

## Niche separation between the cosmopolitan species *Drosophila melanogaster* and the tropical Asian species *Drosophila ananassae* based on larval host-plant species<sup>1</sup>

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**Abstract**— Studies of ecological separation between insects breeding in decaying fruits have rarely determined how generalist feeders can coexist. The present study tests whether two generalist and presently cosmopolitan species of fruitfly, *Drosophila ananassae* and *Drosophila melanogaster*, differ in their ability to breed on a highly acid, and possibly toxic, fruit, cucumberfruit (Oxalidaceae, *Averrhoa bilimbi*). Yeasted *Averrhoa bilimbi* fruits and yeasted banana (*Musa* species) fruit baits were placed out on Guam as oviposition substrates for local *Drosophila* species. An analysis of variance on the number of male flies of each species that eclosed from these two baits revealed a strong statistical interaction between *Drosophila* species and host-plant species (fruit type) ( $p<0.0001$ ). The great majority of male flies eclosing from cucumberfruit were *D. ananassae*. In contrast, *D. melanogaster* was the principal species eclosing from bananas. Possibly, the geographic overlap between cucumberfruit and *D. ananassae* may have allowed *D. ananassae* to evolve a tolerance for cucumberfruit, which contains a high concentration of oxalic acid. The ability of at least some individuals of *D. ananassae* to breed on a common fruit that is not used by most *D. melanogaster* may permit coexistence of these generalist feeders on Guam and possibly other tropical Pacific Islands.

### Introduction

A number of species of fruit fly in the genus *Drosophila* have specific larval habitats that create niche-partitioning among *Drosophila* species, with some *Drosophila* species being restricted to only one host species (Markow & O’Grady 2008). However, the reasons for the coexistence of two abundant species, *Drosophila melanogaster* (cosmopolitan in temperate and tropical areas) and *Drosophila ananassae* (now pantropical), both of which feed on a variety of decaying fruits, have remained elusive (Wallace 1974, Dhananjaya et al. 2012). Studies on Guam and on other tropical Pacific islands (Wheeler & Takada 1964) have often found large swarms of *D. ananassae* attracted to piles of decaying breadfruit (*Artocarpus*), bananas (*Musa* sp.), and other fruits. Both *D. melanogaster* and *D. ananassae* have also been reported by Buruga & Olecko (1971) as being reared from or observed eclosing from *Averrhoa carambola* (starfruit). Adults of these two species of *Drosophila* are the two most common *Drosophila* species on Guam in areas around human habitations (Bohart & Gressitt 1951).

Recently, it was also found that *Drosophila ananassae* from Guam could also breed successfully in a fruit (*Averrhoa bilimbi*, cucumberfruit or “pickle”, in the Oxalidaceae), that is a close relative of starfruit (*A. carambola*) (Lofdahl & Young 2004). *A. bilimbi* is chemically quite

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similar to *A. carambola*, except that *A. bilimbi* juice is even more highly acidic (pH= 0.9-1.5) with even higher concentrations of oxalic acid (Lima et al. 2001, Bhasker & Shantaram 2013).

Preliminary experiments designed to set up isofemale lines of *D. ananassae*, revealed many *D. ananassae* females eclosing from yeasted cucumberfruit baits. However, when a similar number of *D. ananassae* females were sought eclosing from yeasted banana baits, the vast majority of eclosed flies turned out to be *D. melanogaster*. This observation lead to the design of the present experiment to test whether *D. melanogaster* ecloses in high numbers from yeasted banana bait while *D. ananassae* forms the great majority of flies eclosing from *Averrhoa bilimbi*. Such a result might explain how both species could coexist in areas with both decaying cucumberfruit and decaying bananas. This greater reproductive success of *D. ananassae* on cucumberfruit would contrast greatly with the greater breeding success at all life stages of *D. melanogaster* vs. *D. ananassae* when both are reared on a simple yeast-agar medium (Singh & Kumar 2013).

## Materials and Methods

Each of the five replicates for each fruit type consisted of a 739-ml plastic food container half filled with ripe bananas (Cavendish variety) in skins or ripe cucumberfruit that had been gently crushed by hand to provide open surfaces for oviposition by the flies. For each fruit species, this amount of fruit weighed about 400-450 g.

Two packages (7 g each) of live baker's yeast (*Saccharomyces cerevisiae*, Fleischer's Active Dry Yeast) were then sprinkled over the surface of the crushed fruit. The purpose of using so much live yeast was to exclude differences in the microbial flora of the two media as a cause of a difference in the numbers of the *Drosophila* species that eclosed from cucumberfruit vs. banana.

Five replicates of both the yeasted banana and the yeasted cucumberfruit baits were put out in a shaded area near a compost pile until the first pupae appeared on the surface of the fruit baits at ambient temperatures on Guam in July, 2014. After four days, an additional feeding of 7 g of the same yeast was sprinkled over the entire set of 5 banana or 5 cucumberfruit replicate baits. The 10 baits were placed only a few centimeters apart. For the banana replicates, the baits were left out for five days at ambient temperature when the first pupae had appeared on the surface of the banana skins. In contrast, the first pupae appeared about a day later on the cucumberfruit. In all replicates, the skins of the banana or cucumberfruit were almost completely covered with a layer of pupae after 5 or 6 days of bait exposure, respectively.

The fruit with the pupae was placed in several glass bottles per replicate, and each bottle was sealed with cotton to allow all the eclosing flies to be collected and counted. While some pupae died in the wet media during transfer to the eclosion bottles, all eclosing male flies were counted in each replicate. Only male flies were counted to avoid any confusion in species identification. It is important to note that female flies of each species were as abundant as the males and were found eclosing in similar proportions on each fruit type.

All male flies that eclosed from each replicate were counted and assigned to species on each of four successive days. The flies were allowed to eclose in an air-conditioned room that was kept at 25° C. After four days, the number of flies eclosing had diminished considerably. A limit of four days for counting eclosed flies was set to avoid counting flies from a second generation. Other drosophilid males were also counted if they were present among the eclosed flies.

## Results

No drosophilid species other than *D. ananassae* and *D. melanogaster* eclosed from the cucumberfruit medium. Only a few drosophilid males (and a few females) of species other than *D. ananassae* and *D. melanogaster* were observed eclosing from the banana medium replicates. These flies were likely *Drosophila immigrans*, a common species on breeding on decaying fruits and vegetables in temperate and tropical environments.

A two-way ANOVA was conducted on the data with *Drosophila* species and host plant (fruit type) as the factors. The number of male flies eclosing in each treatment replicate were square-root transformed for this ANOVA to equalize the variances, a common method with count data such as number of male flies (Sokal & Rohlf 2012). An ANOVA performed on the raw data gave similar results. The number of male flies eclosing did not differ according to host-plant species, when data are pooled across *Drosophila* species ( $F = 0.07$ , d.f. = 1, (n.s.), see Figure 1). Likewise, the number of flies eclosing for each *Drosophila* species did not vary, pooling across host-plant species ( $F = 0.00$ , d.f. = 1 (n.s.), see Figure 2). In contrast, a very strong statistical interaction exists between *Drosophila* species and host-plant species ( $F = 203.41$ , d.f. = 1,  $p < 0.0001$ ) in determining the number of male flies eclosing. Most male *Drosophila* eclosing from cucumberfruit were *D. ananassae*, while *D. melanogaster* males constituted the great majority of flies that eclosed from banana (Figure 3). The statistical model using transformed data explained 93% of the variance in number of male flies eclosing in this experiment.

A few individuals of *D. melanogaster* eclosed from cucumberfruit on each of the four days during which eclosing flies were counted. Similarly, a few *D. ananassae* had eclosed on each of the four days from banana medium. Thus, no difference in eclosion times for either species was apparently present on a given host plant.

## Discussion

The hypothesis of equal numbers of *D. ananassae* and *D. melanogaster* males eclosing from each of the two host plants can be rejected. The great majority of male flies eclosing from cucumberfruit were *D. ananassae*. *D. melanogaster* was by far the most numerous species eclosing from banana.

*Drosophila ananassae* is a species thought to have originated in Southeast Asia, probably in Java based on genetic variation patterns (Vogl et al. 2003, Matsuda et al. 2009). In contrast, extensive evidence suggests that *Drosophila melanogaster* has an Afrotropical origin (Pool & Aquadro 2006). While the ancestral host plant of *D. melanogaster* is unknown, it is likely that it was one or several native African plant species (Keller 2007). It has recently been argued that the host-plant generalist, *D. melanogaster*, has evolved from a specialist ancestor, expanding out of Africa within the last 60,000 years and acquiring a number of beneficial mutations (Stephan & Li 2007).

Evidence suggests that *Averrhoa bilimbi* evolved in the Moluccas while its close relative, *Averrhoa carambola* (starfruit) originated in the Moluccas or Ceylon (Morton 1987). Both *A. carambola* and *A. bilimbi* have been widely cultivated throughout India, Indonesia, the Philippines, Guam (Stone 1970) and other Pacific Islands. It might be expected that specific physiological adaptations might be needed for an insect such as *Drosophila* to breed successfully in ripe cucumberfruit, given its high concentration of oxalic acid, which is the main acid contributing to the pH of its juice being in the range of 0.9-1.5. *D. melanogaster* is thought to have evolved from a specialist species in Africa, and it may not have adapted to breed effectively on a medium that has a high concentration of oxalic acid. It is likely that *D. ananassae* has had more time to coevolve with both *Averrhoa* species, given the Southeast Asian origin of this species of *Drosophila*.

Interestingly, recent research (Carolino et al. 2005) has demonstrated that fresh or boiled *Averrhoa carambola* juice can drastically reduce the percentage of *D. melanogaster* eclosing. This

starfruit juice was therefore recommended as a possible insecticide useful in controlling *D. melanogaster*. Clearly, given the large numbers of *D. ananassae* eclosing from cucumberfruit, starfruit juice or cucumberfruit juice may not effectively control *D. ananassae* numbers. However, a study of gene expression in those *D. melanogaster* and in those *D. ananassae* genotypes that can successfully eclose when exposed to starfruit or cucumberfruit juice might possibly reveal clues to genes controlling successful eclosion in *Drosophila*.

Further research will be needed to determine the relative tendencies of *D. melanogaster* and *D. ananassae* to oviposit on cucumberfruit, starfruit, and banana. It may be that only specific genotypes of *D. ananassae* or *D. melanogaster* will choose to oviposit on cucumberfruit. Likewise, not all genotypes of *D. ananassae* or of *D. melanogaster* may survive and eclose on *A. bilimbi*. A comparison of the competitive success of *D. ananassae* and *D. melanogaster* at specific larval densities would also help to reveal the mechanisms by which each species breeds successfully on different fruits. It is clear from detailed studies of mating success and larval survival through pupation, that *D. melanogaster* is far more successful than *D. ananassae* in mixed cultures reared on an agar-yeast medium (Singh & Kumar 2013). Future studies might test whether genetic adaptations allow some individuals of *D. melanogaster* or *D. ananassae* to survive on highly acidic cucumberfruit juice, a liquid that is possibly toxic to other conspecifics.

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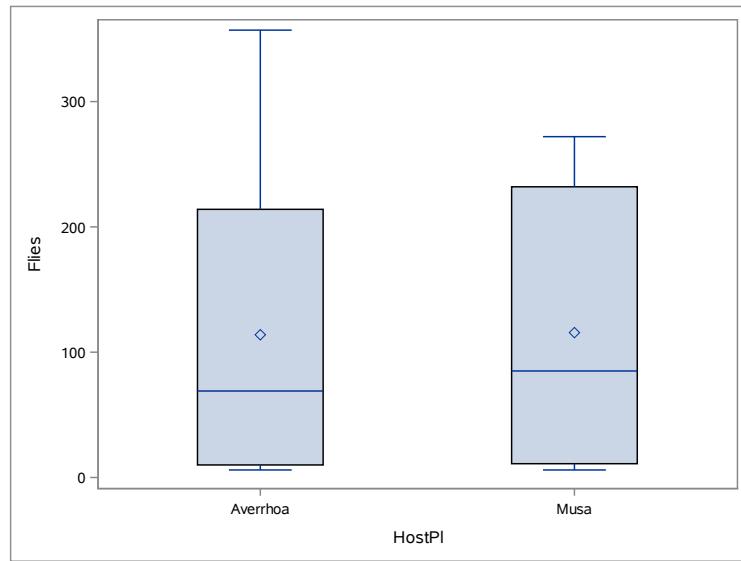


Figure 1. Number of male flies eclosing from cucumberfruit (*Averrhoa bilimbi*) vs. banana (*Musa* species), pooled across *Drosophila* Species.

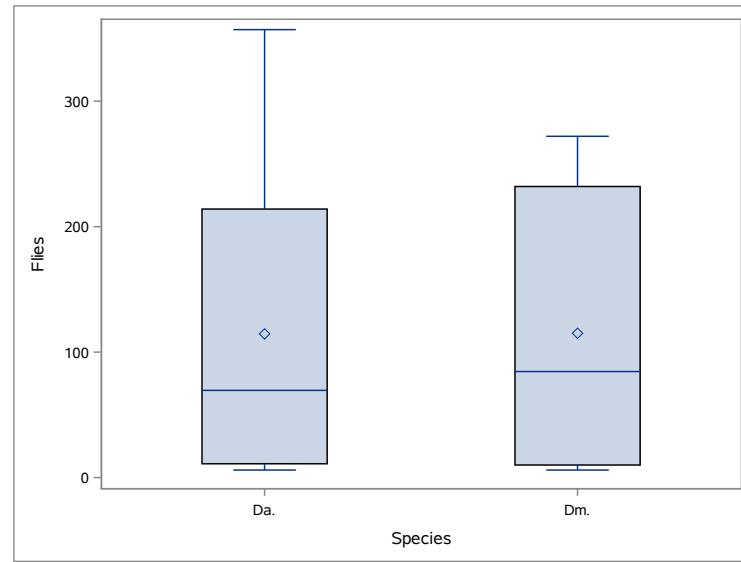


Figure 2. Number of *Drosophila ananassae* and *Drosophila melanogaster* eclosing in the present experiment, pooling across fruit types. Da. = *Drosophila ananassae*, Dm.= *Drosophila melanogaster*.

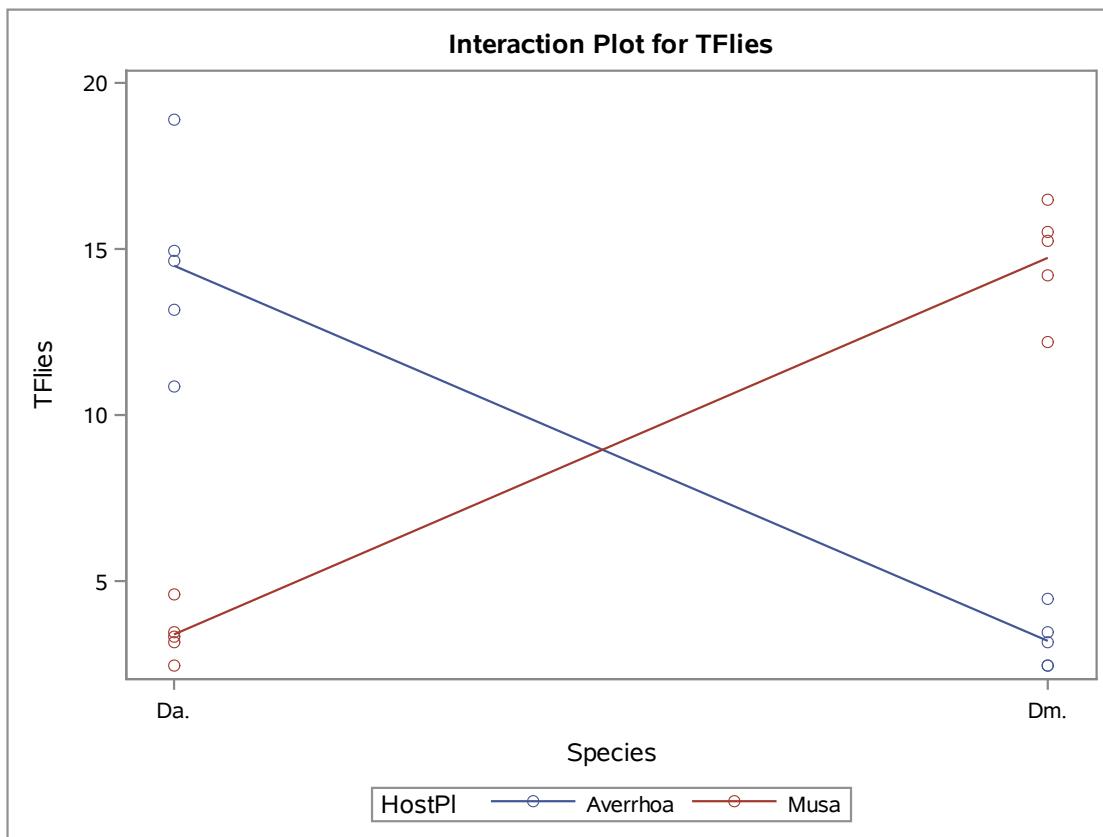


Figure. 3. Statistical interaction between *Drosophila* species and larval host plant in determining the number of male flies eclosing in the present experiment. Blue circles represent replicates for *Drosophila ananassae* and *Drosophila melanogaster* when raised on cucumberfruit (*Averrhoa bilimbi*). Red circles indicate replicates where *D. ananassae* or *D. melanogaster* were raised on banana (*Musa sp.*). The number of male flies eclosing from each replicate are presented as square-root transformed data (variable: TFlies), since the transformed data were used in the ANOVA to determine the statistical significance of this statistical interaction. Da. = *Drosophila ananassae*, Dm.= *Drosophila melanogaster*.