

Figure 1. Scheme of heat shock induced recombination and selection of the recombinants.

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Can *D. simulans* breed on *Morinda citrifolia*, the host plant of *D. sechellia*?

**Liang, Bai-Ling, and Yoshiaki Fuyama.** Department of Biology, Tokyo Metropolitan University, Hachioji, Tokyo 192-0397, Japan

## Introduction

*D. sechellia* is a member of the *melanogaster* species-subgroup and endemic to Seychelles archipelago. Unlike most of the other members of the subgroup, it is monophagous and breeds exclusively on the ripe fruit of an arboreal rubiaceae, *Morinda citrifolia* (Tsacas and Bachli, 1981). The morinda fruit is known to contain several fatty acids such as *n*-caproic acid (hexanoic acid) and *n*-caprylic acid (octanoic acid) which are extremely toxic to the sibling species of *D. sechellia* (Amlou *et al.*, 1997, 1998a, b). Genetic analyses have shown that the tolerance of *D. sechellia* to the toxic substances of the fruit is mainly controlled by genes located on the third chromosome, suggesting that relatively small number of genes are involved in the adaptation to the host plant (Jones, 1998). It may be expected, therefore, that some degree of tolerance to the toxic substances can evolve within the local populations of sibling species where *M. citrifolia* is available for a potential food resource. In the fall of 1998, we happened to find a considerable number of *D. simulans*, one of the sibling species of *D. sechellia*, were gathering on the fallen fruit of *M. citrifolia* in Hahajima Island of the Ogasawara Islands (the Bonin Islands), Tokyo. Surprisingly, a number of adult flies of *D. simulans* emerged from the fruit collected in the field and brought back to the laboratory. The Ogasawara Islands are located about 1000Km south of Tokyo. On these islands, *D. simulans* is known to have been a dominant species for more than 60 years since its first discovery (Kikkawa and Peng, 1938; Okada, 1971). *M. citrifolia* is abundant in some of the islands. We examined for the possibility that some of *D. simulans* on Hahajima Is. might have acquired a

tolerance to *n*-caproic acid, one of the toxic substances contained in the morinda fruit (Legal *et al.*, 1991, 1992).

## Materials and Methods

Isofemale strains of *D. simulans* were established from flies collected by sweeping over the fallen morinda fruit in Hahajima Is. (designated as Mca) and also from those emerged from the fruit (Mcb). For comparison, another collection (Lab) was made on the same occasion using a banana trap in Chichijima Is., an island located about 60Km apart from Hahajima Is., on which morinda is rarely found. Isofemale strains S354 and S357 were derived from a collection in 1979 at Oiso in the mainland of Japan. *D. sechellia* strains SS86 and SS77 were derived from flies collected in Praslin Is., Seychelles in 1987.

Table 1. Tolerance to *n*-caproic acid for populations of *D. simulans* and *D. sechellia*

Species/Population	Survival (%)±S. E.	
	Female	Male
<i>D. simulans</i>		
Mca	30.8 ± 1.5	15.6 ± 1.8
Mcb	25.0 ± 1.8	10.9 ± 1.2
Lab	27.9 ± 2.1	12.2 ± 1.3
Oiso	20.6 ± 2.7	9.4 ± 2.2
<i>D. sechellia</i>	82.7 ± 3.8	69.3 ± 3.8

Table 2. ANOVA for the tolerance of *D. simulans* strains

SV	df	MS	F
Sex	1	2.967	142.99***
Population	3	0.108	3.96*
Sex x Population	3	0.003	0.14
Strain/Population	24	0.027	1.31
Sex x Strain/Population	24	0.022	1.04
Error	224	0.021	

\*  $p < 0.05$

\*\*\*  $p < 0.001$

ANOVA was carried out on angular transformed survival rates.

tolerance among populations of *D. simulans* were barely significant ( $P = 0.02$ ). This is mostly due to a reduced level of tolerance in the two mainland strains. Contrary to the expectation, none of *D. simulans* strains that originated from flies bred on the morinda fruit (Mcb) showed an increased level of tolerance as compared with the other Ogasawara strains (Mca and Lab).

These results seem to give no evidence for adaptation to *M. citrifolia* in *D. simulans* inhabiting the Ogasawara Islands. Genetic mechanisms of the adaptation might be more complex than those anticipated, or fitness trade-off accompanying the host adaptation might be so large that novel adaptive mutations are quickly swept out from the population under a heavy gene flow.

Acknowledgment: We thank Drs. H. Watabe and S. Tanabe for their help in collecting flies at Hahajima Is.

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The tolerance of adult flies to *n*-caproic acid was tested as follows: a piece of filter paper (15 × 25mm) was inserted into a plastic vial (10mm diameter × 80mm long), into which 40 µl of 1% *n*-caproic acid was absorbed. Then, 30 flies, 2-4 day-old, were introduced into the vial and left at 25 °C for 1 hr. Then, the number of flies survived was counted. All tests were carried out within six months after the establishment of the Ogasawara strains.

## Results and Discussion

Figure 1 shows average survival for each strain after 1hr exposure to *n*-caproic acid. Average tolerances for each population of *D. simulans* and *D. sechellia* are summarized in Table 1. As expected, two *D. sechellia* strains showed survival rates much higher than those of *D. simulans* ( $P < 0.001$ ). Table 2 shows the results of ANOVA for *D. simulans* strains. Females are in general more tolerant than males ( $P < 0.001$ ). Differences in

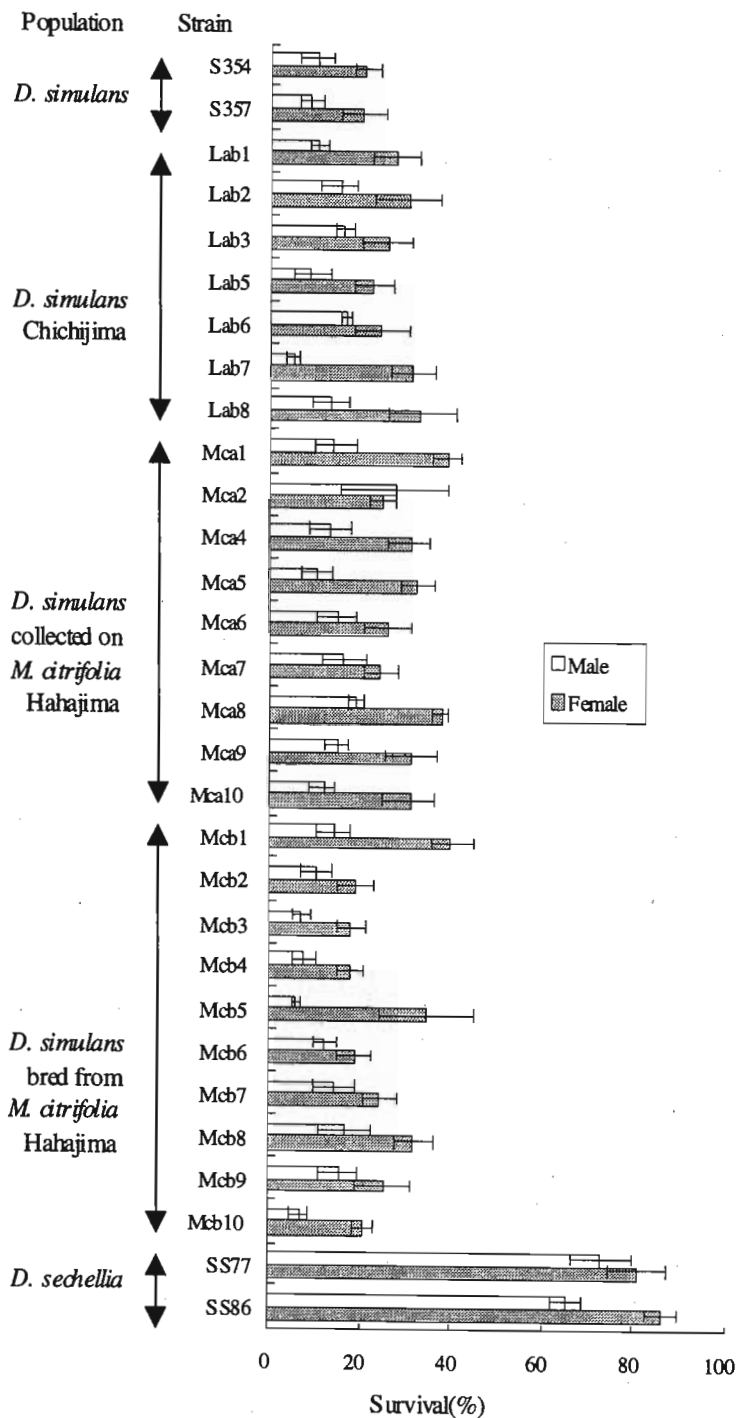


Figure 1. Tolerance of *D. simulans* and *D. sechellia* to *n*-caproic acid. Each bar represents average survival and standard error based on five replicate tests.

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Lack of mite parasitism in natural populations of *Drosophila* of the cactophilic *D. martensis* cluster (*D. repleta* group).

**Cornejo, O., and M. Benado.** Centro de Ecología, IVIC, and Departamento de Estudios Ambientales, Universidad Simón Bolívar, Caracas, Venezuela.

Parasitism is widespread among organisms. Thus, parasite-host associations may affect host population size (Anderson and May, 1979), demographic structure, and host population cycles (Goater and Holmes, 1997). Furthermore, several studies indicate that host-parasite associations influence body size, growth rate, longevity, fecundity and developmental stability (Møller, 1990; Polak, 1993, 1996; Markow and Polak, 1995; Polak and Starmer, 1998).

Mite parasitism studies in *Drosophila* have been restricted to few species. Furthermore, few investigations have characterized the extent of this interaction in natural populations. Thus, Markow and Polak (1995) assessed the dispersion pattern of the parasitic mite *Macrocheles subbadius*, and its impact on host sexual selection in natural populations of the cactophilic *D. nigrospiracula*. They reported nearly random to strongly aggregated mite distributions over the flies, as well as mean infestation intensities of up to 7.8 mites per fly. They further experimentally showed that parasitism by mites affected the copulating success of *D. nigrospiracula*. On the other hand, Polak (1993) found that mite-infested flies did not show significant increases in fluctuating asymmetry, but that nematode infestation increased fluctuating asymmetry above mite-infested and control flies. Valuable as these studies are, it remains an open question the extent of mite parasitism in other natural *Drosophila* populations.

We report here the results of a study aimed at evaluating mite parasitism in natural populations of *Drosophila uniseta* Wasserman, Koepfer & Ward 1973, in the *D. martensis* cluster of the *D. repleta* group. The cluster is endemic to Venezuela and Colombia (Wasserman and Koepfer, 1979), and *D. uniseta* is the only species that exploits the fermenting issues of the columnar cactus *Stenocereus griseus* (Benado and Montero, 1988). We were interested in assessing the impact of mite parasitism on fluctuating asymmetry in the wings as a measure of developmental instability.

**Material and Methods and Results:** We sampled four localities on the North Central Coast of Venezuela. about 25 Km from each other. The flies were netted monthly from May 1999 to December 1999 over banana baits and transferred immediately to vials for further inspection. In

addition, from January 1999 to April 1999 we collected rot pockets of *Stenocereus griseus* and daily harvested the 2018 flies that emerged in the laboratory for up to 10 days. The flies were inspected under the dissecting microscope for mites and/ or mite scars. None were found.

Table 1. Cumulative parasitism over eight months and four localities.

\* *D. sp.*: *D. starmeri* and *D. venezolana*.

Species	Parasitized	Non Parasitized	% Parasitism	Total
<i>D. uniseta</i>	3	215	1.40	218
<i>D. martensis</i>	7	613	1.13	620
<i>D. sp.*</i>	4	600	0.66	604
Total	14	1428	0.97	1442

In Table 1 we give the