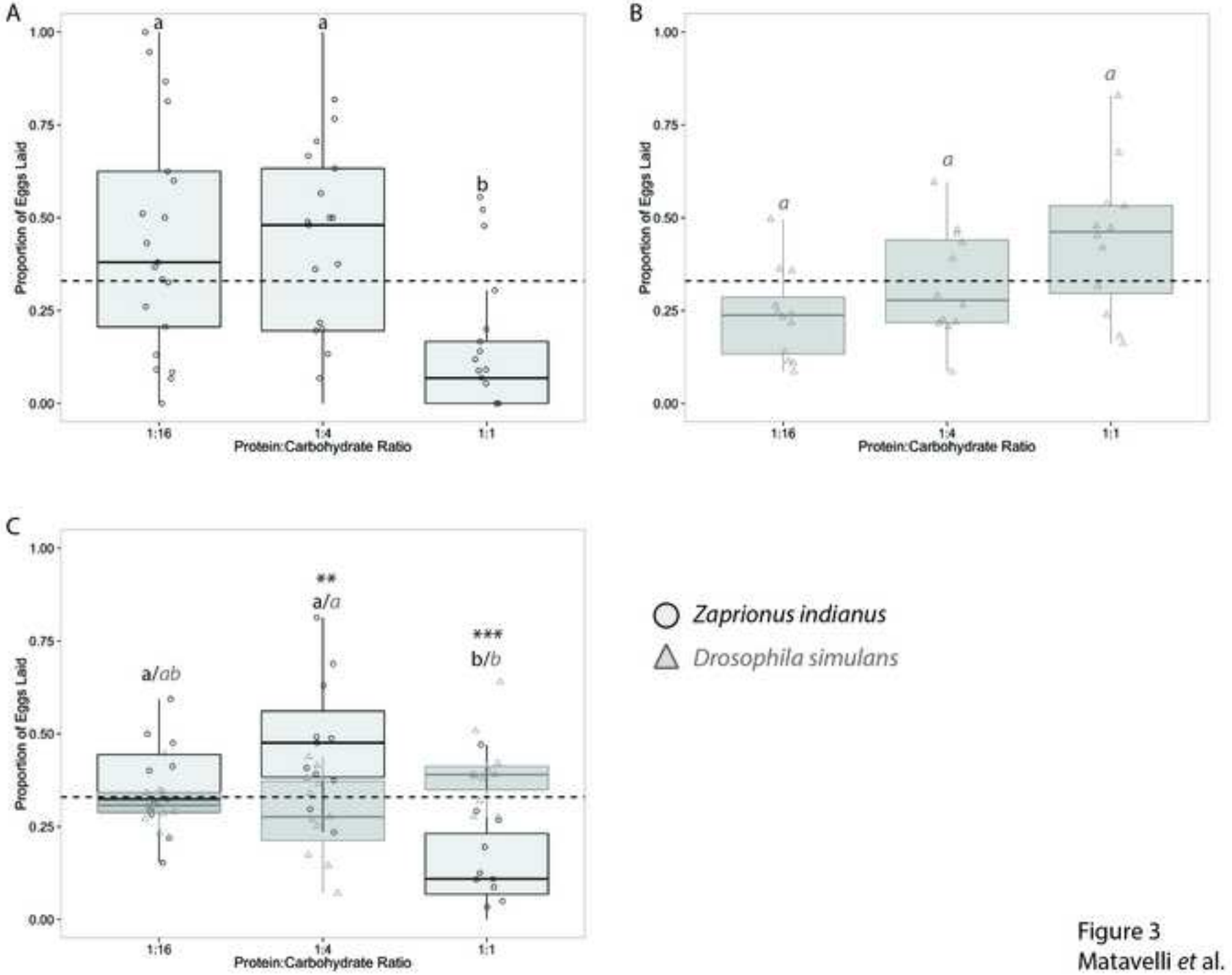

Figure 3
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Colour Choice *Zaprionus indianus*							
Not Competing				Competing			
Food Colour	lsmean	st error	group	Food Colour	lsmean	st error	group
Blue	-0.90	0.293	1	Blue	-0.29	0.244	1
Green	-0.73	0.283	1	Green	-0.81	0.261	1
Red	-0.47	0.273	1	Red	-1.02	0.273	1
	Colour Choice *Drosophila simulans*						
Not Competing				Competing			
Food Colour	lsmean	st error	group	Food Colour	lsmean	st error	group
Blue	-0.66	0.207	1	Blue	-0.68	0.146	1
Green	-0.74	0.209	1	Green	-0.72	0.147	1
Red	-0.66	0.207	1	Red	-0.67	0.145	1

Supplementary Table S1: *Z. indianus* and *D. simulans* females do not show significant preferences for food colour for oviposition. Generalized linear models using a quasibinomial distribution to account for the overdispersion of the data showed no significant differences in the proportion of eggs laid in each colour for either *Z. indianus* (not competing: χ^2= 1.1829, df=2, p-value=0.55, competing: χ^2=4.30, df=2, p-value=0.12) or *D. simulans* (not competing: χ^2=0.078, df=2, p-value=0.96, competing: χ^2=0.96, df=2, p-value=0.95). The table above shows the least squared means (lsmean), standard errors (st error), and groups for each food type, with significant differences denoted by different numbers in the group column (adjusting p-values using the Bonferroni method for a significance level of 0.05).

P:C Ratio	1:1			1:4			1:16		
	lsmean	st error	group	lsmean	st error	group	lsmean	st error	group
Zaprionus indianus	-1.82	0.287	1	-0.13	0.200	1	-0.43	0.125	1
Drosophila simulans	-0.43	0.119	2	-0.87	0.128	2	-0.79	0.204	1

Supplementary Table S2: *Z. indianus* and *D. simulans* females differ in the proportion of eggs that they lay in a given P:C ratio. We fit the data with generalized linear models, using a quasibinomial distribution to account for the overdispersion of the data. Our models showed significant differences in the proportion of eggs laid in each P:C ratio between *Z. indianus* and *D. simulans* (Species: χ^2=0.00, df=1, p-value=1, Food: χ^2=0.081, df=2, p-value=0.96, Food x Species: χ^2=36.16, df=2, p-value<0.0001). The table above shows the least squared means (lsmean), standard errors (st error), and groups for each species in each food type, with significant differences denoted by different numbers in the group column (adjusting p-values using the Bonferroni method for a significance level of 0.05).

Supplementary Figure 1
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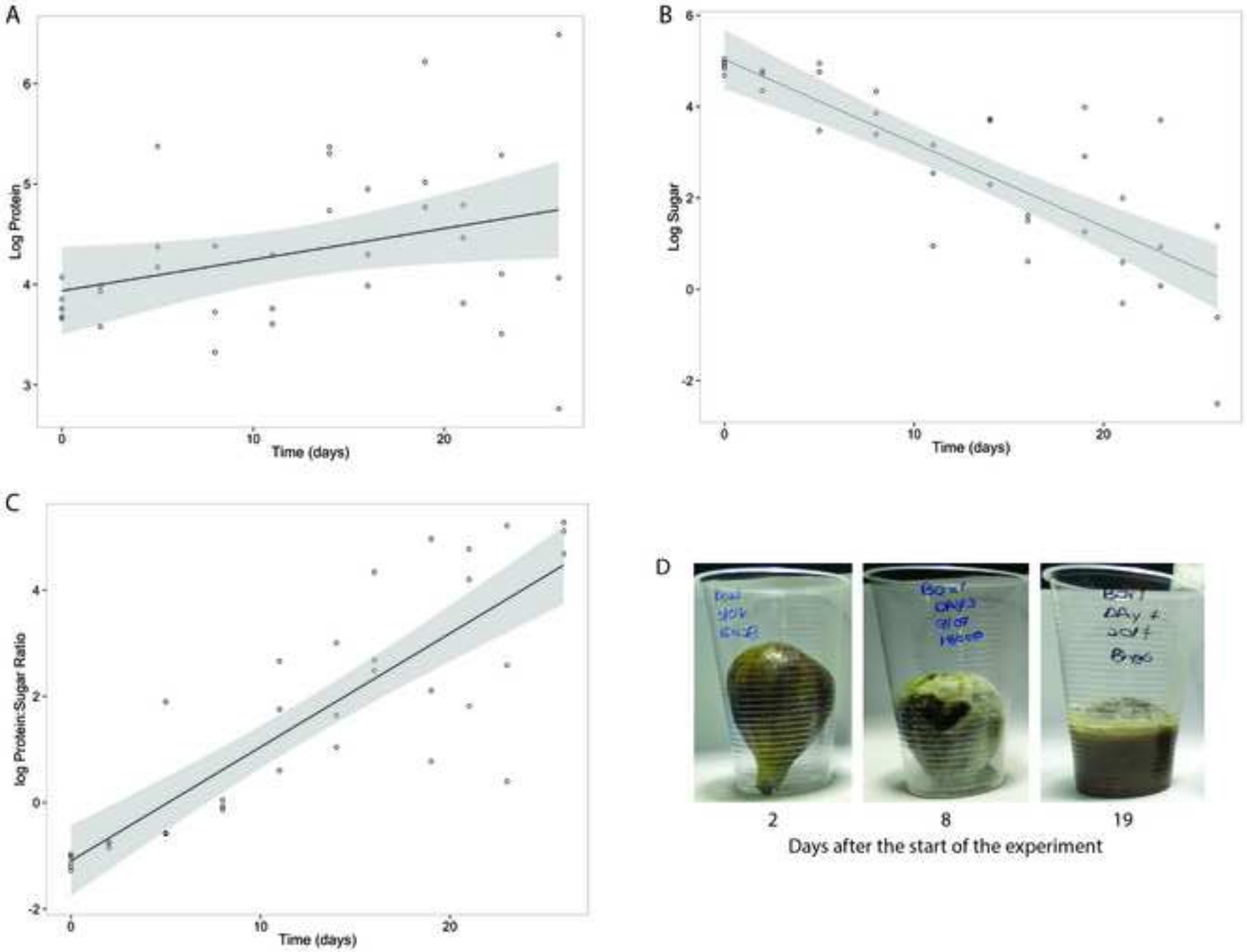

Supplementary Figure 1.