time of hatching was only observable during the first 5 minutes when 57.5 percent males hatched as compared with a 49.6 percent observed for the overall pattern. Although the number of larvae reared for the Lagos strain was only 880 for each time interval, this appears to be sufficient to indicate the existence of some variation between strains for this phenomenon.

The water used in hatching was tested in order to insure uniformity. The dissolved oxygen was 1.06 ± 0.52 ppm, the carbon dioxide was 0.61 ± 0.2 ppm, the temperature was 27.0 ± 0.3° C., and the number of ions was less than 0.2 ppm.

Summary. In laboratory rearing of *Aedes aegypti*, eggs containing male larvae hatched somewhat faster than female. This early rate of hatching of larvae resulted in a predominance of males in the early hatch and females in the later. The results indicated the possibility of some strain variation for this phenomenon.

The majority of the eggs hatched between 10 and 40 minutes after being submerged in the de-oxygenated water. Ninety-six percent of eggs hatched when exposed for 60 minutes.

No significant mortality was observed in the larvae hatched during any specific time interval.

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INSECTICIDE-RESISTANT MOSQUITOES IN CALIFORNIA 1949–1961

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Although chemical control has been an integral part of mosquito abatement in California since the first districts were formed in 1915, resistance problems did not assume prominence until after the introduction of synthetic organic compounds in 1946. At this time DDT began to replace paris green, cresylic acid, the botanicals, and petroleum distillates.

DDT proved to be so effective as a mosquito larvicide that it appeared a panacea in chemical control had been achieved. The intense spray schedules carried out by abatement districts soon revealed, however, that DDT was not the ultimate in chemical control. In some cases only two seasons' control was obtained before resistance problems were encountered.

The first report of DDT resistance in California mosquitoes was by Gordon F. Smith (anonymous, 1949.) Despite several increases in dosage rate, the material was failing to control *Aedes* and *Culex* larvae in Kern County.

Bohart and Murray (1950) confirmed through laboratory tests that larvae of *Aedes nigromaculis* Ludlow had developed resistance to DDT in Tulare County.

Lindane, toxaphene, aldrin, and dieldrin were substituted in larviciding programs by districts that encountered difficulty in achieving control with DDT.

Gjullin and Peters (1952) reported on the degree and extent of resistance to DDT, lindane, toxaphene, and aldrin in several species of mosquitoes in the San Joaquin Valley. In general, it appeared that all chlorinated hydrocarbons were becoming ineffective for larviciding operations. The development of multiresistant strains precluded the further use of chlorinated hydrocarbons by many districts.

Even though mosquito larvae have developed rather extensive resistance to chlorinated hydrocarbons, some districts are still able to maintain control with these materials, particularly in the coastal areas.

Organophosphate insecticides have replaced chlorinated hydrocarbons where resistance problems have become severe. Gjullin, Isaak, and Smith (1953) demonstrated the effectiveness of EPN and other organophosphates in the control of chlorinated hydrocarbon resistant *A. nigromaculis* and *Culex tarsalis* Coq. Ultimately, parathion and malathion became the insecticides of choice in most mosquito abatement districts.

Malathion resistance was first detected in *C. tarsalis* collected in Fresno County by Gjullin and Isaak (1957). After two and one-half years of use, both larvae and adults exhibited considerable resistance to this compound.

Parathion failures against *A. nigromaculis* in the Kings Mosquito Abatement District near Hanford were confirmed in laboratory tests reported by Lewallen and Brawley (1958), Lewallen and Nicholson (1959). Parathion had been used for six years in the control program before resistance developed to a level sufficiently high to interfere with control. Even higher levels of parathion resistance have subsequently been found in *A. nigromaculis* larvae from Tulare County, with a resistance of the magnitude of 660 times being reported (Lewallen, 1961).

DETECTION OF RESISTANCE. When chemical control failures are noted in the field, physiological resistance is not always necessarily responsible. In treating water with a dense overgrowth of vegetation, spray applications sometimes fail to penetrate effectively, resulting in small amounts of toxicant reaching the larval source. Granular formulations are useful in overcoming this difficulty (Whitehead, 1951; Mulla, 1960; Rai and Lewallen, 1960).

Frequently pastures are irrigated in sections so that all stages of *A. nigromaculis* are present from one side of a field to the other. If the entire field is treated with a single application of insecticide, the more tolerant stages (eggs, pupae) survive, particularly with easily hydrolyzed organophosphates. Subsequent emergence of adults makes it appear that the material has failed to control the larvae to which it presumably was applied.

Inadequate coverage and sublethal concentrations can lead to control failures which may be interpreted by some observers as evidence of resistance.

Water with much organic matter, high alkalinity, mats of algae, dense vegetation, extremes in temperature and other factors may interfere with the action of toxicants.

Faulty materials account for some failures. Malathion, for example, must be stored in specially lined drums to prevent deterioration.

Generally, field evidence alone is not sufficient to substantiate the presence of resistance in mosquitoes since the variables mentioned above can result in misleading interpretations.

CROSS-RESISTANCE. DDT-resistance in *C. tarsalis* and *A. nigromaculis* does not induce immediate resistance to lindane, toxaphene, aldrin or dieldrin, but resistance to these compounds appears to develop more rapidly if DDT-resistance has occurred first. Some species of mosquitoes exposed only to dieldrin with subsequent development of dieldrin-resistance, remain susceptible to DDT (Brown, 1958).

Most of the work done on characterizing chlorinated hydrocarbon resistance has been done on the house fly. Chlorinated hydrocarbon resistance in the house fly is manifested in two specific types that are genetically distinct (Busvine, 1954; Metcalf, 1955; Kearns, 1955). One type confers resistance to DDT and its analogs; the other type confers resistance to lindane, dieldrin and other cyclodiene compounds. This separation into two distinct types also applies to mosquitoes (Brown, 1960).

Resistance to chlorinated hydrocarbons does not confer cross-resistance to organophosphorous compounds. House flies that develop a high level of organophosphorous resistance also acquire a cross-resistance to chlorinated hydrocarbons. This phenomenon appears to happen even when there has been no previous history of treatment with chlorinated hydrocarbons (Brown, 1960).

This relationship does not necessarily hold for mosquitoes, as evidenced by findings on the malathion-resistant strain of *C. tarsalis* from Fresno. Malathion was used against this strain because high levels of chlorinated hydrocarbon resistance had developed. Despite pressure from malathion applications, the chlorinated hydrocarbon resistance decreased (Lewallen, 1960).

When resistance has developed to a given organophosphate, cross-resistance to other organophosphates is not necessarily conferred. The malathion-resistant strain of *C. tarsalis* remains susceptible to parathion and other organophosphates (Gjullin and Isaak, 1957). Parathion-resistant *A. nigromaculis* remain susceptible to methyl parathion and other organophosphates (Lewallen and Nicholson, 1959).

NATURAL RESISTANCE VS. ACQUIRED RESISTANCE. In assessing the value of a toxicant for the control of mosquito larvae, it is advantageous to obtain information on more than a single species since the response to synthetic organic compounds varies considerably with different species. A case in point concerned the promotion and development of DDVP as a mosquito larvicide based on information obtained from *Culex* sp. only (Isaak, 1957; McFarland, 1957). Although the dosage recommended for the control of mosquito larvae was adequate for *Culex* sp. it was found that several times the recommended dosage was required for control of *A. nigromaculis* (Lewallen, 1958b). The material proved to be too expensive for economical application at the dosage required. This expression of natural resistance is distinct from acquired resistance where the toxicant works well at first, becoming ineffective after continued usage.

DETERMINATION OF RESISTANCE LEVELS BY LABORATORY TESTS. In order to establish the degree of resistance in a strain, a base line for comparison must be established. Usually this is done by obtaining information on the response of a susceptible strain and comparing the results with those obtained on the suspect resistant strain. The magnitude of resistance will depend on the susceptibility level of the strain used for comparison.

Considerable variation in susceptible strains has been noted. The susceptible strain of *C. tarsalis* maintained at the United States Department of Agriculture laboratory at Corvallis, Oregon, is about ten times more susceptible to DDT than the susceptible strain maintained at the Bureau of Vector Control laboratory at Fresno. The LC_{50} and LC_{90} for DDT in the Corvallis strain is 0.003 ppm and 0.01 ppm, respectively (Eddy *et al.,* 1958). At Fresno the LC_{50} is 0.03 ppm and the LC_{90} equals 0.129 ppm.

In establishing base lines for resistance studies of *A. nigromaculis* it has been necessary to field-collect susceptible strains, since this species has not been colonized. Mosquito abatement and agricultural pest control have become so intense in California that it is difficult to collect material in areas free from insecticide influence. As an illustration, the Pinedale strain of *A. nigromaculis* used as a base line in

1958 for parathion-resistance studies (Lewallen and Brawley, 1958; Lewallen and Nicholson, 1959) has since that time been under treatment with parathion for two seasons. No data were obtained on this strain in 1960 but parathion susceptibility tests were made in 1958, 1959, and 1961. Results of the three years' tests are shown in Table 1 which shows a four-fold LC_{50}

TABLE 1.—Susceptibility levels to parathion of *Aedes nigromaculis*, Pinedale, 4th instar

Year	LC_{50} in ppm	LC_{90} in ppm
1958	0.000035	0.0001
1959	0.000045	0.00013
1960	..	..
1961	0.00013	0.0008

and eight-fold LC_{90} decrease in susceptibility level between 1959 and 1961.

SUBSTITUTE INSECTICIDES FOR RESISTANT STRAINS. The substitution of organophosphates to control chlorinated hydrocarbon-resistant mosquitoes has been complicated by subsequent resistance to the substitute compounds (Gjullin and Isaak, 1957; Lewallen and Nicholson, 1959). Although it appears that continued substitution of other organophosphates will be successful in regaining control, the question remains as to how long the substitutes will remain effective. Methyl parathion has given satisfactory control of parathion-resistant *A. nigromaculis* for two seasons (Lewallen, 1960).

In developing new insecticides the trend has been toward organophosphates; consequently, a large variety of compounds of this type is available to select from in a substitute insecticide program. Relying so heavily on one type of insecticide may impose serious limitations eventually upon the success of substitutes in chemical control programs. For this reason increased attention has been focused recently on the development of other types of compounds. Carbamate insecticides have been tested against mosquito larvae in California with promising results (Mulla *et al.*, 1960). The ease with which resistance develops to this type of compound in house flies, however, may be indicative of its limitations in mosquito control (Georghiou *et al.*, 1961; Georghiou and Metcalf, 1961).

Chrysanthemumic acid esters have also been tested against various species and strains of mosquitoes in California (Gjullin and Lewallen, 1958; Lewallen and Gjullin, 1960). Economic considerations based on necessary high rates of application have restricted the use of this type of material for mosquito control.

RESEARCH ON NEW COMPOUNDS FOR RESISTANT STRAINS. Most of the work on compounds designed specifically against resistant strains has been directed toward studies on the house fly. Cetyl bromoacetate has been reported to be more effective against DDT-resistant house flies than against a susceptible strain (Ascher, 1957, and 1958).

Bold, new approaches such as the search for a negatively correlated insecticide as cited above, the use of chemosterilants (LaBrecque *et al.*, 1960), and application of mitotic poisons (Mitlin *et al.*, 1957) must be pursued if we are to keep ahead of the development of resistant strains. Another possibility that offers promise in the attempt to develop new insecticides for mosquito and fly control may be found in the synthesis of materials similar in nature and action to naturally occurring insect hormones. The feasibility of this approach has been pointed out previously (Knipling, 1958; Lewallen, 1958). Work on naturally occurring hormones has progressed to the point where structures are near the point of elucidation (Karlson, 1959). Naturally occurring hormones have already been demonstrated as having insecticidal potential (Williams, 1956; Gilbert and Schneiderman, 1958).

Based solely on empirical formulae, attempts have been made to synthesize molecules similar in structure to the molting hormone in our laboratory. The literature has indicated that some of the insect hormones may be steroids (Gilbert and Schneiderman, 1958). Oddly enough, one of the materials synthesized that had a fair degree of insecticidal activity was an aliphatic ester. Since the basis for forming this compound was derived solely from empirical considerations, it is not known whether the activity observed was hormonal or not. Tests were performed on mosquito larvae, and a fairly high degree of mortality was observed. Even if the compound does not approximate a naturally occurring growth regulator, it represents an entirely new type of compound that exhibits insecticidal properties that may be improved by a study of synthesized analogs. Details of the synthesis and experimental results will be published elsewhere.

Although there have been numerous setbacks in the chemical control of mosquitoes due to the development of resistance, by and large it has been possible to cope with the problem through substitute insecticides. With the threat of development of resistance to materials now in use against mosquitoes, as well as other arthropods of public health importance, research agencies are hard at work to extend the present array of insecticides. Only through sustained, penetrating research on this problem can the challenge offered by the perplexing problem of resistance be met, and the high standards of mosquito control technology be maintained.

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STUDIES ON THE INHERITANCE OF RESISTANCE TO DDT AND TO MALATHION IN THE MOSQUITO, *CULEX TARSALIS* COQ.

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A colony of *Culex tarsalis* Coq. highly resistant to DDT was collected from a log pond at Oak Ridge, Oregon, in 1956 and has been maintained at the Corvallis laboratory. Data on the amount of resistance to DDT present in the colony and on rearing techniques have been reported (Eddy *et al.* 1958).

A malathion-resistant strain of *tarsalis*, collected near Fresno, Calif., in 1957 is also being reared in the laboratory. The colony was about 100 times resistant to malathion at the time these experiments were undertaken and also about 2 times resistant to DDT. Initially it was about 75 to 80 times resistant to malathion and 5 times to DDT. The extent of cross-resistance to other insecticides present in this colony has been reported (Darrow and Plapp 1960).

In the present work the inheritance of insecticide resistance in these colonies was studied. Data are also presented on levels of cholinesterase (ChE) and aliphatic esterase (Ali-E) activity in the same colonies. Measurements of enzyme levels in resistant strains of insects have been of special interest since the finding that low levels of aliesterase are related to organophosphate resistance in the house fly (*Musca domestica* L.) (Asperen and Oppenoorth 1959, Bigley and Plapp 1960).

MATERIALS AND METHODS. Insects.—The DDT-resistant colony from Oak Ridge and the malathion-resistant colony from Fresno were the two resistant colonies used in these experiments. The insecticide-susceptible colony was obtained from the Rocky Mountain Laboratory, Hamilton, Mont., and has been reared for several years in the laboratory without exposure to insecticides. For the sake of brevity, the three colonies will be referred to as DDT-R, Mal-R, and Reg, respectively.

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