BIOLOGICAL DIFFERENCES BETWEEN TWO GEOGRAPHICALLY ISOLATED, INSECTICIDE-SUSCEPTIBLE, HOUSE FLY (*MUSCA DOMESTICA* L.) POPULATIONS

by

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A THESIS

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MASTER OF SCIENCE

Department of Entomology

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INTRODUCTION

Numerous studies have been conducted on resistance of house flies to insecticides, and a few have considered biological characteristics associated with various resistant strains. Studies on biological characteristics have included differences in the length in life history stages. Metcalf (1955) in summarizing numerous studies by various workers using several different strains, concluded that "there is little evidence of positive correlation between the biological factors responsible for biotic potential or vigor and specific insecticide resistance". Alterations in the biology of an insecticide-susceptible strain of house flies have been demonstrated after one treatment with dieldrin (Afifi and Knutson, 1956). Ouye and Knutson (1957), using the same insecticide-susceptible strain, found reproductive potential and longevity to be altered following treatments of the larvae with malathion.

Most of the strains reported in the literature with respect to biological characteristics have been laboratory strains, sometimes laboratory-selected, and nearly all had been removed from the field for many generations. This study was initiated to determine how much biological variation occurs naturally between two insecticide-susceptible, isolated, field populations.

MATERIALS AND METHODS

After considerable search, two isolated populations of insecticide-susceptible house flies were located. One was collected near Ellsworth, Kansas, northeast of the Kanopolis dam, on a farm at least one and one-half miles from the nearest adjacent house fly breeding place. This population is designated as the Ellsworth population. Numerous small house flies were

detected within this collection; determination was verified by C. W. Sabrosky, U. S. Department of Agriculture, Washington, D. C. The other population was collected near Wilmore, Kansas, on a farm at least one mile from the nearest adjacent house fly breeding place. This population is designated as the Wilmore population.

The last possible contact with insecticides by the Ellsworth population was 1951, when DDT and chlordane were used on range cattle for biting flies, outside the barns, but not within and around the sheds where much residual deposit could have affected house flies. During the past two or three years the barn had been used almost exclusively to raise young calves. No spraying with insecticides had been done at the Wilmore location for at least ten years.

A sample consisting of several hundred flies of each of the two populations, which included most of the adult flies available at time of collection, were brought to the laboratory. The investigation was conducted in an insecticide-free room with a constant temperature of 80° ± 2° F.

The Ellsworth population had an LD_{50} of 3.7 µg per fly and the Wilmore 2.7 ug per fly, as determined by topical application with DDT. This indicated virtually no resistance in both populations as indicated by an LD_{50} of 3.0 in the KUN non-resistant strain maintained in the laboratory.

Mass rearing of both populations was done in order to obtain a large number of flies. The females seemed reluctant to lay on the usual water-soaked cloth, so evaporated milk (½ milk to ½ water) was used to induce oviposition. Eggs were placed in 10 pound waxed paper cheese tubs containing standard CSMA medium. Approximately 1,650 cc. of dry medium was mixed with 32 oz. of water, one package of dry yeast, and 1.5 oz. of diamalt for each tub. Five-tenths ml. of eggs (approximately 3,333 eggs) was then planted

in each tub. Twenty-six tubs were used for the Ellsworth population and 36 tubs were used for the Wilmore population. After eight days, the resulting pupae were separated from the media. These pupae were then placed in cages 10" x 10" x 10". The cages were made with screened tops and sides, a sliding glass panel for a front, and rubber back with a hole through which the arm could be thrust to facilitate feed changing, removing eggs, and other manipulations.

Twenty-four hours after the resulting flies emerged, they were transferred by a moving air stream into one quart cylindrical cardboard cartons with screened ends in preparation for sexing. Carbon dioxide anaesthesia was used to facilitate handling and sexing. The use of CO_2 anaesthesia was kept at a minimum, well within the limits indicated by Williams (1946).

Five hundred males and 500 females were transferred into each cage. Twelve replications of the Ellsworth population were used. Only five replications of the Wilmore population were used since that was all the adults that were available.

Food and water were changed daily. The food consisted of a mixture of one volume granulated sugar to two volumes of powdered milk. Oviposition site and water source consisted of crystallization dishes, 70 mm. in diameter by 50 mm. high, in which cork covered with muslin four inches by six inches was floated on water.

Longevity was determined by making a daily record of the number of dead male and female flies in the cages. They were dragged out of the cages with a looped wire as the front glass panel was raised high enough to permit entry of the wire.

Egg production was recorded daily for each cage. The eggs, which had been deposited on muslin, were washed off with a stream of water from a

polyethylene wash bottle into a 25 ml. beaker. The eggs were concentrated by decanting, then were transferred to a 15 ml. graduated centrifuge tube and measured volumetrically. The volume of eggs was converted to numbers, using 0.3 ml. equals 2,000 eggs ± 180. When eggs were too few to measure volumetrically, actual numbers were counted and recorded.

After egg counts from each cage were recorded each day, the eggs from the replicates were thoroughly mixed. To determine hatchability, moistened blotter paper was fitted into each of three petri dishes. Two hundred eggs from the combined collection were placed in each dish in groups of ten eggs to facilitate counting. The blotter papers were kept saturated by adding water at intervals. Records of hatched and unhatched eggs were made at 24 and 48 hours.

To determine pupation and emergence, approximately 2,000 eggs, taken at random from each day's combined egg collection, were planted in standard CSMA fly larva media in ten pound waxed paper cheese tubs and covered with muslin. Eight days following planting of each daily batch of eggs, the pupae were separated and counted. The pupae from each daily batch of mixed eggs were then placed in cages without food and water. After all flies had emerged and died, the number of emerged flies were sexed and recorded. In a few cases some of the larvae were held until all pupation had occurred.

For weight studies, the adults were sexed and placed in petri dishes in a hot air oven and desiccated at 100°C. for 72 hours. They were then placed in calcium chloride desiccating containers. When cooled, they were weighed with a Christian Becker Chainomatic balance. The dishes with flies were then replaced in the hot air oven and the process repeated for 12 hours to derive a constant weight. It was necessary, in some instances, to combine flies for two or more consecutive days, in order to have sufficient numbers for weighing.

For statistical analysis of egg production, the number of eggs from each replicate was cumulated each successive day. Eggs from both strains were then analyzed for significance by the Wilcoxon (1945) and Mann and Whitney (1947) ranking test. A similar analysis was made for female fly-days (the cumulative number of females remaining alive each day) and for average number of eggs per female fly-day. This figure on any given day was derived by dividing the cumulated egg total by the respective female fly-day.

Hatchability, pupation and emergence data were analyzed for significance by chi-squaring the weekly totals and the 24 day summary. A similar procedure was followed for analyzing the potential adult progeny.

In the weight studies, the flies were analyzed for significance by the "Student's t-test".

RESULTS

Egg Production

Table 1 indicates that the cumulative egg production in the Ellsworth population was 78 per cent greater during the first nine-day period, but 81 per cent less during the second nine-day period, and 72 per cent less during the third nine-day period, compared to the Wilmore population. Total eggs of the Ellsworth population exceeded the Wilmore population by five per cent, over the entire 27 days of the parents' life span, which was not statistically significant. Statistical analysis indicated egg-producing superiority in the Ellsworth population from the fifth through the tenth day. From the eleventh day through the twenty-seventh day, no significant difference between the two populations was indicated.

Table 1. Cumulative egg production of flies from Wilmore (W)[a] and Ellsworth (E) house fly populations during successive periods of parents' lives.

Successive Periods, Days		Cumulative Total Eggs by Periods	Conclusions	Per cent E/W	Per cent Total[b]
1– 2[c]	E	1,805		178	0
	W	56,424			3
3– 9	E	808,922	E > W[d]	178	46
	W	455,314			27
10–18	E	842,247	E = W[e]	82	48
	W	1,029,406			62
19–27	E	91,390	E = W	72	5
	W	126,863			8
Total	E	1,744,364	E = W	105	
	W	1,668,007			

(a) Converted to 12 replicates to be comparable with (E).
(b) Combined totals of eggs by periods, indicating relative importance of each period in productive life.
(c) Eggs layed by (E) during first and second day were not sufficient in number to allow statistical analysis.
(d) E = W the third, fourth and sixth day, E ≅ W the seventh day, E > W the fifth, eighth and ninth day.
(e) E > W the tenth day, E = W the eleventh to twenty-seventh day.

Longevity

These data were obtained by cumulating the totals of females remaining alive each day and then grouping them in successive nine-day periods. Table 2 indicates that the Ellsworth population exceeded the Wilmore population by 39, 23 and 15 per cent for the first, second, and third nine-day periods, respectively. Statistically, the Ellsworth population exceeded the Wilmore population for the first 25 days. By the twenty-sixth and twenty-seventh days, however, there was little or no significant difference.

Table 2. Longevity expressed as cumulative female fly-days. Wilmore (W)[a] and Ellsworth (E) populations during successive nine-day periods of the parents' lives.

Successive Periods, Days		Cumulative Female Fly-Days	Conclusions	Per cent E/W
1- 9	E	65,869	E≫W	139
	W	47,453		
10-18	E	104,267	E≫W	123
	W	85,046		
19-27	E	116,817	____[b]	115
	W	101,546		

(a) Converted to 12 replicates to be comparable with (E).
(b) E > W from twenty-second through twenty-fifth day; E ≅ W twenty-sixth and twenty-seventh day.

Cumulative Average Number of Eggs Per Female Fly-Day

To obtain these data, the cumulated eggs totals (Table 1) were divided by the corresponding parent female fly-days (Table 2). These data (Table 3) indicate the Ellsworth population to have been 14 per cent greater during the first nine-day period than the Wilmore population. However, during the second nine-day period the Ellsworth egg production was only 88 per cent of the Wilmore egg production, and during the third nine-day period the Ellsworth production was 91 per cent of the Wilmore production.

Individual daily data, as indicated in footnote (b) of Table 3, showed the Wilmore population to have averaged significantly more eggs per female fly-day through the fourth day, but this difference disappeared by the fifth day. At the end of the third nine-day period, there was no significant difference between the two populations.

Table 3. Cumulative average number of eggs per female fly-day for Wilmore (W) and Ellsworth (E) house fly populations during successive periods of the parents' lives.

Successive Periods, Days		Number of Eggs	Conclusions	Per cent E/W
4– 9[a]	E	12.3	E=W[b]	114
	W	10.8		
10–18	E	15.9	E=W	88
	W	18.1		
19–27	E	15.0	E=W	91
	W	16.4		

(a) Insufficient number of eggs layed before fourth day by the Ellsworth flies for statistical analysis.
(b) W>E through fourth day.

Correlation Between Data on Cumulative Egg Production, Female Fly-Days and Cumulative Average Number of Eggs Per Female Fly-Day

The relationship between egg production, longevity, and average number of eggs per female fly-day is attained by comparing Tables 1, 2 and 3. Table 1 shows the cumulative egg production in the Ellsworth population to be significantly (78 per cent) greater than the Wilmore population at the end of the first nine-day period, but there was no significant difference between the two populations during the last two nine-day periods, indicating a much greater egg production of the Ellsworth population earlier in life, and of the Wilmore population later in life. Table 3 shows the two groups to be approximately equal by the end of their productive life, but with some tendency for the Ellsworth population to exceed the Wilmore population during early life, and the reverse to occur later in life. Table 2 shows the Ellsworth population to be much greater in number of females available to lay

eggs than the Wilmore population especially during the first nine-days.

The overall indication is that the Ellsworth population laid a substantially greater number of eggs (Table 1) earlier in life (46 per cent vs. 27 per cent during the first nine-day period) to a slight extent due to the greater number of eggs per female (Table 3), but largely because of the lower death rate during that period (Table 2). By the same token, the Wilmore population exceeded the Ellsworth population in egg production later in life (62 per cent vs. 48 per cent during the second nine-day period) to a slight extent due to a greater production of eggs per female fly-day, accompanied by a lower death rate during that period.

Hatchability, Pupation and Emergence

The data presented in Table 4 were based on a standard number of eggs, and, therefore, represent only differences in percentage of survival in the various life history stages; they do not take into consideration natural differences in egg production between the two groups.

During the first (four to ten-day) period, the progeny of the Wilmore population exhibited a greater survival rate than the Ellsworth population in hatchability (81 per cent vs. 77 per cent). In pupation, the Wilmore population was slightly more than the Ellsworth population (14 per cent vs. 12 per cent). In the adult studies, the Wilmore population exceeded the Ellsworth population (9 per cent vs. 7 per cent).

During the second period, hatchability in the Wilmore population was less than that of the Ellsworth population (74 per cent vs. 79 per cent). In this same period, however, the Wilmore population exceeded the Ellsworth population in number of pupae (28 per cent vs. 12 per cent) and adults (22 per cent vs. 8 per cent).

Table 4. Hatchability, pupation and emergence of Wilmore (W) and Ellsworth (E) house fly populations during successive periods.

Successive Periods, Days		Hatchability: Hatched[a]	Conclusion	Per cent: Hatched	Per cent: Total[b] Eggs
4–10[c]	E[d]	3,234	W>E	77	33
	W	3,384		81	35
11–17	E	3,304	E>W	79	33
	W	3,074		74	31
18–26	E	3,390	E≅W	63	34
	W[e]	3,348		62	34
Total	E	9,928	E≅W	72	
	W	9,806		71	

Successive Periods, Days		Pupation: Pupated[f]	Conclusion	Per cent: Pupation	Per cent: Total Pupated
4–10	E	1,611	W>E	12	25
	W	1,990		14	24
11–17	E	1,696	W>E	12	26
	W	3,858		28	47
18–26	E	3,210	E>W	18	49
	W[g]	2,340		13	29
Total	E	6,517	W>E	14	
	W	8,188		18	

Table 4. (concl.)

Successive Periods, Days		Emergence: Adults[h]	Conclusion	Per cent: Emergence	Per cent: Total Adults
4–10	E	990	W > E	7	21
	W	1,276		9	21
11–17	E	1,130	W > E	8	24
	W	3,032		22	50
18–26	E	2,638	E > W	15	55
	W[i]	1,800		10	29
Total	E	4,758	W > E		
	W	6,108			

(a) Based upon 4,200 eggs the first two periods and 5,400 eggs the third period.
(b) Combined total by periods, indicating relative importance of each period in production life of parents.
(c) No substantial number of eggs layed before fourth day.
(d) Eggs converted from 3,990 to 4,200 to be comparable.
(e) Eggs converted from 3,600 to 5,400 to be comparable.
(f) Based on 14,000 eggs for first and second periods; 18,000 for third period.
(g) Eggs were converted from 14,900 to 18,000 to be comparable.
(h) Based on 14,000 eggs for first and second period, 18,000 for third period.
(i) Eggs converted from 14,900 to 18,000 to be comparable.

During the third period, the hatchability of the two populations was about equal (63 per cent vs. 62 per cent). The pupae and adults were, however, reversed in trend from the first two periods in that the Ellsworth population (18 and 15 per cent respectively) was greater than the Wilmore population (13 and 10 per cent respectively). Overall hatchability was about equal (71 per cent vs. 72 per cent), but the Wilmore population produced significantly more pupae (18 per cent vs. 14 per cent) and adults (13 per cent vs.

10 per cent) than the Ellsworth population.

Total eggs hatched between the Wilmore and Ellsworth populations was about uniformly distributed throughout the three periods (33 vs. 35, 33 vs. 31, and 34 vs. 34 per cant), but the Ellsworth population showed a substantial portion of its pupation (49 per cent) and emergence (55 per cent) to have occurred during the third period, while the Wilmore population showed a substantial portion of the pupation (47 per cent) and emergence (50 per cent) to have occurred during the second period.

It was noted in the pupa study that many small pupae occurred in both populations. These small pupae were measured and found to be 0.17 cm. in diameter compared with 0.27 cm. for the normal-sized pupae. Less than one per cent of these small pupae reached adulthood.

Calculated Total Adult Progeny to Have Been Expected

The ultimate evaluation of the difference between the two populations lies in the potential adult progeny given in Table 5 in which the relative number of eggs produced (Table 1) is multiplied by the relative adult emergence rates (Table 4).

The Ellsworth population was 1.47 that of the Wilmore population during the fourth through the tenth days because of the greater number of eggs laid, rather than because of emergence rates, since the latter was actually seven per cent compared to nine per cent in the Ellsworth population. However, the Wilmore population greatly exceeded the Ellsworth population from the eleventh through the seventeenth days because of the larger number of eggs laid in combination with a much higher emergence rate (22 per cent vs. 8 per cent). Although the Wilmore population exceeded the Ellsworth population from the eighteenth through the twenty-sixth days in eggs laid, the survival

Table 5. Average totals by successive periods of potential number of adult progeny based on actual number of eggs produced by successive periods and corresponding emergence rate. Ellsworth (E) vs. Wilmore (W).[a]

Successive Periods, Days		Total Eggs	Adult Progeny	Conclusion	Per cent E/W	Per cent Adults[b]
1–3[c]	E	2,888	202[d]			>0
	W	148,452	44,198[e]			15
4–10	E	956,024	66,922	E > W	147	47
	W	504,756	45,428			16
11–17	E	625,773	50,062	W > E	30	35
	W	797,628	175,478			61
18–26	E	158,662	23,799	E > W	110	17
	W	216,276	21,628			8
27[f]	E	783	117		13	>0
	W	895	90			>0
Total	E	1,744,364	141,102	W > E		
	W	1,668,007	286,822			

(a) Converted to 12 replicates to be comparable.
(b) Combine total by periods, indicating relative importance of each period in productive life of parents.
(c) Eggs layed first three days not significant to allow statistical analysis.
(d) Actual eggs too few for comparison with (W) population. Adult progeny based upon per cent emergence during subsequent seven days.
(e) Actual eggs too few for comparison on first day. Adult progeny first day was based upon per cent emergence during subsequent seven days. Emergence for first three days was 29.7 per cent.
(f) Based on per cent of above period.

rate (10 per cent vs. 15 per cent) was lower with the overall result that the Ellsworth population exceeded the Wilmore population. The overall result was more than twice that of the Ellsworth population (286,822 vs. 141,102) largely because of higher egg production and adult emergence rates during the first through third and tenth through the seventeenth day period.

Weight Study

The weights of male and female progeny, based upon mean weight for 16 days (Table 6), was not significantly different.

Table 6. Mean weights (in mg.) per 100 male and female progeny for the Ellsworth (E) and Wilmore (W) house fly populations over a 16 day period.

	(E)	(W)	Conclusion
Females	395.3	428.9	E=W
Males	408.6	404.3	E=W

DISCUSSION

The results of this work indicate that a highly significant difference in reproductive potential occurred between these two populations. Evidence of further differences between populations are shown when comparing the data with that of Ouye and Knutson (1957) in which they used an insecticide-susceptible but laboratory-reared strain. Using identical techniques, they found an adult emergence rate of 56 per cent as compared to 10 per cent in the Ellsworth and 13 per cent in the Wilmore population (Table 4).

The work reported herein may explain some of the variations in results of control measures, when presumably the same control techniques were used.

It also emphasizes the innate differences between field populations and between strains, so that correlation between biological variations and such characteristics as resistance need not necessarily be expected.

CONCLUSIONS AND SUMMARY

Laboratory studies of certain aspects of the biology of the house fly, Musca domestica L., were conducted to determine possible differences in the biology of two isolated, insecticide-susceptible populations. The studies were conducted on flies collected directly from the field.

On a daily cumulative total basis superiority in the Ellsworth population appeared from the third day through the ninth day, at which time it was 78 per cent greater. Conversely, the Wilmore population excelled during the second and third day and from the tenth day on throughout the life of the parents. The Ellsworth population produced five per cent more eggs than the Wilmore population.

Longevity of the Ellsworth population on a daily cumulative basis exceeded the Wilmore population for the first 25 days. During the twenty-sixth and twenty-seventh days, the two populations were equal.

The cumulative number of eggs per female fly-day for the Wilmore population averaged more than the Ellsworth population during the first four days but this difference gradually disappeared as the parents grew older.

The overall trend was that the Ellsworth population layed a greater number of eggs earlier in life, with the exception of the first three days. The Wilmore population produced a greater number of eggs very early in life and again later in life, due to a greater number of eggs per female fly-day and because of a lower female death rate for those periods, respectively.

The Wilmore population produced eggs with a greater percentage of hatch-

ability from the fourth to the tenth day of the parents' lives and greater pupation and adult emergence rates from the fourth to the eighteenth day. The total hatchability for the Wilmore population was about equal to the Ellsworth population but the Wilmore population had four per cent greater survival from egg to pupa and three per cent greater survival from egg to adult.

The potential adult progeny to have been expected from actual number of eggs layed, indicated the Wilmore population would have exceeded the Ellsworth population by more than two times because of the higher survival rate of adults from eggs layed during the (1) first three days, and (2) the 11 to 17 day period of the Wilmore parents' lives, which was when a substantial portion of the Wilmore eggs were layed.

Weights were based upon constant dry weight of adult progeny. The mean weight of 100 flies from sixteen days' collection showed the Ellsworth flies (395.3 mg. for females and 408.6 mg. for males) to be about equal to the Wilmore flies (428.9 mg. for the females and 404.3 mg. for the males).

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After considerable search, two isolated populations of insecticide-susceptible house flies were located near Ellsworth and Wilmore, Kansas. The LD_{50} for DDT on these flies was 3.7 ug per fly and 2.7 μg per fly, respectively, indicating little or no resistance.

After mass rearing in the laboratory, the adults were anaesthesized with carbon dioxide, sexed and placed in cages. Five hundred males and 500 females were placed in each cage. Twelve replicates of the Ellsworth population, but only five replicates of the Wilmore population were used as only this number was available because of the small number of flies which could be collected at the Wilmore location.

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Longevity was determined by making a daily record of the number of dead females and converting this to female fly-days (cumulative number of females alive each successive day).

To record egg production in each cage, the eggs which had been oviposited on muslin were concentrated and measured in a 15 ml. graduated centrifuge tube. The volume of eggs was converted to number of eggs since

each 0.3 ml. equals 2,000 eggs ± 180 and daily egg counts were recorded.

The eggs from all replicates were then thoroughly mixed.

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To determine pupation and emergence, approximately 2,000 eggs from mixed eggs of the replicates were transferred daily into standard CSMA fly larvae medium. The resulting pupae and emerging adults were recorded.

For weight studies, the resulting adult progeny was placed in petri dishes, and desiccated for 72 hours. They were then cooled in desiccating containers and weighed. This procedure was found to give a constant weight.

The investigation was discontinued after the parent flies were 27 days old, because egg production was negligible thereafter.

Both populations were treated the same and all data were statistically analyzed for significance.

On a daily cumulative total basis, superiority in the Ellsworth population appeared from the third day through the ninth day, at which time it was 78 per cent greater. Conversely, the Wilmore population excelled during the second and third day and from the tenth day on throughout the life of the parents. The Ellsworth population produced five per cent more eggs than the Wilmore population.

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The Wilmore population produced eggs with a greater percentage of hatchability from the fourth to the tenth day of the parents' lives and greater pupation and adult emergence rates from the fourth to the eighteenth day. The total hatchability for the Wilmore population was about equal to the Ellsworth population but the Wilmore population had four per cent greater survival from egg to pupa and three per cent greater survival from egg to adult.

The potential adult progeny to have been expected from actual number of eggs layed, indicated the Wilmore population would have exceeded the Ellsworth population more than two times because of the higher survival rate of adults from eggs layed during the (1) first three days, and (2) the 11 to 17 day period of the Wilmore parents' lives, which was when a substantial portion of the Wilmore eggs were layed.

Weights were based upon constant dry weight of adult progeny. Two or more consecutive days were combined for significant weighing. The mean weight of 100 flies from sixteen days' collection showed the Ellsworth flies (395.3 mg. for females and 408.6 mg. for males) to be about equal to the Wilmore flies (428.9 mg. for the females and 404.3 mg. for the males).