Allozyme Differentiation Correlated with Host-Plant Use in the Pantropical Species, *Drosophila ananassae*

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Abstract—Populations of the pantropical species, *Drosophila ananassae,* were reared from a variety of temperate and tropical fruits on the island of Guam and genotyped at three allozyme loci [malate dehydrogenase (MDH-1), malic enzyme (ME), and isocitrate dehydrogenase (IDH)]. Significant differentiation was found in both MDH-1 and IDH allele frequencies between populations breeding on the fruit of the cucumber tree *(Averrhoa bilimbi)* versus all other fruits tested [including starfruit *(Averrhoa carambola)*]. Genotype frequencies for these two enzymes deviated strongly from Hardy-Weinberg frequencies. This suggests possible population subdivision along host-plant lines in sympatric populations of this widely-distributed *Drosophila* species.

Introduction

Host-plant specific genotypes have been identified in a wide array of insect groups (Bernays & Chapman 1994). However, it is rare to find such differences between populations isolated from two closely-related plant species. Such local genetic differentiation is also uncommon in insects, such as *Drosophila ananassae,* that exhibit a wide geographic distribution (Tobari 1993). One important exception to this pattern is the alcohol dehydrogenase (ADH) polymorphism that has been so well-studied in *Drosophila melanogaster* with respect to the influence of natural selection on the maintenance of genetic polymorphism (Kreitman 1991).

The purpose of the present study is to report a case of strong genetic differentiation in allozyme frequencies between subpopulations of the pantropical species, *Drosophila ananassae,* breeding on two closely-related tropical fruits: starfruit [or carambola *(Averrhoa carambola)*] and cucumber tree fruit [also known as bilimbi *(Averrhoa bilimbi)*]. Since the difference in the chemical compositions of these two host plants is well known (Nagy et al. 1990, Verheij and Coronel 1991), this genetic difference in flies raised on these two *Averrhoa* species may also be a promising case for studies of the importance of natural selection in maintaining differences in allozyme frequencies in *D. ananassae.*

Methods

Yeasted fruit baits of several species of tropical and subtropical fruits were placed in containers on the ground in several locations in northern and central Guam. The species used included starfruit *(A. carambola)*, cucumber tree fruit *(A. bilimbi)*, banana (*Musa* sp.), pineapple *(Ananas comosus)*, orange *(Citrus sinensis)*, and kiwi fruit *(Actinidia chinensis)*. First-generation adults reared from each fruit were used for protein electrophoresis using starch gels and methods outlined in Pasteur et al. (1988). Each individual was analyzed for three different enzymes: malate dehydrogenase [MDH-1 (EC 1.1.1.37)], malic enzyme [ME (EC1.1.1.40)], and isocitrate dehydrogenase [IDH (EC 1.1.1.42)]. Samples of *Drosophila melanogaster* were genotyped as controls alongside *D. ananassae* individuals on each gel run.

Since the distributions of starfruit trees and cucumber trees did not always coincide on Guam, a second experiment tested whether the difference observed in MDH-1 allele frequencies was due to a host-plant effect or microgeographic spatial differentiation. This involved placing yeasted baits of both starfruit and cucumberfruit in a single location in central Guam (Chaot River site). The two fruit types were placed approximately 10 meters apart to prevent the odors of each fruit from mixing. Several days later, the baits were brought into the laboratory where the larvae were reared to adulthood. These flies were then subjected to allozyme analysis for MDH-1. Some flies from each fruit type were frozen, or preserved in ethanol or as pinned specimens to voucher the species identity of the *Drosophila* studied. No reason presently exists, however, to suspect that a sibling species of *D. ananassae* is present on any Micronesian island, including Guam (Wheeler & Takada 1964).

Results

Chi-square tests of allele frequencies showed significant differences based on host plant species in the case of both MDH-1 and IDH (Tables 1 and 2). No such host plant differences were found in ME (Chi-square = 3.92, n.s.). For MDH-1, flies reared from cucumber tree fruit had two unique alleles (designated F and M) in comparison with the monomorphic SS genotype of flies reared from all other fruits. In the case of IDH, the difference was due to a high frequency of a slow (S) allele in cucumberfruit that was present only as a rare allele in the other fruit types.

When flies reared from cucumber tree fruit and starfruit at a single site were assayed for MDH-1, the same pattern of allozyme frequencies was found as in the earlier experiment (Table 3). The unique F and M alleles found in cucumberfruit-reared flies from other sites were again found when cucumberfruit and starfruit baits were presented at a single location.

Tests for deviations from Hardy-Weinberg equilibrium were significant for both MDH-1 (Chi-square=281.8, p<0.001) and IDH (Chi-square=142.1,

Table 1. Chi-square test of independence of malate dehydrogenase (MDH-1) gene frequency from host plant species in *Drosophila ananassae*.

Host Plant	Allele Frequency		
	F	M	S
Cucumber fruit *(Averrhoa bilimbi)*	32	28	26
Star fruit *(Averrhoa carambola)*	0	0	54
All other fruits tested (banana, pineapple, orange, kiwi fruit)	0	0	104

$\chi^2 = 145.7$ (P < 0.001)

Table 2. Chi-square test of independence of isocitrate dehydrogenase (IDH) gene frequency from host plant species in *Drosophila ananassae*.

Host Plant	Allele Frequency			
	F	M	S	S'
Cucumber fruit *(Averrhoa bilimbi)*	2	0	36	0
Star fruit *(Averrhoa carambola)* +All other fruits tested (banana, pineapple, orange, kiwi fruit)	42	49	32	1

$\chi^2 = 107.8$ (P < 0.001)

Table 3. Chi-square test of independence of malate dehydrogenase (MDH-1) gene frequency from host plant species in *Drosophila ananassae* collected from a single site (Choat River, Guam).

Host Plant	Allele Frequency		
	F	M	S
Cucumber fruit *(Averrhoa bilimbi)*	26	20	22
Star fruit *(Averrhoa carambola)*	0	0	30

$\chi^2 = 38.4$ (P < 0.001)

p < 0.001). In both cases, the deviation was due to a great decrease in the number of heterozygotes observed compared to the number expected under a Hardy-Weinberg distribution. Such an observed lack of heterozygotes (often referred to as the Wahlund Effect) is frequently taken as evidence of population subdivision (Wright 1978).

Discussion

The allozyme differentiation observed between *D. ananassae* breeding on cucumber tree fruit and conspecifics breeding on all other fruits (including starfruit) is quite strong for both MDH-1 and IDH. One of the principal differences between cucumberfruit and all other fruit types tested is the much greater acidity of the fruit of the cucumber tree, which is produced by a high amount of citric acid [Verheij & Coronel 1991]. Both cucumber tree fruit and starfruit also contain oxalic acid, a characteristic of their family (Oxalidaceae). Since cucumber tree fruit has more oxalic acid than starfruit (especially the domesticated varieties of *A. carambola* such as the ones found on Guam) [Nagy et al. 1990], this chemical difference may also influence the allozyme genotypes of flies reared from these two fruits.

One possible explanation for the significant allozyme differences detected between the two species of *Averrhoa* is host plant selection by ovipositing females. If females carrying the F and M alleles at MDH-1 preferred to lay eggs on cucumber tree fruit and those with the S allele preferred starfruit, oviposition preference might explain the present results. It is likely, however, that this is not the main explanation for the genotypic differentiation, since females reared from either fruit will oviposit on both cucumberfruit and starfruit (Pablo & Lofdahl unpublished).

Both cucumberfruit and starfruit are introduced species on Guam as they are not among the plant species listed by Stone (1970) as of pre-European aboriginal introduction. Both fruits, however, are probably native to the Indo-Malayan region (Nagy et al. 1990; Verheij & Coronel 1991). Starfruit seems mainly confined to local gardens on Guam, while cucumberfruit tends to also become naturalized in secondary growth forests on the island (L. Raulerson pers. comm.). It is unknown whether other populations of *D. ananassae* breeding on cucumber tree fruit and starfruit show the same genotypic differentiation as found on Guam. Previous allozyme studies of *D. ananassae* on other Pacific islands (Johnson 1971, Futch 1973) have used native breeding substrates [e.g., breadfruit (*Artocarpus* sp.)] or non-native fruits such as papaya *(Carica papaya)*. These earlier works also did not assay malate dehydrogenase, malic enzyme or isocitrate dehydrogenase allele frequencies.

Tests of genotype frequencies based on Hardy-Weinberg expectations suggest that true population subdivision based on host plant identity is present in the Guam population of *D. ananassae*. This raises the possibility that we are dealing with two populations that may be partially reproductively isolated. Alternatively,

the cucumber tree fruit-breeding population might be a separate species from the population breeding on the other fruit types, although no fixed genetic differences have yet been detected among these populations.

In fact, a sibling species of *D. ananassae*, which was later named *D. pallidosa* for its paler body color, was detected in an allozyme survey on Samoa (Futch 1973). However, *D. pallidosa* is apparently confined to the Pacific islands near Samoa (Evenhuis 1989). Extensive trapping of flies on Guam using yeasted banana baits as well as by sweeping other native and non-native fruits has shown that *D. ananassae* is by far the most abundant species of *Drosophila* on Guam [and on most other Pacific Islands surveyed (Wheeler & Takada 1964)]. In a collection of over 5,000 *Drosophila* from Micronesia, *D. ananassae* greatly outnumbered other species, and it was also found to be abundant and widespread on Guam (Wheeler & Takada 1964). No evidence was found of a sibling species that was morphologically distinguishable from *D. ananassae* in Wheeler and Takada's (1964) survey. Further research is needed, however, to determine whether the two host-associated genotypes identified here as *D. ananassae* do indeed represent populations of a single biological species.

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