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

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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.

1. Introduction

Species that use the same ecological niche are faced with the problem of interspecific competition, which affects their fitness and population structure. Studies of priority effects between two fungal-breeding *Drosophila* species, *Drosophila phalerata* and *Drosophila subobscura*, show that the species that arrives late at a patch has decreased survival, decreased body size (measured by wing length), and increased mean developmental time (Shorrocks and Bingley, 1994), thus lowering their fitness. Exploiting specific nutritional niches decreases competition among closely-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 amounts of protein and carbohydrate from their environment (Behmer and Joern, 2008). Another way species can avoid competition is by partitioning their resource, either spatially or temporally. Homogeneous niches can be partitioned in space through low interspecific and high intraspecific aggregation (Shorrocks, 1975; Atkinson and Shorrocks, 1981, 1984). Alternatively, in more heterogeneous environments different species can 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 and Fogleman, 1986). Yeasts also show a species-specific pattern of succession in their colonization of decaying fruits (Morais et al., 1995), wood (Gonzalez et al., 1989), and logs of *Pseudotsuga menziesii* (Crawford et al., 1990).

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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 Drosophilids and other insects sharing this ephemeral substrate (Morais et al., 1995). Importantly, yeast succession allows 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 hydrolyzes during ripening to produce sugars in various fruits (Pech and 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), presumably resulting in changes in the ratio of protein to carbohydrate (P:C) depending on the stage of fruit decay (Tournas and 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 Drosophilids emerges 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 *Zaprionus indianus* 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 and 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, thereby colonizing 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 is on 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 parameters varied across a broad range of values, an approach termed nutritional geometry (Raubenheimer and Simpson, 1997; Simpson and 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 and Simpson, 1997; Simpson and Raubenheimer, 1999). Nutritional geometry has been used to explore the response of life history traits and behavioral strategies to the macronutrients in a broad range of animals (Kohler et al., 2012; Simpson et al., 2015; Rothman et al., 2014).

Previous studies in *Drosophila melanogaster* show that the protein content of the larval diet regulates their growth (Bakker, 1959; Tu and Tatar, 2003), their development time (Beadle et al., 1938), their body and organ sizes (Tu and 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 and 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 play important 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 for oviposition. Overall, our results indicate that differences in the nutritional requirements of larvae and oviposition preference of the females allow resource partitioning between species.

2. Material and methods

2.1. 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 °C, in a 12 h light: 12 h 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.

2.2. 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 ($11 \times 20.5 \times 27$ 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 standard food and 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 distributed 2 ml of the blended fig into one of three Eppendorfs. Samples were lysed with metal beads using a Qiagen TissueLyzer for 10 min at maximum speed. Samples were centrifuged for 10 min at 6189*g*. Supernatant was collected and used for protein quantification using a Pierce BCA protein assay kit (Thermo Scientific #23227) and for glucose/sucrose quantification using a Glucose and Sucrose Colorimetric/Fluorimetric Assay Kit (Sigma #MAK013).

2.3. 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 (Lesaffre SAF-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