The figure shows the urate uptake in the liver slices, and the difference in the uptake rates between beagle and Dalmatian dog must originate from a defective transport system. From the finding that the hepatic 'clearance' of uric acid in the non-Dalmatian dog was greater than in the Dalmatian dog[3,10] it has been suggested that the hepatic cellular membrane prevents in some way the oxidation of uric acid in the Dalmatian liver. The data in this paper demonstrate that the uptake rate into Dalmatian hepatocytes is lower by a factor of about 3 as compared to that in the beagle. This result appears to prove that the genetic abnormality of uric acid metabolism in the Dalmatian coach hound is due to a change in urate transport through the membranes of hepatocytes.

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0014-4754/84/121415-02$1.50 + 0.20/0

Genetic analysis of permethrin resistance in the house fly, *Musca domestica* L.

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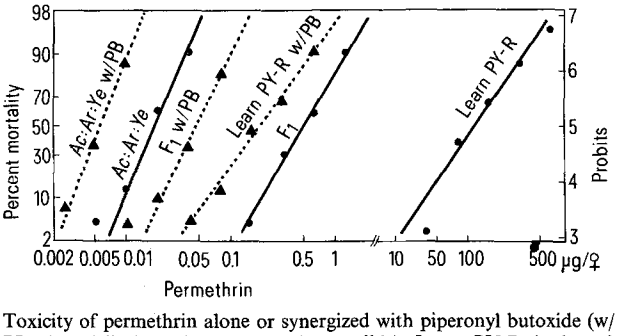
Summary. The genetic control of mechanisms conferring resistance to permethrin was investigated in *Musca domestica* L. Factors conferring resistance were found on autosomes 1, 2 and 3, with their relative dominant effects being 3 > 1 > 2. Three combinations of chromosomes (i.e. 2+5, 3+5 and 1+3+5) were found to interact, but in a less than additive manner. Data obtained with synergists suggest that the mixed function oxidase system is responsible for part of the resistance. Pyrethroid resistance on chromosome 1 has not been previously reported.
Key words. Fly, house; *Musca domestica;* permethrin resistance; resistance, genetic control.

Pyrethroid insecticides have emerged into prominence largely as a result of the successful modification of the structure of the natural pyrethrins to increase stability and insecticidal activity[2]. Although these new field-stable compounds have been used for only a few years, cases of control failures due to the development of resistance have been reported[3]. Earlier studies of the genetics of resistance to the natural pyrethrins have shown that four factors, one each on chromosomes 2 and 5 and two on chromosome 3, confer the resistance[4]. Due to changes in the structure of pyrethroids, reports that the newer pyrethroids may possess a different type of action [5-7] and the great economic importance of these insecticides, we conducted a chromosomal analysis of resistance in the house fly using the widely used pyrethroid, permethrin, in an attempt to identify the chromosomes involved and to quantify their respective contribution to resistance.

Materials and methods. House flies, *Musca domestica* L., were collected from a dairy (Learn) near Ithaca, New York, following three years of limited spraying with permethrin, and were subsequently selected with this insecticide in the laboratory for 18 generations to achieve a circa 6000-fold level of resistance (Georghiou et al., unpublished data). This strain (Learn PY-R) is cross-resistant to all pyrethroids tested to date and has a low to moderate resistance to several organophosphates and carbamates (Scott and Georghiou, unpublished data). Three susceptible strains were also used; *ac;ar;ye* (bearing the morphological markers *ali-curve, aristapedia* and *yellow eyes* on chromosomes 1, 2 and 4 respectively), *ac;ar;bwb;ocra* (bearing the morphological markers *ali-curve, aristapedia, brown body* and *ocra eyes* on chromosomes 1, 2, 3 and 5, respectively) and NAIDM (non-marked strain). Bioassays involved application of 1 µl acetone (controls) or permethrin in acetone to the thoracic notum of 3-day-old female flies under CO_2 anesthesia.



Toxicity of permethrin alone or synergized with piperonyl butoxide (w/PB, dotted line) to the *ac;ar;ye* (susceptible), Learn PY-R (resistant) and F_1 (*ac;ar;ye* ♀ × Learn PY-R♂) strains of house fly.

The genetic analysis was based on the F_1 male backcross method of Tsukamoto[9] and involves crossing susceptible marker strain females with resistant (R) males, backcrossing the F_1 males to susceptible females and testing separately each phenotypic class of the progeny with a diagnostic dose of the insecticide. Since no crossing over occurs in male house flies, this method permits the detection and measurement of the 'heterozygous'effect[8] ('dominant' effect[9]) of each chromosome (i.e. the resistance contributed by individual R chromosomes). In this type of analysis the percent survival is converted to an arc-sin unit, the 'effect' of each chromosome or combination of chromosomes is calculated[9], subjected to an analysis of variance[10] and significance is determined by an F test[9]. The dose of permethrin (*cis:trans* ratio 41.9:53.6) employed was 80 ng/fly ($LD_{99.9}$ of the susceptible strains). Three and four replications were used for the experiments reported in tables 1 and 2, respectively.

Results and discussion. The dose-response relationships of permethrin to the *ac;ar;ye* and Learn PY-R strains and their F_1 offspring (*ac;ar;ye* ♀ × Learn PY-R ♂) are shown in the figure. They indicate that the level of resistance to permethrin in the Learn PY-R strain was greater than 6000-fold by comparison to the susceptible (ac;ar;ye) strain.

Resistance in the F_1 is seen as nearly co-dominant with respect to the parental strains. In similar tests involving reciprocal crosses between the susceptible (NAIDM) and the Learn PY-R strain the LD_{50} values of the F_1 progeny (Learn PY-R ♀ × NAIDM ♂ and NAIDM ♀ × Learn PY-R ♂) were not significantly different (870 and 833 ng, respectively), indicating the absence of cytoplasmic influences on the level of resistance.

Table 1. Factorial analysis of permethrin resistance effects on chromosomes 1, 2 and 4 using *ac*; *ar*; *ye* multichromosomal marker strain

Chromosome	Effect	Degrees of freedom	Mean square	F value
1	98.73	1	3655	203.3**
2	31.21	1	365.3	20.32**
4	6.61	1	16.4	0.91
1+2	6.33	1	15.0	0.84
1+4	−10.39	1	40.5	2.25
2+4	2.77	1	2.88	0.16
1+2+4	1.89	1	1.34	0.07
Error		7	17.98	

** Significant at the 1.0% level.

Table 2. Factorial analysis of permethrin resistance effects on chromosomes 1, 2, 3 and 5 using the *ac*; *ar*; *bwb*; *ocra* multichromosomal marker strain

Chromosome	Effect	Degrees of freedom	Mean square	F value
1	152.5	1	5816	57.96***
2	98.4	1	2421	24.13***
1+2	−9.02	1	20.3	0.20
3	258.64	1	16724	166.69***
1+3	−11.32	1	32.0	0.32
2+3	−76.42	1	1460	14.55***
1+2+3	−33.08	1	273.6	2.73
5	4.84	1	5.9	0.06
1+5	−8.94	1	20.0	0.20
2+5	−37.3	1	347.8	3.47*
1+2+5	−3.52	1	3.1	0.03
3+5	−16.3	1	66.4	0.66
1+3+5	−56.9	1	808.3	8.06**
2+3+5	23.6	1	139.2	1.39
1+2+3+5	0.06	1	0	0
Error		15	100.33	

* Significant at the 5.0% level. ** Significant at the 1.0% level. *** Significant at the 0.1% level.

The results of genetic analysis of the 'heterozygous' resistance effects are shown in tables 1 and 2. In the tests reported in table 1, the *ac;ar;ye* strain was utilized in order to examine the contribution to resistance by factors on chromosomes 1, 2 and 4. One or more factors on chromosome 1 contribute the largest part of the resistance, and factors on chromosome 2 also play a significant role. Table 2 shows the analysis of chromosomes 1, 2, 3 and 5 using the *ac;ar;bwb;ocra* strain. In this case, the overall effects of the chromosomes were 3 > 1 > 2. As no pyrethroid resistance factor on chromosome 1 has previously been detected[14], this constitutes the first report of such a gene or genes. Use of the esterase inhibitor DEF (S,S,S-tributyl phosphorotrithioate) with permethrin had no significant effect on the resistance level of the Learn PY-R strain. However, piperonyl butoxide (PB), a mixed function oxidase (MFO) inhibitor, decreased resistance to a 33-fold level (w/PB in the figure), thus implicating increased oxidase activity (MFO) as a resistance mechanism. One or more additional resistance mechanism(s) are presumed to be responsible for the 33-fold resistance remaining after synergism by PB. This could be 'target site insensitivity' (i.e. *kdr* or super *kdr*), a mechanism known to exist in house flies in Europe (*kdr*[4] and super *kdr*[11]) as well as in the US (*kdr*[12,13]). Farnham has shown that resistance to pyrethrins was due to mechanisms on chromosomes 2, 3 and 5[4]. The mechanisms on chromosome 3 were shown to be reduced penetration *(pen)* and target site insensitivity *(kdr)*. The pyrethrin resistance mechanism on chromosome 5 *(py-ses)* was suppressed by sesamex while the mechanism on chromosome 2 *(py-ex)* was detected only with sesamex-synergized pyrethrins[4]. It seems reasonable that target site insensitivity with or without *pen* may be the mechanism(s) which we detected on chromosome 3. This implies that the factors on chromosomes 1 and 2 control a mechanism(s) which can be suppressed by PB (i.e. increased oxidase activity). This contrasts with the mechanism described by Farnham on chromosome 2 *(py-ex)*, but is consistent with other reports of genes for increased oxidase activity on chromosome 2[19,20]. No significant 'heterozygous' effect was detected on chromosome 5 of the Learn PY-R strain. However, chromosome 5 does not appear to be inert with regard to resistance since weak but significant interactions were observed in certain combinations with other chromosomes (table 2). It is quite possible that a nearly completely recessive factor may exist on this chromosome.

Although there are three possible types of interactions between insecticide resistance mechanisms (less-than-additive, additive or greater-than-additive)[15], only additive[16] or greater-than-additive[16-18] effects have been previously reported. Our data show that there was a significant less-than-additive effect among three combinations of chromosomes (table 2). One possible explanation for this less-than-additive effect would be that different genes control detoxification enzymes with overlapping action.

From our data it appears that the genetics of resistance to permethrin is different from the genetics of resistance to the natural pyrethrins. However, to fully characterize the resistance found in the Learn PY-R strain it will be necessary to also examine the 'homozygous'[8] effect of each chromosome. Such studies are now underway and will be reported at a later date.

1 The authors would like to express their sincere thanks to Professor M. Tsukamoto, University of Occupational and Environmental Health, Kitakyushu, Japan, for his critical reading of the manuscript.

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0014-4754/84/121416-03$1.50 + 0.20/0

Sexual behavior without adult morphogenesis in *Locusta migratoria*

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Summary. Injection of azadirachtin into young Vth (= last) instar *Locusta* hoppers inhibits molt and a considerable portion of the animals survive for more than 40 days as 'over-aged nymphs'; in contrast, the duration of the Vth instar is 8–10 days in normal controls. Males of over-aged nymphs exhibit adult sexual behavior, and injection of juvenile hormone intensifies this behavior. The results demonstrate that the 'terminal' molt leading to morphogenetic adult differentiation is not necessary for an adult behavioral pattern to develop, and/or to become overt, in a hemimetabolous insect.
Key words. Azadirachtin; sexual behavior; ontogenetic relations between morphology and behavior; juvenile hormone; *Locusta migratoria.*

Azadirachtin, extracted from neem seeds[2–4], acts as an anti-feedant and/or a growth regulator in many insects. It inhibits feeding, disrupts growth, delays or inhibits molt, interferes with completion of the molt and/or induces morphogenetic disturbances in various species[2, 5–12], and often causes high mortality. It also reduces ecdysteroid titre in the hemolymph[12–14]. We injected azadirachtin into Vth (= last) instar *Locusta* hoppers, in order to inhibit the molt and to obtain 'over-aged' nymphs. Then we assessed the male mating behavior of the over-aged nymphs in order to investigate whether the ontogenetic development of an adult behavioral pattern depends on overt morphogenetic adult differentiation, and to what extent adult behavior is correlated with adult morphology.

Crowded nymphs of *Locusta migratoria migratorioides*, from the Jerusalem stock culture[15], were kept under previously described conditions[16]. Azadirachtin, kindly supplied by Prof. H. Rembold, was injected in 2 µl ethanol 90%, through the lateral side of the abdomen, into Vth (= last) instar nymphs, 2–8 h after the molt to this instar.

Following injection of 1.6 µg azadirachtin per nymph, 2 major kinds of mortality were observed (table). A portion of the nymphs showed markedly delayed apolysis and actually started to molt, but were unable to shed the exuvia and died in the course of this unsuccessful molt. Such 'death in molt' became less frequent after the age of 30 days (here and below the age refers to the time elapsed after the molt to the Vth instar) and did not occur after 40 days. Another portion of the animals died as Vth instar nymphs, either without apolysis, or after apolysis but without attempting to molt. This kind of mortality, termed 'death not in molt', was common up to the age of 20 days, but became less frequent later, though it occurred up to 50 days and even beyond this age (see footnotes to table). In spite of all the mortality, about 25% (table, exp. 1) or 48% (table, exp. 2) of the locusts survived more than 20 days as over-aged Vth instar nymphs and respectively 16% and 20% of them survived more than 40 days in this state; the duration of the Vth instar was 8–10 days in the controls. The last azadirachtin-injected nymphs, out of those observed for their whole life span, died at the age of 69 and 71 days, not in molt. This age approaches the life span of normal adults[17, 18]. Very recently Sieber and Rembold[12] described the lethal and molt delaying/inhibiting effects of azadirachtin injected into IVth and Vth instar *Locusta* hoppers. Their presentation of the results is different from that employed by us in the table (our aim was to obtain a maximum number of over-aged nymphs for behavioral studies), but insofar as the findings are comparable, they agree well. The dose of 1.6 µg azadirachtin was optimal for obtaining the highest proportion of over-aged Vth instar nymphs. With lower doses of 1.2 and 0.8 µg azadirachtin, the proportion of nymphs showing death in the molt markedly increased, and with 0.4 µg about 45% of the nymphs completed the moult to normal adults. Higher doses, 3.2 and

Mortality and effect on delay/inhibition of the molt caused by injection of 1.6 µg azadirachtin (in 2 µl ethanol 90%) to 2–8-h-old Vth instar *Locusta* nymphs*. In parenthesis: % of No. treated

Experiment	No. treated	No. died in molt within the age of			No. died not in molt within the age of				No. survived as Vth instar nymph over 40 days
		9–20 days	21–30 days	31–40 days	1–10 days	11–20 days	21–30 days	31–40 days	
1	24	1 (4.2)	2 (8.3)	0 (0.0)	14 (58.3)	3 (12.5)	0 (0.0)	0 (0.0)	4** (16.7)
2	115	8 (7.0)	22 (19.1)	2 (1.7)	24 (20.9)	27 (23.5)	3 (2.6)	6 (5.2)	23*** (20.0)

* Out of 24 controls injected with 2 µl ethanol, 23 molted to normal adults within the age of 8–10 days and 1 died at the age of 3 days; ** 1 died at the age of 42 days, 2 at the age of 69 days and the last one at the age of 71 days; none of these deaths occurred in the molt; *** 9 died between the age of 41–48 days, none of them in the molt. The remaining 14 were not followed over 48 days.