A COMPARISON OF METHODS OF SELECTING FOR INCREASED EGG SIZE IN *DROSOPHILA MELANOGASTER*

by

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INTRODUCTION

Differences in rates of reproduction within a population whereby animals with certain advantageous characteristics tend to have more off-spring than animals without those characteristics are brought about by means of selection. The aim of selection by man is to make the representatives in the next generation as good as, or better, than those of the present one. By selection, the genes responsible for the favored characteristics tend to become more abundant in the population and those of the less favored ones less abundant. Selection has always been practiced by animal breeders, the degree depending upon the individual breeder. Selection may be based either upon the characteristics of individuals, ancestors, sibs, progeny, or upon various combinations of the four.

Artificial selection is usually more intense than natural selection since less of the decision is left to chance. Among domesticated animals, natural selection is merely supplemented by man's selections. Domestication intensified processes which already existed in nature. Early domestication brought about the development of more uniform stock by the practice of discarding undesirable individuals appearing in the stock, which was usually inbred to a degree. This early inbreeding was fortunately unavoidable because of the close association of breeders within a neighborhood and the lack of systematic record keeping. Inbreeding may well have been the most potent force leading to the production of distinct races among domesticated animals.

In artificial selection there are two methods employed, individual or phenotypic selection and family selection, which are used either individually or in combination. Individual selection has been used by man since animals were first domesticated thousands of years ago. Centuries before the genetic principles were known, animal breeders strove to produce animals that would best suit their use under domestication. The early breeding efforts were of this nature. Early poultry fanciers and fighting cock breeders used this method of selection for many years before the economic value of the chicken was emphasized. Family selection to produce change is used quite extensively at present and has been used many years in selecting individuals in which the factor, or factors, under study are expressed in one sex only. Proverbs such as "Like father like son", are found in every language. People have always known that offspring tend to resemble their parents and that brothers and sisters show some of the same family characteristics. This principle is used in selecting breeding cocks on the laying performance of their sibs and progeny. In family selection, there is usually a certain degree of individual selection, the most desirable individuals being selected from within a superior family.

Much progress has been made in recent years by different types of selection, but information concerning the merits of one selection system over another is lacking. Since there is a place for the study of the effectiveness of different selection methods, this problem was undertaken. The purpose was to compare the rate of increase in egg size by individual or phenotypic selection and by family selection. The study was undertaken with the aim in mind of duplicating with the fruit fly, Drosophila melanogaster, a project which would have taken several years in poultry breeding.

REVIEW OF LITERATURE

Warren (1924) was the first who used Drosophila eggs for genetic study and suggested that the constancy of the egg size made this character an excellent one for study. He found factors for egg size in all four linkage groups of D. melanogaster. In a preliminary test, Warren studied the influence of environmental and physiological factors on the egg size and concluded that moisture and temperature to which the flies were subjected during their development had no effect on the size of the eggs produced. From a test on starvation of the larvae during their development, Warren found a decrease in number of eggs produced, but no noticeable decrease in size of eggs produced by flies developed in the undernourished condition.

Gause (1931) in his experiment on starvation, found in *Drosophila funebris* and in *D. melanogaster* that the shortening of the feeding period during the larval stage caused a decrease in egg length. The extent of starvation applied by both Warren and Gause was not stated, so there is reason to believe that the degree of starvation could account in part for some of the differences in results. Different strains of flies could also have played a part in the variation, since a factor affecting egg shape known as "Spheroidal" was discovered in *D. funebris* by Crew and Averbach (1937). This characteristic was found by the same workers to be more sensitive to environmental change than normal shaped eggs.

Imai (1935) suggested that food conditions, so far as they are modified by the number of flies in a culture, had an influence on length of eggs. Imai, in his experiment on temperature variations, found a difference in the size of eggs produced by individuals developing under various temperature conditions. He found the size of egg produced by flies developing in different temperature conditions was affected only for the first few days of laying after which the flies began to produce eggs of normal length for the stock. This factor may account for some of the differences in results obtained by Warren since no mention was given as to the age of the flies when their eggs were measured.

All those working on egg size of *D. melanogaster* are in agreement that the age of the fly does not influence the size of the eggs produced if the temperature remains constant.

MATERIALS AND METHODS

The stock for this study was a strain of wild type fruit fly, D. melanogaster, collected in Manhattan, Kansas and carried for several generations of mass matings without the practice of any type of artificial selection. Stock for each selection method, together with stock to be carried as unselected controls, was of the common origin.

All flies carried as controls as well as those in the selection lines were reared in one-half pint milk bottles. This type of bottle proved satisfactory from the standpoint of ease in handling both mass cultures and single pair matings. The hazard of escape of flies when feeding single pair matings was kept at a minimum by use of one-half pint bottles. Milk bottle cases, holding 30 bottles each, were used in the storage of surplus bottles and in transferring breeding flies to and from a specially constructed incubator. The incubator was heated with electric light bulbs and controlled with a mercuromatic thermostat. The temperature within the incubator was regulated as near to 23° C. as possible.

An egg laying medium, consisting of 175 cc water, 200 g mashed banana, 5 g agar, and 2.5 cc "Moldex" (10 percent in alcohol) was used. This is basically the banana food formula suggested by Sinnott and Dunn (1939, p. 397). In order to supply sufficient food for three or four days (during which time the flies were maturing, eggs were being measured, and being made) approximately three g of the food mixture were placed on the under side of each milk bottle cap. The methods employed by Gowen and Johnson (1946) in placing the food on the bottle caps and in supplying the food with a yeast suspension were used. The eggs were easily picked up with a scalpel from the bottle caps carrying the food mixture and removed to a slide where they were placed in rows for measurement under a compound microscope. Ten eggs taken at random on the first or second day of laying were measured for obtaining a female's mean egg size.

Eggs of D. melanogaster are white in color and have an outer membrane, the chorion, composed of hexagonal cells. Near the anterior end, on the dorsal surface, a pair of filaments extend and serve in keeping the eggs from sinking into soft food on which they may be laid. At the anterior end of the egg is a micropyle through which the spermatozoa enter as the egg passes through the uterus (Demerec and Kaufman, 1945). The eggs are elongate with a flattened dorsal surface and a somewhat rounded venteral surface. In this study, eggs were placed on their sides and size was determined by measuring the long dimension. For measurement, a 20X eye piece combined with a 4X, 32 mm objective was used.

After the flies to be used as breeders had been chosen, they were provided with food consisting entirely of banana sprinkled with dry yeast, since this type of food material seemed to give a large number of vigorous offspring. A small amount of banana, approximately 4 g, was used until larvae appeared and then extra food of the same type was added. The presence of the larvae in the food kept mold growth under control without the use of "Moldex". Paper toweling in strips, two inches by eight inches, was used in the breeding stock bottles to absorb the excess moisture from the fermenting bananas. This size paper did not absorb all the moisture but prevented the flies from drowning in excess fluid. As soon as the larvae began to pupate the parents were removed. Flies of all groups were raised under similar food conditions in order to minimize the effects of nutrition on egg size. The food for males being held as prospective breeders consisted of the egg laying medium since it was drier than the pure banana and since "Moldex" was necessary to prevent mold growth where larvae were not present. Approximately 20 g of the food mixture was used for each stock bottle.

The two methods of selection were used for six consecutive generations in the males and seven generations in the females.

In the individual or phenotypic selection group, the female breeders were selected solely on the basis of their mean egg size. Those ranking within the upper 20 to 25 percent of the population were used. The breeding males for this group were selected as sons of females whose egg size ranked within the upper 6 to 10 percent of their population, regardless of the average for the family from which they came.

In the family selection group, the female breeders were selected as outstanding individuals within outstanding families. In this study, the term "family" was applied to individuals which were the product of a single pair mating. The female breeders in family selection ranked in egg size within the upper 20 to 25 percent of the population, but also have been included within the upper 40 percent of the families tested. The male breeders for this group were selected on the egg size of their dams and sisters. They came from females within the upper 6 to 10 percent of the entire tested population and were sons of females ranking in the upper 30 percent of those tested. Each generation in both types of selection was made up of five daughters from each of 25 females of the preceding generation.

In order that breeding males in the family selection group could be selected on their combined dam and sister records, their utilization was accomplished one generation behind the females; namely, sons of the first generation females were mated with the daughters of the second generation females. This delay in use of males was practiced in each type of selection so that mating methods would be comparable.

By observing Fig. 1, one will note the practice followed in selecting breeding stock for the two types of selection employed. Males and females used in generation A and males used in generation B were from the unselected stock. Females used in generation B of both selection methods were daughters of generation A females having the largest mean egg size. A consistent selection program was followed for each type of selection in generation C and successive generations.

Females that died or escaped, or that were removed for any reason before their egg size records were complete, were replaced with full sisters. Male replacements were full brothers of the original males. Females to which the original males were mated were replaced in order to avoid any possible error in determining the sire of the offspring produced.

For the control cultures, four groups of six pairs of flies each were randomly selected each generation. The mass cultures were mixed every third generation to keep inbreeding at a minimum.

RESULTS

The mean egg length of females for generation A, 18.6 units, was taken to be the mean for the unselected stock. A unit equals .027 mm. There was not a significant change in mean egg size for generation B, compared with generation A. This fact may be noted in Table 1, in which mean egg size is recorded by generations for the two types of selection and

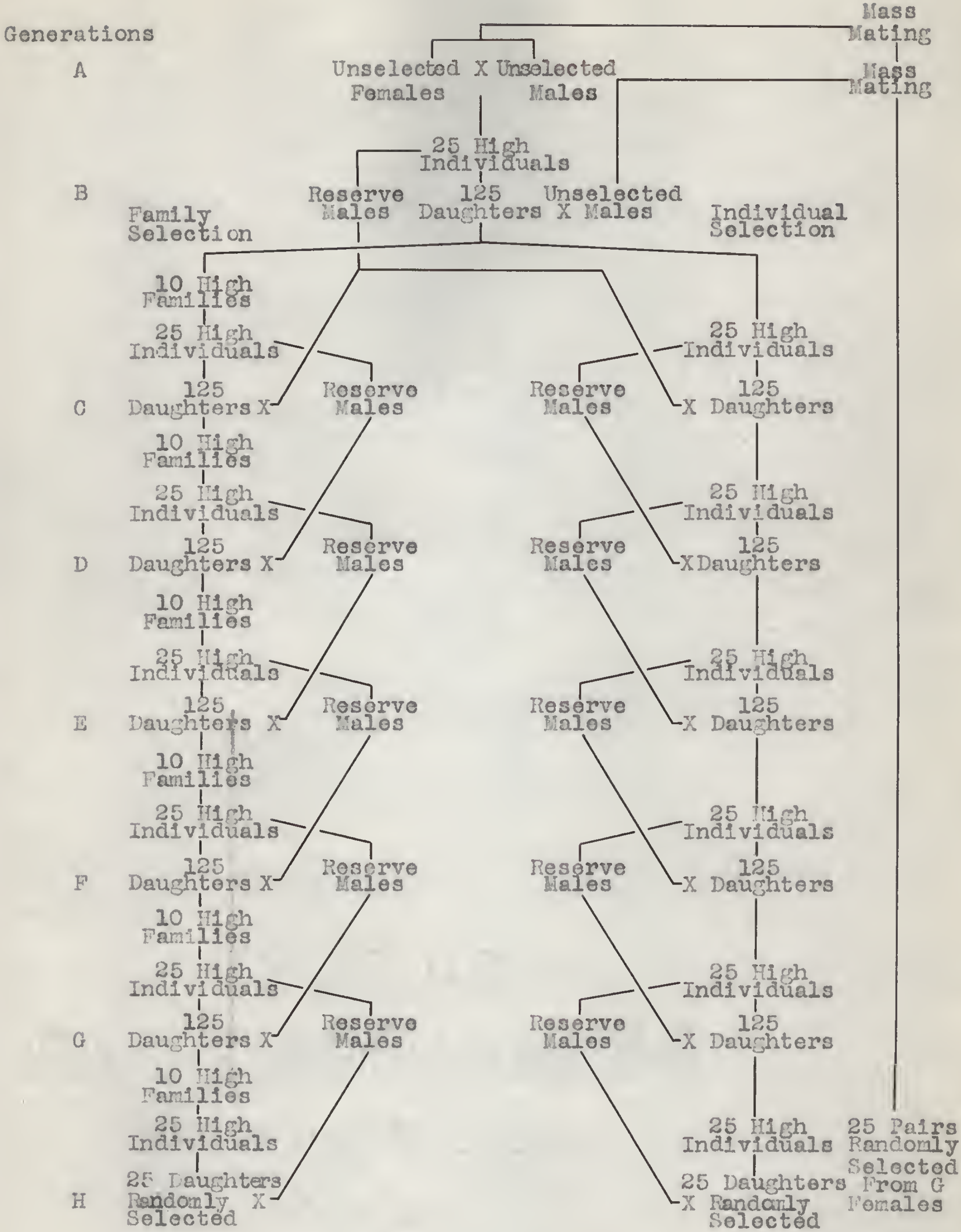

Fig. 1. Diagram of the methods used in selecting breeding stock for the two types of selection employed.

for the unselected control and in Fig. 2 where a linear representation of mean egg size is presented by generations for the two types of selection. For statistical significance, a change of .3 unit or more was necessary in each case whether between methods of selection for a generation or between two generations in the same selection method. For generation C, the first generation in which the selection methods were different, there was a highly significant increase in mean egg size by both selection methods. This increase was caused,in part, by a reduction in mean egg size variation for the individual flies as shown in Fig. 3 and in Table 2. A similar increase may be noted in the family selection group for generation D. This increase in generation D was probably due to the fact that a small percentage of the females had mean egg sizes considerably above the average for the population and to a change in the frequency distribution. The variation noted in this generation for the family selection group was unexplainable. A smaller increase, due almost entirely to a change in the frequency distribution, was noted for the individual or phenotypic selection group in generation D. In generations E. F. and G for both types of selection, there was not a significant increase in mean egg size. The very slight increase observed was due almost entirely to the reduction of variability in the stock as shown in Fig. 3 and in Table 2. There was no production of larger individual eggs noted for generations E, F. and G.

A comparative test consisting of 25 females taken at random from each selection stock and from the unselected control was conducted in generation H. Smaller numbers of flies were used in the H generation than in previous generations in order that all groups might be carried simultaneously to prevent environmental differences from entering into the test. By observing Table 1, one may note a non-significant difference in mean egg length for both types of selection over the preceding generation, generation G, and a non-significant change in mean egg size for the unselected control, 18.7 units, over the A generations, 18.6 units, for the unselected control.

Even though statistical significance was lacking between selection methods for most generations, one will note in Fig. 2 and in Table 1 that the family selection group was consistently higher than the phenotypic selection group in average egg length beginning with generation D and proceeding to the end of the selection test.

Table 1. Mean egg size by generations and a comparative test in generation H for the two types of selection and the unselected control.

Mating methods	Generations							
	A	B	C	D	E	F	G	Comparative test
Family	18.6	18.5	19.0	19.5	19.6	19.6+	19.7	19.9
Individual	18.6	18.5	19.0+	19.2	19.3	19.4	19.5	19.6
Control	18.6	-	-	-	-	-	-	18.7

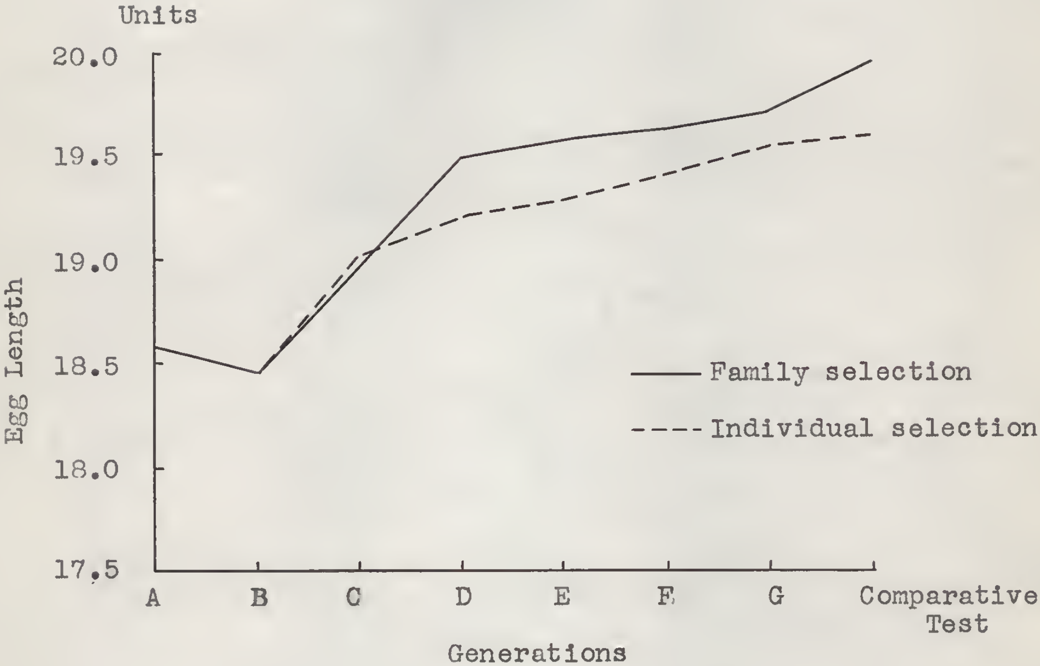

Fig. 2. A linear representation of mean egg size by generations for the family and individual types of selection.

Table 2. A frequency distribution of mean egg size for individual flies by generations for the two types of selection.

	Units and frequencies											
	Family selection						Individual selection					
Generation	17.6 : 18.0	18.1 : 18.5	18.6 : 19.0	19.1 : 19.5	19.6 : 20.0	20.1 : 20.5	17.6 : 18.0	18.1 : 18.5	18.6 : 19.0	19.1 : 19.5	19.6 : 20.0	20.1 : 20.5
A	25	32	50	13	5		25	32	50	13	5	
B	31	36	44	10	4		31	36	44	10	4	
C	1	19	58	37	10		3	15	57	30	18	2
D	1	2	21	40	46	15	1	7	34	50	31	2
E	1		13	41	63	7		8	31	55	29	2
F			8	45	69	3			23	52	49	1
G			3	27	92	3			9	52	61	3
H				1	19	5				10	15	

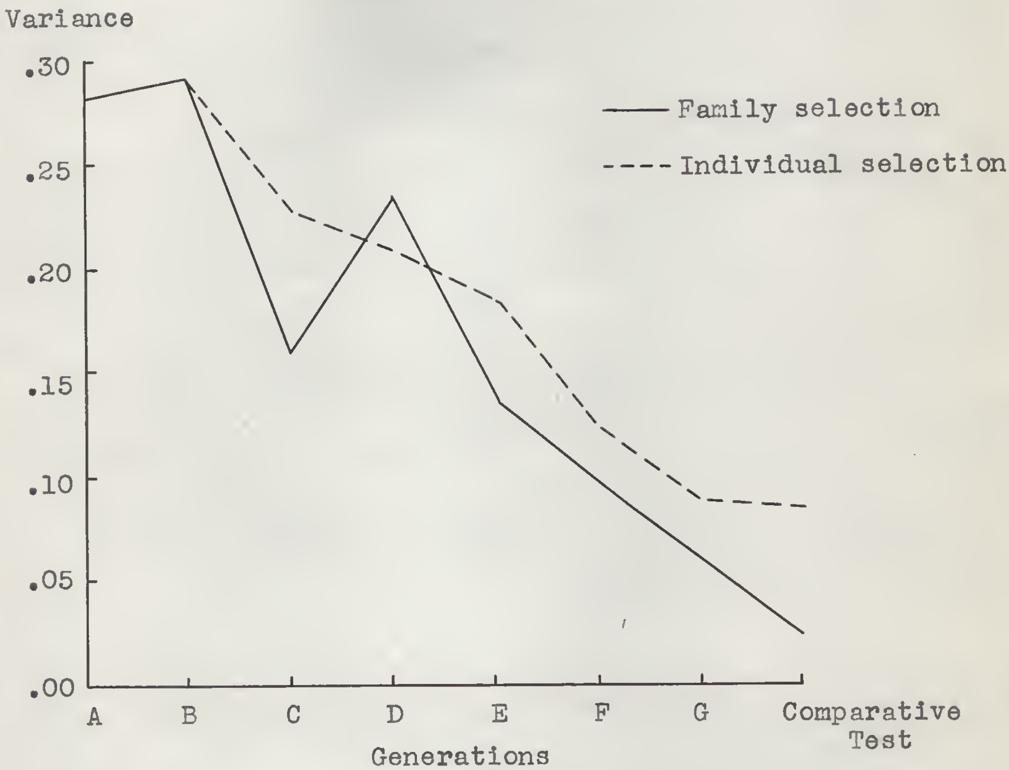

Fig. 3. A linear representation of variations from the mean by generations for the two types of selection.

DISCUSSION

One of the early experiments on artificial selection in poultry was conducted by Gowell (1902, p. 26-40) in which the phenotypic method was employed for nine generations with the object in mind of increasing egg numbers in the fowl. The failure of this experiment to produce much change in the egg production may be due to the fact that phenotypic selection is not too effective after a certain degree of change is made in a population. This theory is supported by an experiment by Warren (1924) in which selection for increased egg size was practiced for 10 generations in a stock of D. melanogaster in which the egg size was exceptionally large for the original, unselected population. The selection in this stock showed no increase in egg size.

Payne (1920) reports results from individual selection for increased numbers of bristles on the scutellum of D. melanogaster in which he increased the mean number of bristles from 1.95 in the unselected parent stock to 3.25 after 18 generations of brother sister matings.

Goodale (1937) selected for larger numbers of white hairs in the foreheads of mice and increased the number from a few hairs in the unselected stock to white spots covering as much as 165 square millimeters after 17 generations of selection. Goodale and Payne were working with factors having phenotypic expression in both sexes.

Knox and Olsen (1938) progeny and sib tested for increased egg weight in Single Comb White Leghorns. After four selective generations, these workers had increased the mean egg weight in the stock from 54.7 g to 57.3 g.

In the problem under stody and in the selection experiments conducted by the various workers mentioned above, there is agreement that family selection may be utilized in the production of a change in a wild type population. The rapidity of change brought about by family selection seems to be due to the rigidity of the selection practices and to the expression of the characteristics; namely, whether there is phenotypic expression in one or in both sexes. Data concerning the effectiveness of phenotypic selection are lacking, especially for a factor having phenotypic expression in only one sex, but data obtained from the present problem seem to point to the fact that phenotypic selection could be quite effective in selecting for change in a wild type population.

SUMMARY

1. An increase of seven percent in mean egg size was accomplished in seven generations of family selection in *D. melanogaster*.

2. An increase of five percent in mean egg size was accomplished in seven generations of individuals of phenotypic selection in *D. melanogaster*.

3. The increase in mean egg size by both types of selection was largely due to the reduction of variability within the stock, the individuals with smallest egg size failing to appear after the third generation.

4. Control flies originating from the same stock as the two selected lines remained unchanged in egg size during the experiment.

5. The difference in effectiveness of the two methods of selection employed is questionable.

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