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Effect of Mechanical Sifting of Pupae on the α-Glycerophosphate Dehydrogenase Activity in Adults of the Mediterranean Fruit Fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae)[1]

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ABSTRACT

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Agitation caused by the sifting used to separate pupae of tephritid flies from their pupation medium during a critical period in the pupal cycle may result in adults which cannot fly. The number of flies capable of flight increases with a progressive decrease in sifting intensities or duration. Alpha-glycerophosphate dehydrogenase (α-GPD) activity in fliers from unsifted pupae and in those from pupae sifted for 2 revolutions was >10-fold higher than that in nonfliers sifted for 15 revolutions. A progressively lower α-GPD activity was found in fliers sifted as pupae for 5, 10, and 15 revolutions, respectively. "Sifted" fliers appear to be affected with subacute forms of the droopy wing syndrome.

The separation of mass-reared tephritid pupae from their pupation medium by mechanical sifting through a rotating, screened drum has been standard practice in laboratories in Hawaii and elsewhere. Two of us (Ozaki and Kobayashi 1981) discovered that agitation of pupae by sifting was responsible for the so-called "droopy wing syndrome" characterized by an inability to fly and the abnormal "drooping" position of the wings at rest (see the report by Hart[4]). This condition developed only when sifting was conducted during a critical period in pupal development, and the droopy wing condition represented only one manifestation of an inability to fly (Ozaki and Kobayashi 1981). Little and Cunningham (1978) reported that major groups of indirect flight muscles were absent or deficient in droopy-winged adults of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), commonly called medflies. Further studies revealed that injury was due to muscle detachment caused by agitation from the sifting process (Little et al. 1980). Sensitivity to breakage depended on the physiological age of the pupae when sifted and intensity or duration of the sifting (Ozaki and Kobayashi, unpublished data). The question arose whether adult flies obtained from pupae produced under a reduced sifting regimen during the critical period, and thus capable of flight, are the same as those obtained by sifting during the noncritical period, or in the absence of sifting.

The medfly was selected for this study since most of the previous work on the droopy wing syndrome was conducted with this species and since quality control of mass-reared medflies for the sterile-fly release eradication program has become increasingly important, due to plans to eradicate this fly in Hawaii and the recent outbreaks of the pest in California and Florida. To this end, we have attempted to test if any differences existed among laboratory-reared, sifted and nonsifted medflies by measuring the activity of flight muscle α-glycerophosphate dehydrogenase (α-GPD) (1-glycero-3-phosphate: NAD oxidoreductase, EC 1.1.1.8), an enzyme which plays a central role in flight metabolism, and to ascertain whether forms of subacute droopy wing syndrome existed. We here report results from this study.

Materials and Methods

Mass-reared medfly larvae were allowed to pupate in fine vermiculite at 24°C (Mitchell et al. 1965, Tanaka et al. 1970). After 24 h, pupae were gently hand-sifted from their pupation medium and placed in 10-ml lots, and held at 24°C and 70 to 96% relative humidity (RH) until treatment on day 5 (the most sensitive age of pupae to sifting when held at ca. 24°C [Ozaki and Kobayashi 1981]). Sifting was simulated by mechanically agitating pupae in a sifting drum set at 20 rpm for 0 (control), 2, 5, 10, and 15 revolutions. Treated pupae were then held in 30-dm^3 cages at 18.9 to 27.8°C and 70 to 90% RH until adult eclosion, whereupon the adults were fed cube sugar and water ad lib. Twenty-four hours after eclosion, we separated fliers from nonfliers by vigorously tapping the cage; all flies dropped to the bottom, and we collected only those that immediately flew to the top of the cage. Enzyme assays were carried out on adults from three different larval batches. Eclosion rates for all treatments were above 90%, whereas the percentages of nonfliers sharply increased, from less than 3% for the unsifted control and those sifted for two revolutions, to 40, 94, and 97% for those sifted at 5, 10, and 15 revolutions, respectively.

Twenty thoraces weighing ca. 80 mg (wet weight) were obtained within 24 h after emergence from each of the preceding treatments and homogenized in cold 50 mM Tris-hydrochloride buffer (pH 7.8), followed by centrifugation at 3,000 × *g* for 15 min at

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[4]Richard Hart. 1967. "Droopy wing, a disease of Tephritidae." Unpublished Quarterly Reports, Hawaiian Fruit Flies Investigations, Agric. Res. Serv., USDA, Honolulu, Hawaii.

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5°C. The supernatant was used as the source of the enzyme (α-GPD) and assayed by a modification of the method of White and Kaplan (1969). The final concentrations of the reaction mixture were as follows: 1 ml of 0.1 mM NADH (P-L Biochemicals, Inc., Milwaukee, Wis.) and 1 ml of 0.5 mM dihydroxyacetone phosphate (DHAP) as the lithium salt (Sigma Chemical Co., St. Louis, Mo.) in 50 mM Tris-hydrochloride (pH 7.8). To this mixture in a quartz cuvet (5-mm light path) was added 10 μl of enzyme extract; the mixture was shaken, and the reduction in absorbancy (NADH oxidation) was followed at 340 nm in a double-beam Beckman 26 spectrophotometer against a Tris-hydrochloride blank. Values obtained from the reaction mixture in the absence of NADH were subtracted from values obtained in its presence to correct for nonrelated NADH coupled reactions. All assays were run at 23 ± 1°C. At least five assays were performed for each test. Protein determinations were made on the enzyme extract, using the microbiuret reagent by the method of Goa (1953). α-GPD-specific activities were determined, and comparisons were made between batches and within groups by the *t* test (P = 0.05) for significance.

Results and Discussion

All data are presented in Table 1. Flight muscle α-GPD from laboratory-reared, unsifted controls showed considerable activity, although a crude extract was used as the source of the enzyme. However, α-GPD specific activity in nonfliers from sifted pupae was 10.5-fold lower than the controls among the three batches tested. The degree of α-GPD activity in sifted flies capable of flight appeared to depend on the number of revolutions to which they were subjected during the sifting process at 20 rpm, with α-GPD activity higher in those sifted at lower numbers of revolutions. Adult fliers from pupae sifted for two revolutions showed comparable α-GPD activity with the controls from all three batches tested. Significantly higher (P = 0.05) α-GPD activity was found in fliers sifted for 2 revolutions than in those sifted for 5, 10, and 15 revolutions. A 1.2-, 1.4,- and 2-fold lower α-GPD activity was realized in flies sifted for 5, 10, and 15 revolutions, respectively. The pattern of α-GPD activity among the groups did not significantly differ (P = 0.05) among the three batches tested.

Table 1.—Comparison of the specific activities of flight muscle α-glycerophosphate dehydrogenase among adults of the Mediterranean fruit fly, *C. capitata*, obtained from pupae sifted for different durations at 20 rpm

No. of revolutions at 20 rpm	Sp act (μmol of DHAP × 10^{-3} min^{-1} · mg of protein^{-1})[a]			
	Batch 1	Batch 2	Batch 3	$\bar{x}$
Fliers				
0	10.1	11.2	10.1	10.5
2	10.8	11.6	9.0	10.5
5	9.0	9.0	5.0	7.7
10	9.2	8.5	5.0	7.6
15	5.7	6.4	4.0	5.4
Nonfliers				
15	0.9	1.1	1.0	1.0

[a]Mean enzyme specific activities calculated from at least five assay determinations ±SE = 0.05, n = 5. See text for assay procedures.

It appears the injury to adult flies resulting from pupae sifted during the critical period in the pupal cycle is progressive, i.e., gradations of damage may be seen ranging from loss of flight capability without any outward abnormalities, through aberrant changes in wing position, to the most acute state where wings are permanently held in the droopy position. If the activity of α-GPD is an indication of flight capability or maintenance, then adults from pupae handled at the critical period may not be normal, in spite of normal external appearance and flight capability, but may well be affected with subacute forms of the droopy wing syndrome.

It is clear that α-GPD activity is sharply reduced in nonflying, droopy-winged adults. The cause of this loss of enzyme activity is not presently understood but may be related to certain flight muscles missing in droopy-winged individuals (Little and Cunningham 1978, Little et al. 1981). A correlation between muscle loss and decline in α-GPD as well as other enzyme activity has been shown by Rockstein (1963, 1966) who investigated the correlation between enzyme changes in the flight muscle with aging and flight ability in the house fly, *Musca domestica* L. Young male house flies begin to lose the capability to fly by the middle to end of week 1 postemergence and total capability by week 3, due to actual loss of wing surface by abrasion. Measurement of α-GPD revealed a decline in enzyme activity before the onset of wing loss, and a concomitant loss of Mg-activated ATPase activity with the onset of wing loss. Sohal and Allison (1971) observed the fusion and degeneration of mitochondria and dissolution of myofibrils in flight muscles of 7- to 11-year-old house flies, whereas both striation pattern and myofibrillar arrangement in 25- to 30-day-old flies were lost. Relationships between enzyme activity and structural changes in muscle are not unique to insects. Rockstein and Brandt (1961) have shown that a loss of muscle mass from 4.58 to 2.45 g in aging male Sprague-Dawley rats resulted in a loss of ATPase activity on a muscle mass basis.

The potential use of flight muscle α-GPD levels as a biochemical indicator of flight capability is due to the central role that the enzyme plays in flight metabolism (see Sacktor 1975). The absence or low titer of lactate dehydrogenase and the presence of a high titer of α-GPD in the flight muscles of species from several orders attests to the importance of α-GPD in insect flight energetics (Beenakkers et al. 1975).

Since measurement of flight muscle α-GPD from a crude extract of medfly thoraces is a rather simple and rapid procedure, its use as a tool in monitoring the quality of medflies produced under mass-rearing conditions appears promising.

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