have rarely been considered. Such species are likely to be constrained in their evolutionary responses to future climate changes (Kellermann *et al.*, 2009).

At first sight the flies collected from Madhuvana are increasingly higher in number, as it contains fruiting vegetation. But in Ghandhibhavana the number of flies collected was less as there is very little fruiting vegetation. But as far as the species diversity are connected the Madhuvana and Ghandhibhavana consists in a total of 9 species, which are common in both the collected localities of the present study. A better understanding of how different species are affected by current climates and why they sometimes respond differently to climate change is necessary for predicting future effects of climate change (Weatherhead, 2005).

Interestingly, it was also observed that the flies were recorded more in number during rainy season when compared to summer and winter. However, in winter season flies were least recorded. This ensures that the distribution of the flies is mainly effected in nature due to the variation in the temperature. The present study also implies that the climatic variables such as humidity, rainfall, and temperature are determining factors in the occurrence of Drosophilid species as suggested (Pavan, 1959). The diversity and distribution of the Drosophilids have been affected enormously where human habitat is frequently sensed in Gandhibhavana when compared to Madhuvana. Irrespective of vegetation, seasonal variations also have an impact on population density of Drosophilids. Thus assemblages of Drosophilids are less frequent in numbers at Gandhibhavana, which means that it is prone to be a disturbed gradient with human habitat than Madhuvana.

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Reduced male fertility of the Canton-S strain due to spermiogenic failure.

Kanamori, T.[§], Y. Yasuno[§], T. Tomaru, and T. Takano-Shimizu-Kouno*. Drosophila Genetic Resource Center, Kyoto Institute of Technology, Saga Ippongi-cho, Ukyo-ku, Kyoto 616-8354, Japan; [§]These two authors contributed equally; *author for correspondence (E-mail: fruitfly@kit.ac.jp).

Introduction

Male fertility is a quantitative trait composed of several components and appears to vary considerably among individuals; therefore, it is not a simple matter to quantitatively define the wild type. On the other hand, for a detailed analysis of the reproductive process, standard and marker strains with a normal phenotype are essential. Here, to test the adequacy of strains often used in the study of spermatogenesis, we studied the male fertility of eight strains of *Drosophila melanogaster*, finding a significantly reduced fertility of the Canton-S strain.

Materials and Methods

Flies

We studied eight strains for male fertility: three strains from Bloomington Drosophila Stock Center, w^{1118} (BDSC stock number 5905) and two strains from the *Drosophila melanogaster* Genetic Reference Panel (DGRP 208, BDSC # 25174, and DGRP 301, BDSC # 25175; Mackay *et al.*, 2012); four strains from Drosophila Genetic Resource Center, Kyoto Institute of Technology, Canton-S (DGRC# 105666), Canton-S-brn (DGRC# 109019), *w[*]; P{w[+mC]=dj-GFP.S}AS1/CyO, P{ry[+t7.2]=sevRas1.V12}FK1* (abbreviated here as *dj-GFP*, DGRC # 108217), and *w*; P{ProtamineB-eGFP}1/CyO* (*ProtamineB-eGFP*, DGRC # 109173); and a highly inbred *y w* strain (TT16). *dj-GFP* and *ProtamineB-eGFP* are used as a sperm tail and a sperm nucleus marker, respectively.

Male fertility test

Three- to five-day-old males were individually placed with a single female of the same age in a vial (day 0) and males were removed the next day (day 1). The females were transferred to new vials on days 2, 4, 7, 10, 14, 18, and 22 and then removed on day 29. They were thus allowed to lay eggs for 29 days in a total of eight vials, and all offspring were counted. We used DGRP 208 and *y w* strains as female parents and made twelve replicate crosses for each combination of female and male strains. All crosses (2 female strains × 8 male strains × 12 replicates = 192) were done simultaneously.

Microscopic examination

Squashed testes were prepared essentially as described in Pisano *et al*. (1993). Adult testes were dissected in 0.7% NaCl and squashed with a coverslip. Sample slides were quickly frozen in liquid nitrogen and the coverslips were removed with a razor blade. Samples were fixed by methanol at –20°C for 5 min and then by acetone at –20°C for 1 min. They were immersed in PBST, washed twice in PBS, and mounted with Vectashield containing DAPI (Vector Laboratories). Microscopic examination of testes was performed under a Nikon Eclipse 80i microscope, and micrographs were processed with Adobe Photoshop CS6.

Results and Discussion

The numbers of male and female offspring were not significantly different in any combination of female and male strains, and we pooled them. In general, the number of offspring from *y w* females was smaller than that of DGRP 208 females (Figure 1); the average number of offspring per cross was 128.1 ± 7.0 in *y w* and 174.1 ± 9.3 in DGRP 208. Indeed, the two-way analysis of variance revealed a highly significant effect of female parents (F = 23.1, $d.f.$ = 1/176, P < 0.001). Offspring were not observed at all in the last vials of 160 out of 192 crosses, and only 32 crosses, including 4 crosses in which parental females died

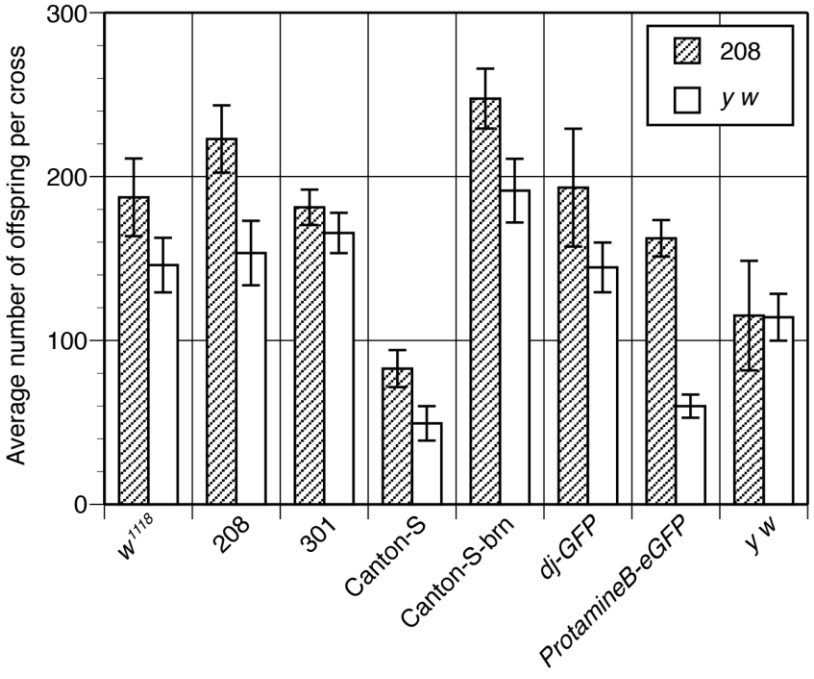

Figure 1. Variation in male fertility among eight strains. The male fertility is defined as the total number of offspring of a single-pair cross, where DGRP 208 (hatched bars) and *y w* (open bars) were used as the female parents. Error bars shown are standard error of mean.

before making the eighth vial, produced offspring in the last vials with an average of 8.3. Therefore, we counted most, if not all, of the offspring, presumably from a single mating. The total number of offspring per cross was used as an index of the male fertility.

The male fertility varied among the eight strains (two-way ANOVA: F = 13.41, *d.f.* = 7/176, P < 0.001); specifically, Canton-S had a reduced fertility. Indeed, the male fertility of the Canton-S strain was significantly lower than *ProtamineB-eGFP* (approximate test of equality of means using the Games-Howell method, the actual difference of 79.5 > the minimum significant difference, MSD, of 74.9 at a 5% experiment-wise level of significance), DGRP 301 (98.4 > MSD of 73.8), DGRP 208 (140.1 > MSD of 113.7) and Canton-S-brn (164.8 > MSD of 103.4) in crosses with DGRP 208 females, and *dj-GFP* (95.2 > MSD of 88.2), w^{1118} (96.6 > MSD of 94.7), DGRP 301 (116.2 > MSD of 76.5), and Canton-S-brn (142.0 > MSD of 107.4) in crosses with *y w* females.

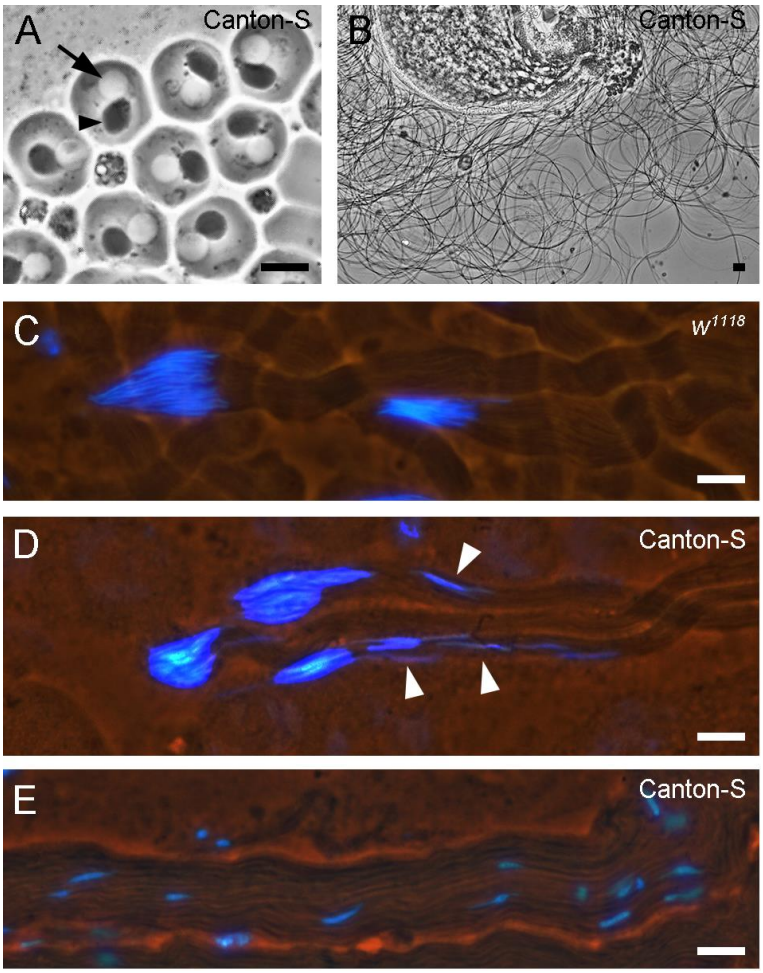

Figure 2. Post-meiotic spermatid differentiation is impaired in Canton-S males. (A–B) are phase-contrast images and (C–E) are DAPI-stained images with illumination of weak transmitted light (phase-contrast). (A) Canton-S onion-stage spermatids showing normal size nuclei (arrow) and nebenkern (arrowhead). (B) Canton-S motile mature sperm dissected from a seminal vesicle. (C) Normal elongated spermatid of w^{1118}, showing aligned nuclei at the head end of the cysts. (D) Abnormal elongated spermatid of Canton-S, showing disorganized alignment of nuclei in the head region. Arrowheads indicate spermatid nuclei that were scattered caudally. (E) Abnormal elongated spermatid of Canton-S, showing scattered and irregular-shaped nuclei in the tail end. Scale bars = 10 μm.

The male fertility still varied significantly even if the Canton-S strain was removed from the analysis (F = 7.45, *d.f.* = 6/154, P < 0.001), although individual comparisons were not statistically significant except for three cases of *y w* females (DGRP 301 *vs. ProtamineB-eGFP*, 105.7 > MSD of 68.6; Canton-S-brn *vs. ProtamineB-eGFP*, 131.5 > MSD of 103.4; *dj-GFP vs. ProtamineB-eGFP*, 84.7 > MSD of 82.3).

To investigate the cause of fertility reduction in Canton-S males, live and fixed testes were examined under light microscopy. Germ cell development of Canton-S males appeared to be normal under phase-contrast optics; spermatocyte growth, meiosis, and spermatid elongation occurred properly, and motile mature sperm were observed in the seminal vesicles (Figure 2 A–B). However, DAPI staining showed conspicuous abnormalities during spermiogenesis. While spermatid nuclei from normal males such as w^{1118} elongated synchronously and were aligned at the head end of the elongated cysts (Figure 2C), spermatid nuclei from Canton-S males were often misaligned and scattered in the tail region of the cyst (Figure 2D). The caudally displaced nuclei were irregular in shape (Figure 2E). This suggests that the reduced fertility of Canton-S males can be attributed, at least partly, to the spermiogenic failure.

Conclusion

The male fertility significantly varied among the eight strains studied here. In particular, the fertility of Canton-S, which has been used as a wild-type control in many studies, was reduced to one-half to one-third of those of most strains. Because Canton-S-brn has a high male fertility, a mutation responsible for the reduced male fertility likely occurred after the Canton-S strain and the Canton-S-brn diverged from each other in about 1980 (Boussy *et al.*, 1998). In contrast, *dj-GFP* and *ProtamineB-eGFP* had the normal range of male fertility, although the fertility of *ProtamineB-eGFP* was lower than normal when crossed with *y w* females. The reduced fertility of the Canton-S strain can be explained, at least partly, by the spermiogenic failure.

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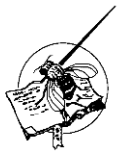

Flavors supplemented in diet regulate the hatchability and viability in *Drosophila.*

Neethu, B.K.[1], Yarajarla Ramesh Babu[2], and B.P. Harini[1*]. [1]Department of Zoology, Drosophila Culture Laboratory, Bangalore University, Bangalore-560 056, Karnataka, India; [2]Centre for Applied Genetics, Bangalore University, Bangalore-560 056, Karnataka, India; E.mail- dr.bpharini@gmail.com.

Abstract

Food additives are substances added to food to preserve flavor or enhance its taste and appearance. The ways of food additives classification, source of nature, food coloring, flavors, taste, which were collected from literature based on structural and biochemical characteristics with description of source and possible effects on *Drosophila* organisms, have been presented. The study reveals significant differences with reference to hatchability and viability on exposure to variant food additives with varying concentrations in *Drosophila melanogaster*. Of the three additives used namely, ajinomoto, turmeric, and vanilla, vanilla has a significant effect on hatchability and viability. Keywords: Food additives, *Drosophila melanogaster*, hatchability, and viability.

Introduction

Food additive is any substance, which is added to, or used as food at any stage to affect its keeping quality, texture, consistency, taste, color, alkalinity or acidity, or to serve any other technological function in relation to food, and includes processing aids in so far as they are added to food, which are common in the food production, have been described in the present review. Food additives and preservatives have been used for thousands of years. In industrialized nations, the last 50 years have seen a significant increase in the number of preservatives and additives introduced to foods before they go to market. The growth in the use of food additives has increased enormously in the past 30 years, totaling now over 200,000 tons per year. Therefore, it has been estimated that today about 75% of the Western diet is made up of various processed foods. Each person is now consuming on average 3.6-4.5 kg of food additives per year. With the great increase in the use of food additives, there also has emerged considerable scientific data linking food additive intolerance with various physical and mental disorders, particularly with childhood hyperactivity and hypersensitivity (Smith, 1991).

To regulate these additives and inform consumers, each additive is assigned a unique number, termed as "E numbers", which is used in Europe for all approved additives. This numbering scheme has now been adopted and extended by the Codex Alimentarius Commission to internationally identify all additives (Bucci, 1995) regardless of whether they are approved for use. This is usually done for the purpose of enriching the