A COMPARISON OF METHODS OF SELECTING FOR INCREASED
EGG PRODUCTION IN DROSOPHILA MELANOGASTER

by

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A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

Department of Poultry Husbandry

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1948

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INTRODUCTION

"Select" is a word of Latin origin, a combination of _se_, meaning aside and _legere_ meaning to gather. Select is defined by Webster as, "Taken from a number of the same or analogous kind by preference." In the field of animal breeding, Lush (1945) defines selection as, "causing or permitting some kinds of individuals to produce more offspring than other kinds."

In the field of animal breeding man has, consciously or unconsciously, selected his animals on the basis of ornamental or utilitarian requirements or a combination of the two. The difficulties encountered in meeting either the utilitarian or the ornamental standards are not nearly so great as the attempt to combine these two qualities in the same individual. So great is this difficulty, that it has either brought about compromises in many of our standards or has resulted in two sets of standards, one fitted to the ornamental types and one to the economic types.

Although animal breeding is a relatively new science, many of its principles have been practiced for hundreds of years. The Arabian horse and the game cock are examples of early attempts, with a great measure of success, to direct hereditary characters according to man's desires. It is well to note that the Arabian horse was bred for utilitarian purposes, principally speed and stamina, while the game cock was bred only for his ability to fight. The simplicity of the desired goals was probably the

greatest asset in the success of these early attempts. It also might be due to the fact that the desired characteristics were not far removed from those of the original wild stock.

Robert Bakewell, 1725-1795, as reported by Lush (1945), is referred to as the founder of animal breeding. His early work with cattle and sheep brought into play many methods employed by present-day successful breeders. His pioneer work was an example to other breeders of what could be done by combining selection with judicious inbreeding. After this time scientific methods of breeding, whether recognized as such or not, were applied to all kinds of domestic animals.

The problem of improvement of some economic qualities is further complicated by restriction of the expression of the trait to one sex. This is well illustrated by milk production in the dairy cow and egg production in the hen. Not only is the economic quality limited to one sex, but it is also intimately connected with reproduction which brings in additional factors to consider. The external body conformation of meat animals and length and quality of hair and wool in sheep and goats give a fairly reliable estimate of the value of the animal concerned. In the hen and the dairy cow, the individual must be sexually mature before the product becomes available. It is obvious that products as complex in structure as eggs and milk require the action and interaction of many organs, glands, and physiological processes. Since their production is so complex, the inheritance controlling their production is also complex, as has been shown by

numerous experiments in animal breeding. The male adds to the
complexity of the picture because no method has been found to
measure his potentialities in regard to production; yet this is
more important in the case of the male, because he becomes the
parent of more individuals than the average hen or cow.

The simplest method of breeding with the use of records is
the method used by Gowell at the Maine Agricultural Experiment
Station, and described and summarized by Woods (1908). By this
method, females with high production records were mated with males
that were the sons of females with high production records. This
method of individual or phenotypic selection would appear to offer
an excellent basis for increasing the productive ability of the
offspring. However, the experiment, which extended over a period
of nine years, failed to produce a significant increase in the
mean egg production. Unfortunately, the effects of segregation,
recombination, epistasis, and other complicating factors tend to
produce individuals that may vary widely from the expected results.

From the methods of predicting the quality of the progeny,
attention was directed toward testing the progeny to determine
what the parents were actually transmitting. This has proved to
be a reliable method, but the progeny are exposed to many environ-
mental factors which may somewhat confuse the records. Time and
mortality are two factors that tend to reduce the effectiveness
of this method of breeding. The progeny must reach sexual
maturity, and the parents must either be held in reserve, or
used to produce additional offspring of yet unknown quality,
during the period while the first progeny are maturing and are
being tested. This great expanse of time allows mortality to

affect both parents and progeny. Increased age will also affect the reproductive ability of the parents while the test is in operation, and in subsequent matings.

Another method employed in an attempt to compromise between the phenotypic, or individual selection method and the progeny test is the family or sib test. The family test, or sib test, consists of the selection of breeding stock because they belonged to a group of brothers and sisters whose mean performance was better than that of other groups of brothers and sisters in the same generation. The use of sisters' records reduces the time necessary for the progeny test and gives a more accurate measure of the individual's transmitting ability than the production record of the individual in question. This is of value to the poultryman because it avoids the decline in reproductive ability due to increasing age and the ever present possibility of mortality in reserve breeding stock.

In this experiment, the common fruit fly, Drosophila melanogaster, has been used in an attempt to compare the family selection and individual selection methods for increased egg production under conditions simulating those found in a commercial poultry breeding program. The fruit fly was chosen because several generations could be completed in a short period of time and a relatively small amount of equipment is necessary for handling large numbers of individuals.

EXPERIMENTAL PROCEDURE

The source of the flies used in this experiment was wild stock collected at two different points in the vicinity of Manhattan, Kansas in the autumn of 1947. Half-pint milk bottles were used for holding the flies. This type of container proved to be convenient for storage, handling, and observation. The bottles were stored in ordinary milk bottle cases containing thirty bottles each. The cases were kept in a cabinet heated by electric light bulbs and controlled by a mercuromatic thermostat. The bottles were closed by plain, unwaxed, nonperforated milk bottle caps. The food for the females being tested was placed on the caps in the manner described by Gowen and Johnson (1946). The use of caps equipped with pull tabs greatly facilitated changing food and transferring flies without injuring the flies or permitting them to escape.

The food used for collecting eggs was mixed according to the banana formula suggested by Sinnott and Dunn (1939) with approximately 3 g of animal charcoal added to each 100 g of food mixture to provide a contrasting background to facilitate the counting of the eggs. Approximately 1 cc of the food material was placed on each bottle cap by means of a piece of glass tubing which served as a pipette. One drop of a yeast suspension, made by dissolving approximately 0.3 g of yeast in approximately 7 cc of water was placed on each cap with an ordinary eye-dropper. The food is more uniform and easier to handle if the bananas are pushed through a strainer or prepared by one of the other devices suggested by

Demerec and Kaufmann (1945). In order to obtain faster development and a more abundant production of eggs, adult flies kept for breeders were placed in bottles containing slices of banana. The slices averaged about 4 g in weight, and were liberally sprinkled with dry yeast. Small pieces of paper toweling (approximately 3" x 4") were added to absorb the excess moisture. Very thin slices of banana were used at the start and larger amounts were added as soon as the larvae appeared. This method was fairly successful in preventing mold growth. After the larvae are present in considerable numbers, their activity will inhibit mold growth.

The methods and equipment used in handling the flies were essentially the same as those described by Demerec and Kaufmann (1945). A binocular microscope with 10X oculars and 0.7X objective was used for observing etherized flies and counting eggs.

The two groups of original stocks were combined and were multiplied until enough flies were available to supply 125 females of the same age. These 125 females served as the source of both the family selection and the individual selection lines. The flies remaining in the flock bottles after the 125 females were removed, comprised the unselected control group. The latter was maintained as a flock mating in four bottles. They were transferred once each generation and at each transfer the flies were divided among four bottles. As soon as larvae or pupae appeared, the adult flies were released.

After the 125 females were selected they were placed in individual bottles and mated with flock males. This group made

up generation A. Each day the bottle caps were replaced so that
the flies would have fresh food. Egg laying commences on the
second or third day of imago life, rises rapidly to a maximum in
the next few days and then declines at a fairly constant rate until
the fly's death. By using a short period during the time of
maximum egg production, selection could be accelerated and a large
measure of accuracy retained with a great saving in labor. In this
experiment, the eggs laid by each female were counted on the sixth
and seventh day of imago life and the number for the two days was
totaled. Gowen and Johnson (1946) showed that the choice of any
particular day to represent the lifetime record has no marked ad-
vantage, the correlation being about 0.75 with random variation
around that point. Accuracy was apparently increased with each
additional day's record. The 25 highest yielding females were
chosen to furnish the progeny that would comprise the next gen-
eration. The bottles for these females were prepared in the manner
previously described for breeders, and as soon as the pupal cases
began to appear, the adults were released. Five daughters were
taken from each of the 25 females, selected as previously described,
to serve as the progenitors of the family selection line. The male
offspring were held in reserve in the same bottles. Mating is
necessary to bring about high egg production early in life and
the females store the semen in small sacs so that the first male
to which they are mated dominates the fertilization of eggs laid
during the next few days. In order to insure good production and
at the same time provide males selected from outstanding families,
it was necessary to take males from the preceding generation.

Generation B of the family selection line was made up of 125 females, five daughters from each of the 25 females with the highest individual records in generation A. Generation A also furnished the females for generation B in the individual selection line. The females in generation B were mated to unselected flock males and the total egg production for the sixth and seventh days of imago life was used as a measure of fecundity. The brothers of the females in generation B were held in reserve. The mean production for the five daughters of each female in generation A was determined and the ten highest families were selected. The term family, in this experiment, refers to all of the offspring of a single pair. The ten families with highest mean egg production contained 50 females, and from these 50 females, 25 were selected to be the parents of generation C. When the 125 females for generation C were selected, they were mated with the males held in reserve at the time the females were selected for generation B. From this point on the females were selected in the same manner, up to and including generation G, and were mated with males from the best families of the preceding generation. Matings were made more or less at random, but an effort was made to avoid any inbreeding as close as uncle and niece.

In the individual selection line, 125 females were selected from the 25 most productive females in generation A and mated with flock males. When these 125 had been tested in the same manner as those in the family selection line, the 25 highest producing females were chosen, irregardless of their relationship, and were used to produce the flies for generation C. In generation C, 125 females

were again selected (five daughters from each female in generation
B) and were mated with males held in reserve in generation A. This
procedure was repeated for each generation up to and including
generation G. No effort was made to avoid inbreeding and matings
were made at random. The two methods of selection are illustrated
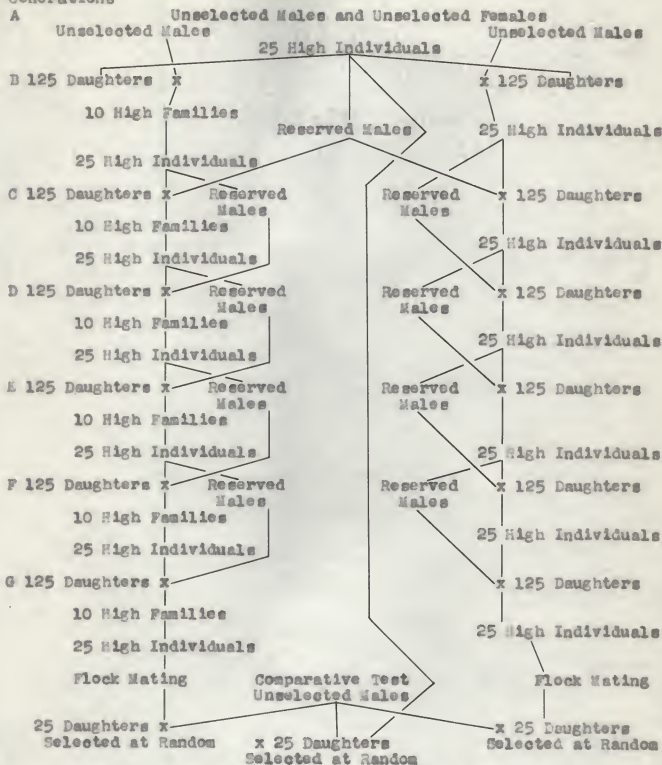in Fig. 1.

Fig. 1   Diagram of the plan for selecting breeding stock.

This experiment was started in late October of 1947 and ended in late April of 1948. During this period seven generations of _Drosophila_ _melanogaster_ were tested, consisting of the generation which provided the source for the two selection lines and six generations in each selection line. At the conclusion of the last generation in the individual selection line, a comparative test was arranged by taking 25 females from each of the selection lines and 25 females from the unselected control. These females, which were of equivalent ages, were mated individually and were taken at random from flock matings that would have comprised the breeding stock for the next generation.

Due to the heavy requirements of time and labor, it was necessary to alternate the generations in the two selection lines. For example, the egg production for generation B was measured on November 21 and 22 for the family selection line and on December 1 and 2 for the individual selection line. These differences in time brought into consideration the effects of temperature and qualitative differences in food material. The cabinet, with its thermostatic control, maintained a fairly constant temperature. However, as no means were available for circulating the air, the temperature varied as much as five degrees from top to bottom and also varied somewhat less from the center toward the sides. The cases were shifted from top to bottom each day in an attempt to alleviate these effects. A more serious influence was the heating system of the building in which the cabinet was located. This heat was turned off at night, which allowed the room temperature to fall

extremely low. On some extremely cold nights the heat was left on. The difference in time for measuring the egg production also allowed sufficient time for a complete turnover in the stock of bananas available at the local stores. There were no storage facilities readily available so that it was impossible to maintain a large food reserve.

The cabinet with a means of thermostatic control was not completed until the experiment had been started. For this reason the egg production records of generation A and generation B of the family line were extremely low. The use of the cabinet was introduced at this point and was used for the remainder of the experiment. The females were kept in the cabinet until their egg production test was completed and then the females selected as breeders were generally removed from the cabinet and held at room temperature while producing eggs to provide flies for the next generation.

At the beginning of the experiment, dead flies were replaced with a full brother or sister, as required, up to the day when the eggs were to be counted. This procedure was abandoned starting with generation C of the individual selection line because it was difficult to secure individuals of equivalent age and it did not give a true picture of vitality. As previously mentioned, the males were mated with the females more or less at random, with the exception that inbreeding was avoided, insofar as possible, in the family selection line. Up to and including generation C of the family selection line, the general practice was to distribute the males through the bottles in order, as for example, 1-2-3-4 ... 15.
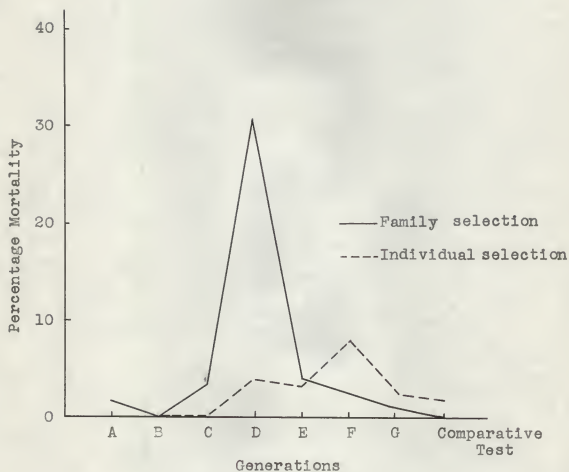
Fig. 2. Percentage mortality in females by generations for different methods of selection.

In the remainder of the experiment the practice was adopted of mating the males to females 1-6-11-16 or 2-7-12-17 etc. The reason for making this change was to secure more variety in the genetic possibilities and reduce the inbreeding because all of the daughters of one female were numbered 1-5, and of another, 6-10, etc.

It has been shown by Hanson and Ferris (1929) that high egg production early in life and high lifetime egg production in Drosophila is more regularly obtained from mated females. The males can be sterile, but their presence is necessary to obtain the best results. The method of using males produced in the preceding generation required them to be held for a period of about three weeks. This resulted in some mortality so matings were delayed until a short time before the actual testing period.

RESULTS

The mortality, shown in Fig. 2, never reached serious proportions with the exception of generation D in the family selection line. A combination of a defective thermostat and building heat produced temperatures high enough to bring about severe mortality. The average egg production remained high, but families selected for breeders necessarily contained as few as three living daughters.

In generation F of the individual selection line, the males were mated with the females not more than twelve hours prior to the first day of the testing period. This in part may explain the sudden drop in production experienced at that time (Fig. 3). At the same time the F generation of the family line also experienced a drop in egg production. This drop might be accounted for in part
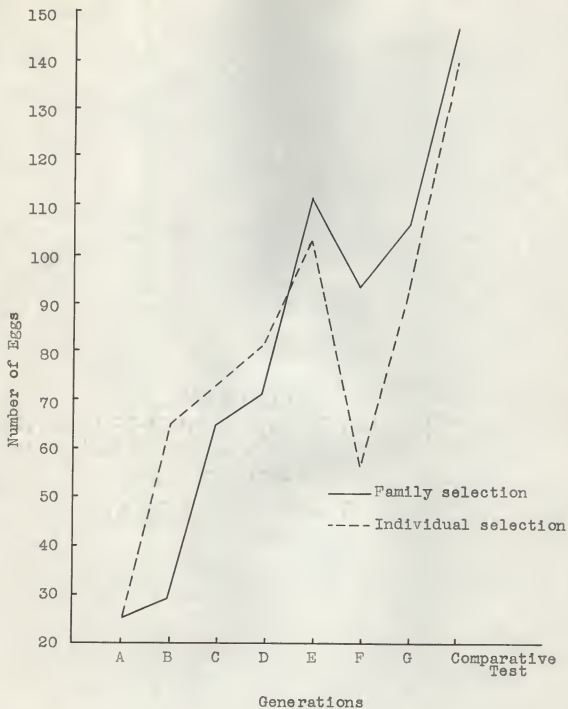
Fig. 3. Mean number of eggs per female for two day periods per generation as obtained by different methods of selection.

by the use of bananas that were not sufficiently ripened.

Table 1. Average two-day egg production per female by generations.

Methods of selection	Generations							Comparative Test
	A	B	C	D	E	F	G	
Family	25.14	29.23	65.11	71.52	110.72	92.83	106.22	146.7
Individual	25.14	64.81	72.24	81.64	103.09	56.18	91.11	139.3
Mass	25.14							144.8

Table 1 shows the mean egg production per female for the entire experiment, including the comparative test. Table 2 shows the distribution of egg production with the number of individuals listed under each class interval. The statistical summary is completed by the analyses of variance in Tables 3 and 4. The analysis of variance in Table 3 does not include generation B because generation B of the family selection line was not kept in the controlled temperature cabinet while generation B of the individual selection line was. The analysis of variance for the comparative test (Table 4) also includes the unselected control group, since all three lines were run simultaneously under identical conditions.

As shown in both Table 1 and Fig. 3, there was a consistent increase by both methods of selection during most of the experiment. Fig. 3 also showed very clearly the marked reversal of superiority near the halfway point. It can be seen that each system had a period in which it was consistently superior.

Table 2. Frequency distribution of individual egg production records by generations.

Generation	Method of selection	Egg Production, number										
		0	1-25	26-50	51-75	76-100	101-125	126-150	151-175	176-200	201-225	226-250
A	Individual	13.	57	42	10	3						
B	Individual	4	8	27	41	39	6					
	Family	45	70	10	23	26	20	6	5			
C	Individual	10	9	14	37	29	16	3				
	Family	10	13	21	16	22	20	16	6			
D	Individual	12	11	12	16	20	20	16	6	4		
	Family	5	13	7	15	18	17	7	1	1		
E	Individual	17	9	8	7	12	12	14	25	8	6	1
	Family	3	8	7	5	19	30	24	13	8	3	
F	Individual	18	12	17	26	29	10	2	1			
	Family	11	13	6	10	12	24	24	11	7	1	
G	Individual	2	10	6	20	29	25	23	7			
	Family	12	5	9	9	18	18	22	16	12	3	
	Mass	0		3	3		3	6	7	6		
	Individual	0	1	1	1	3	2	6	7	4		
Comparative Test	Family	0	1	1		1	5	2	7	5	3	3

In the present experiment, the class interval from generation to generation showed a gradual shift of the predominant group toward higher production, but also showed a gradual widening of the range of variation and an increase in the number of individuals laying no eggs (Table 2). The average production for both family and individual selection lines and the unselected control group was increased by approximately the same amount during the experiment (Fig. 1).

Table 3. Analysis of variance for generations and method of selection.

Source of variation	D/F	Sums of squares	Mean square	F
Systems	1	5076.1	5076.0	
Generations	4	51825.6	12956.4	
GXS	4	18448.8	4612.2	**5.120
Individuals	240	216181.0	900.8	
Total	249	291531.5		

The analysis of variance in Table 3 shows that a significant difference exists between systems and generations. This is indicated by the highly significant F value for interaction. The marked reversal of superiority shown in Fig. 3, indicates that such an interaction might have been expected. The data included in Table 1 show that the unselected stock improved by approximately the same amount as the selected strains. The analysis of variance in Table 4 shows that the differences between systems in the comparative test are not significant.

Table 4. Analysis of variance for the comparative test.

Source of variation	D/F	Sums of squares	Mean squares	F
Systems	2	727.3	363.7	
Individuals	71	131483.9	1851.9	0.196
Total	73	132211.2		

Because of the similiarity of results for all three lines in the comparative test and the failure of any system to be consistently superior, no definite conclusions regarding the value of the selection systems used could be drawn.

DISCUSSION

Four experiments stand out in extensive tests of the value of phenotypic, or individual selection. Gowell, as described by Woods (1908), attempted to increase the mean egg production of the Barred Plymouth Rock flock at the Maine Agricultural Experiment Station by using only females with annual records of 150 eggs or better and males from dams who had annual records of 200 eggs or better. After nine years the experiment failed to produce any significant change. It is well to note that the expression of this character (egg production) is limited to the female, which is the situation in the present experiment. Goodale (1937) has carried out two extensive selection experiments with mice. In the first experiment he selected for increased amounts of white, starting with animals that showed a few white hairs in the center of the forehead. After seventeen generations, he increased the amount of white to as much

as 165 sq mm. Goodale (1938) also reported on an experiment in
which selection was made for increased body weight in albino mice.
After 12 to 16 generations of selection, allowing for some over-
lapping, the males increased from an average of approximately
26.0 g to 36.4 g and the females increased from an average of
approximately 21.3 g to 29.3 g. Payne (1918) selected for increased
bristle number on the scutellum of Drosophila melanogaster for 38
generations and increased the number from an average of four in the
original stock to an average of almost nine in the thirty-eighth
generation. These last three experiments were all concerned with
factors that were expressed in both sexes.

It might be noted that the number of generations in the last
three experiments described greatly exceed the number used in the
present experiment. The number of generations in which selection
is practiced may in some measure influence the amount of change
that occurs.

The results of this experiment do not indicate what might
have been done with this same stock if the experiment had been
continued for a longer period of time and all three groups were
tested simultaneously. The results of the comparative test also
indicated the presence of other factors that could not be controlled
by selection alone.

Differences were clearly shown to be present, but the failure
of any method to be consistently superior and the improvement of
the unselected control by approximately the same amount as the
selected lines did not definite conclusions as to the most valuable
method of selection to be drawn.

SUMMARY

The results of selecting for increased egg production for six generations by both the family test and individual selection followed by a comparative test which included an unselected control group are presented. The egg production of the unselected control group improved by approximately the same amount as that of the flies in the two types of selection and the controls had virtually the same egg production at the end of the experiment as did the selected stocks. Consequently, it cannot be stated that selection accomplished anything. In the comparisons made at the end of the selection period, the family selection had effected slightly superior egg production, but the difference was not statistically significant.

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