workers are capable of skewing investment between female and male brood in the ratio that is optimal for them. This may or may not hold. If it does not, then I am giving an unfair advantage to the haplodiploidy hypothesis. But that is just as well because it makes my falsification of the haplodiploidy hypothesis more robust.

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Effect of dietary citrate in reducing housefly resistance to insecticides

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Toxicity of four insecticides, permethrin, bromophos, malathion and lindane, to citric acid-fed houseflies was studied up to F_2 generation using topical application. The results indicated a gradual fall in LD_{50} values of all the four insecticides thereby suggesting a role of citric acid in reducing insect resistance to insecticides.

CHEMICAL substances fed to insects through diet have a bearing on insect life-span and agility which can alter the insect resistance to insecticides. Also marked age and stage variations in resistance to insecticides are noted in insects. Thus the resistance is negligible in embryonic stage, high in larval stage and again low in pupal stage. After adult emergence, depending upon the species, the resistance increases initially, remains stable and declines with age[1,2]. Since the resistance declines with age and an age-associated increase in citric acid has been reported in musca and ceralitis[3], we decided to study the effect of citric acid feeding in altering housefly resistance to insecticides. We fed citric acid to houseflies through diet up to F_2 generation and studied the toxicity of four insecticides, permethrin (Indian Explosives), malathion (Cyanamid India) bromophos and lindane (E. Merck, India), to these citric acid-fed

Table 1. Toxicity of four insecticides to citric acid-fed houseflies.

Insecticides	Houseflies*	Regression equation	LD_{50} (ng/fly)
Permethrin	Normal	$y = 1.1319 + 2.6131\,x$	30.0
	Fed CA for 3 days	$y = 0.8021 + 3.149\,x$	21.53
	CA-fed F_1 generation	$y = 3.2806 + 1.3876\,x$	17.35
	CA-fed F_2 generation	$y = 0.188 + 5.0305\,x$	10.75
Bromophos	Normal	$y = 0.3323 + 2.145\,x$	150.0
	Fed CA for 3 days	$y = 1.6481 + 1.7021\,x$	93.15
	CA-fed F_1 generation	$y = 1.2128 + 2.1167\,x$	61.55
	CA-fed F_2 generation	$y = 0.1963 + 3.015\,x$	39.19
Malathion	Normal	$y = 2.1718 + 0.8773\,x$	1674.0
	Fed CA for 3 days	$y = 1.0627 + 1.3374\,x$	878.8
	CA-fed F_1 generation	$y = 2.2678 + 0.9826\,x$	603.3
	CA-fed F_2 generation	$y = 2.086 + 1.1132\,x$	414.6
Lindane	Normal	$y = 1.1976 + 1.1888\,x$	1580.0
	Fed CA for 3 days	$y = 1.1835 + 1.3136\,x$	804.2
	CA-fed F_1 generation	$y = 0.7989 + 1.5512\,x$	510.9
	CA-fed F_2 generation	$y = 1.3845 + 1.4225\,x$	348.1

*3 to 4 days old female flies were used.
CA, Citric acid.

flies by topical application. The results of our toxicity studies are presented in this paper.

All insecticides used were of technical grade having purity above 90%. Solutions of these insecticides of desired strength were prepared in acetone and applied topically to 3–4-days-old female houseflies using an aglamicrometer syringe as described earlier[4]. The housefly culture which we are rearing in our laboratory for the past 30 years was used for the toxicity studies. The normal houseflies were fed citric acid through diet (0.1%) from the day of emergence right up to F_2 generation. In each generation the toxicity of the four insecticides permethrin, bromophos, malathion and lindane was tested against 3–4-days-old female flies. The results were subjected to probit analysis and from the regression equations obtained, LD_{50} values were calculated. These are presented in Table 1. For citric acid determination the flies were homogenized with 5% trichloroacetic acid and citric acid was determined by colorimetric method of Natelson *et al.*[5]

Table 1 indicates a gradual fall in LD_{50} values of all the four insecticides right from the present up to F_2 generation. This fall in LD_{50} values of the insecticides was associated with a simultaneous increase in the citric acid content of the flies. The citric acid content of the normal houseflies was 0.68 μM/g. This quantity increased gradually, and in F_2 generation the citric acid content was 1.976 μM/g (Table 2), almost 3 times that of normal flies. The increase in citric acid content was presumed to be due to citric acid feeding. Interestingly, when citric acid mixed with the insecticides was applied topically to normal flies no significant change in the LD_{50} values of insecticides was observed suggesting that citric acid did not act as a synergist. The other changes which were produced presumably due to citric acid feeding during the experimental period, were delayed egg laying, reduction in the number of eggs laid and a slight prolongation of the life cycle.

The mechanism by which citrate feeding reduces insect resistance to insecticides is difficult to explain. Zahavi and Tahori[3] reported that an age-associated citrate increase in houseflies and ceratitis in their work was probably due to discrepancy in the rate of citrate production and utilization at different stages. Since citrate is not acting as a synergist in the present case, the decrease in resistance must be due to some metabolic changes or hindrance. It is possible that the formation of normal metabolic citrate may be suppressed due to higher citrate concentration produced by citric acid feeding. This will naturally create a sort of hindrance or slow down the glycolysis rate thus producing less energy than normal for work. The ultimate result of this low energy production will be the reduced resistance of the insects to insecticides.

The present finding that dietary citrate reduces housefly resistance to insecticides provides useful information for pesticide scientists. This suggests that the development of resistance by insects to insecticides may be reduced possibly by including in the pesticide formulation a chemical like citric acid or a member of citric acid cycle (a citrate former) or some other similar chemical. However, a lot more experimental work has to be done before drawing any conclusion in t. s matter.

Table 2. Citric acid content of 3-days-old female houseflies.

Housefly	Citric acid μM/g
Normal housefly	0.68
Fed Citric acid for 3 days	1.237
Citric acid-fed-F_1 generation	1.613
Citric acid-fed-F_2 generation	1.976

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C3-like carbon isotope discrimination in C3–C4 intermediate *Alternanthera* and *Parthenium* species

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Carbon isotope discrimination and leaf anatomy in relation to leaf position on the stem were studied for the two recently identified naturally occurring C_3–C_4 intermediate dicot plants, *Alternanthera ficoides* and *Parthenium hysterophorus*. The carbon isotope ratios ($\delta^{13}C$ values) for the two C_3–C_4 intermediate species did not change much with the leaf age but were similar to C_3 plants. Although variation in leaf anatomy with respect to leaf age was noted, the carbon isotope ratios were similar at all stages of leaf growth. These results also confirm that *A. ficoides* and *P. hysterophorus* possess leaf anatomical characteristics intermediate to those of C_3 and C_4 dicot plants but exhibit C_3-like carbon isotope discrimination.

THE existence of naturally occurring plant species showing intermediate characteristics to those of C_3 and