PERICARP STUDIES IN MAIZE. III. THE FREQUENCY OF MUTATION IN VARIEGATED MAIZE PERICARP[1]

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TABLE OF CONTENTS

INTRODUCTION

Variegated maize pericarp offers good material for studies on mutation as the mutaton rate is very high and significant data can readily be obtained. The studies herein reported were undertaken to determine whether the mutaton rate remained constant through that period in the life history of the plant for which comparative data could be obtained.

MATERIAL AND METHODS

The variegated plants used in these studies were all obtained by outcrossing plants heterozygous for variegated (P^{vv}) and red-cob white (P^{wr}) to white (P^{ww}). Half of the resulting plants were variegated of the factorial constitution $P^{vv}P^{ww}$. The other half were red-cob white and were discarded.

The mutations studied were the mutations from variegated to red. These mutations were tabulated from their somatic expression on the plants themselves. The relative stage in the life history at which the

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mutation took place is indicated by the area affected. Previous work on variegated pericarp (EMERSON 1914, 1917) had shown the visible patches of color on the ear to be due to the same mutational changes which also affected the germ cells. That the changes involved are gene mutations is indicated by the fact that the new forms produced behave as allelomorphs to the parental type and to other members of the same allelomorphic series (ANDERSON 1924).

As described by EMERSON (1917) the mutations observed are of two types:

1. The near-self type in which the color is located in the deeper lying tissue of the pericarp. The glumes of the cob and a patch at the tip of the grain remain white or nearly so. These mutations are heritable and give rise to self-red progeny.

2. The dark-crown type. In this type the color seems to be confined to the epidermis except the tip of the grain which becomes deeply colored. The glumes of the cob, which are made up largely of epidermis, also become red. This type is not inherited presumably because the mutation has taken place in cells which do not give rise to germ cells.

The variegated ears were harvested and examined for mutation areas. Areas covering one grain or more were classified and recorded. Grains with near-self mutation areas covering a considerable fraction of the grain were saved and classified. The remaining grain was shelled and weighed. The number of grains without mutation areas of recorded size was computed from weight and counts of weighed samples.

The plants used in these studies were grown at the Plant Breeding Department of CORNELL UNIVERSITY in 1919 and 1920.

DATA

A tabular presentation of the data on near-self mutation is given in table 1. The original data were tabulated in classes of $\frac{1}{2}$ grain intervals, but the data have been grouped to reduce the number of classes. A similar presentation of the dark-crown data is given in table 2. Dark-crown areas affecting only a fraction of a grain are more difficult to classify so were not recorded.

Many of the larger areas are of indeterminate size because their boundaries do not fall entirely within the areas under observation. They are parts of larger mutation areas including tissue outside of the seed-producing part of the ear. They are the result of mutations taking place earlier than their recorded size would indicate.

TABLE 1

Data on frequency of near-self (heritable) mutations.

PEDIGREE	TOTAL NUMBER OF GRAINS	NUMBER OF GRAINS IN MUTATION AREA														
		1/8–1/4	1/4–1/2	1/2–1	1	2	3	4	5	6	7	8	9–16	17–32	33–64	65–128
A 1506	37983	968	188	78	39	20		1		1			1			
A 1507	6009	99	42	16	10	1										
A 1508	11185	228	82	39	17	4	1									
A 1510	32383	834*	229	76	39	15				1						
A 1512	7299	264	25	21	5	3		1								
A 1513	8028	193	20	15	10	3										
A 1514	13993	389	70	28	7	2	1				1					
A 1515	14922	378	124	44	20	5	3									
A 1516	25001	467	112	72	23	2	3	1								
A 1517	25204	1041	140	48	17	13	1									
A 1518	25706	700	111	51	33	10										
A 1519	29790	884	148	86	34	8	3		1							
A 1520	37265	774	173	56	36	8	1	2					2			
A 1740	33159	412	214	105	31	14			1			1	1			
A 1742	1792	15	12	2	1											
A 1746	29768	502	265	98	37	11	1	1		1						
A 1747	15089	214	107	35	17	5	1						1		1	
A 1757	8686	94	32	13	9	1										
A 1762	1067	11	6	1	1	1										
A 1763	4012	55	36	14	6	1										
A 1765	13173	201	70	38	1	7							1			
A 1770	41325	368	133	14	46	7	3						2	1	1	
Ey 843	37580	2008	247	145	68	9	4	1				1	3	1		
Ey 844	782	29	5	0	1											
Ey 847	50102	808	183	64	20	4	3					1				
Ey 848	7631	136	25	11	7	2			1							
Ey 851	48147	1509	386	164	88	23	2	3	3	1		1	3			
Ey 852	3754	115	23	12	6	2	2									
Ey 855	4124	163	25	16	7											
Ey 856	39757	740	195	59	45	6	1				1		1			
Ey 860	901	32	8	3	2											
Ey 862	2052	86	19	7	4	1										
Ey 864	2123	77	19	10	5	1							1			
Ey 868	1191	41	18	2	9	1										
Ey 871	1062	34	7	1	1	1										
Ey 874	33233	1281	299	122	72	20	4	1	1		1			1		
Ey 875	1580	38	11	4	1											
Ey 876	543	26	2	1	0											
Ey 877	2296	67	10	7	8	4		1								
Ey 878	29541	1428	181	67	50	13	6	2								
Ey 883	654	19	3	2	0											
Ey 886	4513	160	32	10	10	5	2									1
Ey 890	1277	33	8	4	3											
Total	695682	17921	4045	1661	846	233	42	14	7	4	3	4	16	3	2	1

* Estimated. Observed number not recorded.

TABLE 2

Data on frequency of dark-crown (non-heritable) mutations.

PEDIGREE	TOTAL NUMBER OF GRAINS	NUMBER OF GRAINS IN MUTATION AREA																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17-32	33-64	65-128	129-256	257-512
A 1506	37983	748	42	12	10	4	4			2	1				1		1	3	2			
A 1507	6009	130	2		1		2															
A 1508	11185	278	20	7	3			1												1		
A 1510	32383	808	41	14	6	6		2		1					2			1				
A 1512	7299	93	10	1							1											
A 1513	8028	68	6	1																		
A 1514	13993	139	12	1	1	1	1	1														
A 1515	14922	187	4	2		1	1	1										2				1
A 1516	25001	377	11	4	3	1	1	1	3	1					1			1			1	1
A 1517	25204	447	23	7		1	1			1			1	1		1			1			
A 1518	25706	525	29	4	1	2	1	1		1	1			1	1	1				1		2
A 1519	29790	498	31	6	2					2				1	1				1	1		
A 1520	37265	588	25	10	4	4	3	1			1		1	1				3				
A 1740	33159	484	32	11	4	3	1		1	1					1		1	1				
A 1742	1792	6	1		1			1			1							2				
A 1746	29768	318	13	4	3	2	3			1								1				
A 1747	15089	188	14	3	1				1													2
A 1757	8686	45	4	1	1														2			1
A 1762	1067	8	1																			
A 1763	4012	38	1				1															
A 1765	13173	152	6	6		1	1															
A 1770	41325	526	31	6	3	2	1		1	1		1						2				

TABLE 2 (continued)

Data on frequency of dark-crown (non-heritable) mutations.

PEDIGREE	TOTAL NUMBER OF GRAINS	NUMBER OE GRAINS JN MUTATION AREA																				
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17-32	33-64	65-128	129-256	257-512
Ey 843	37580	291	35	41	7	2	6	1	1	1			1	1	1		1	3	1			
Ey 844	782	2																				
Ey 847	50102	214	13	3	2	1	2								2	1						
Ey 848	7631	35	3	2	1								1									
Ey 851	48147	783	65	26	17	6	6	2		1	3	3				1		1	2		2	
Ey 852	3754	59	6	4		1	1															
Ey 855	4124	94	6		3	1										1						
Ey 856	39757	334	51	9	5	4	2	1			1	1	1	1				2	1			
Ey 860	901	4	1																			
Ey 862	2052	38	7	3																		
Ey 864	2123	31	4										1									
Ey 868	1191	7	1			1																
Ey 871	1062	13	1																			
Ey 874	33233	658	36	15	6	2	3	2	2	1	1	2			1			4	2			
Ey 875	1580	26																				
Ey 876	543	6																				
Ey 877	2296	28	4	3			1															
Ey 878	29541	313	35	10	7	3	1	3		1	1	1	3			1		1		1		
Ey 883	654	3	4	1																		
Ey 886	4513	54	7	2		2	1	1		2												
Ey 890	1277	21	2										1									
Total	695682	9665	640	192	92	51	44	19	9	17	11	8	10	6	11	6	3	27	12	4	3	7

COMPARISON OF NEAR-SELF AND DARK-CROWN FREQUENCIES

Table 3 gives a comparison of the frequency of occurrence of equivalent sized near-self and dark-crown mutation areas. The frequencies from tables 1 and 2 are grouped into classes such that the limits of one class are twice those of the preceding. This is done to avoid the extremely low frequencies of the individual classes of large area.

TABLE 3

Comparison of frequencies of near-self (heritable) and dark-crown (non-heritable) mutations.

MUTATION AREA	NEAR-SELF	DARK-CROWN
0.5– 1	2509	9665
1 – 2	233	640
3 – 4	56	284
5 – 8	18	123
9 – 16	16	72
17 – 32	3	27
33 – 64	2	12
64 –128	1	4
129 –256	0	3
257 –512	0	7
Total	2838	10837

The frequencies for the dark-crown series average about 3.8 times as great as those for the corresponding near-self classes. This may mean that the mutation rate is actually this much greater in those meristem cells which give rise to the tissues affected by the dark-crown mutations. But since the method of growth of the tissues concerned may not be comparable, this cannot be considered proved.

THE THEORETICAL FREQUENCY RELATION BETWEEN DIFFERENT CLASSES FOR A CONSTANT MUTATION RATE

If the mutation rate were constant the number of mutation areas of small size would be more frequent than those of large because there would have been more cells present which might have mutated. Also the numerical class interval chosen is not one that gives an equivalent length of time for mutations to take place. The exact numerical relation cannot be calculated due to the irregularity of growth. But a calculation based on regular multiplication of cells will approximate the true relationship sufficiently closely for comparison with the observed data. A mutation

affecting twice the area of another may be assumed to have occurred one cell generation earlier when the number of cells was only one-half as great. The time interval, expressed in cell generations, may be calculated on the same basis. An error, probably very small, is introduced in the near-self series by growth in the third dimension during the period under observation.

On the above basis, the relative number of available cells at the time of mutation varies inversely as the size of the mutation area, and the time interval varies as the differences between the logarithms of the numerical sizes of the mutation areas.

CALCULATION OF COMPARATIVE MUTATION RATE

The ratios of the observed frequencies to the calculated relative frequencies give us a measure of the relative mutation rate for the period of several cell generations during which the recorded mutations took place. The calculation of this comparative mutation rate may be simplified by a process which is equivalent to making the theoretical equal to unity and multiplying the observed frequencies by the appropriate factor. The simplified calculation is made as follows: (a) The observed frequencies are multiplied by their respective class values. (b) These products are then summed into classes having equal logarithmic intervals. The interval chosen is such that the numerical limits of one class are twice those of the preceding. This interval is such that each class then represents a cell generation. This summation greatly condenses the long series of low frequency classes.

The number of units of area available for mutation is slightly altered by the fact that once an area has mutated it is no longer available for mutation. This error can be corrected by dividing each product by the difference between the total number of grains and the number included in the larger mutation areas. The change due to this correction is not sufficient to alter the calculated rates more than a few percent but for the sake of completeness both the uncorrected and corrected figures are given in the tables and graphs which follow.

COMPARATIVE RATE OF MUTATION

The comparative rate of mutation calculated for the near-self series is given in table 4. The rate of mutation is expressed as the number of cells mutating per hundred thousand during a period equivalent to one cell generation. A graph of the mutation rate for the near-self series is given

TABLE 4

Mutation rate of near-self (heritable) mutations expressed as the number of mutations per hundred thousand cells during one cell generation.

MUTATION AREA	RATE	CORRECTED RATE
0.12– 0.25	644.0	668.7
0.25– 0.5	290.7	300.0
0.5 – 1.0	360.4	365.4
1.0 – 2.0	67.0	67.7
2.0 – 4.0	26.2	26.4
4.0 – 8.0	16.1	16.2
8.0 –16.0	26.6	26.8
16.0 –32.0	9.5	9.5
32.0 –64.0	11.4	11.4

in figure 1. The graph is arranged so that successive cell generations read from left to right. The last class is of little value because of difficulty of classifying. The irregularity in the curve near the right end is largely due to the fact that the class limits adopted do not conform to the original

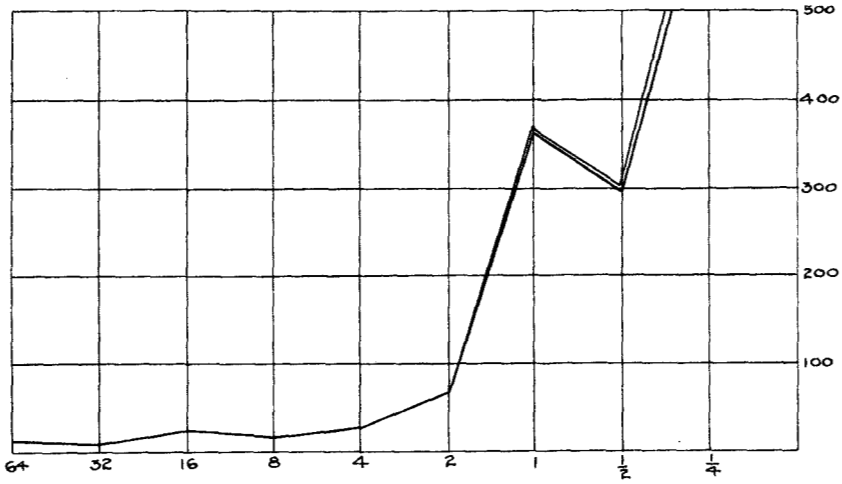

FIGURE 1.—Graph of near-self mutation rate from table 4. Size of mutation areas plotted as abscissae, mutation rates as ordinates.

classification. Table 5 and figure 2 give the corresponding data for the dark-crown series. The curve is smoother because there is no discrepancy in the summation of classes. Mutation areas affecting more than 64 grains for the near-self and more than 128 grains for the dark-crown series are omitted as most of these extended beyond the limits of the ear on which they occurred and were consequently of indeterminate size.

TABLE 5

Mutation rate of dark-crown (non-heritable) mutations expressed as the number of mutations per hundred thousand cells during one cell generation.

MUTATION AREA	RATE	CORRECTED RATE
1	1389.3	1408.7
1 - 2	184.0	190.4
2 - 4	135.7	137.1
4 - 8	104.1	105.0
8 - 16	120.9	121.8
16 - 32	93.1	93.7
32 - 64	82.7	83.1
64-128	58.5	58.8

The rate of mutation for both series shows a great increase within the period covered by these observations. The increase is probably a gradual one, as the sharp changes in the graphs are at least largely due to difficulties encountered in the classification of mutation areas of small size. The

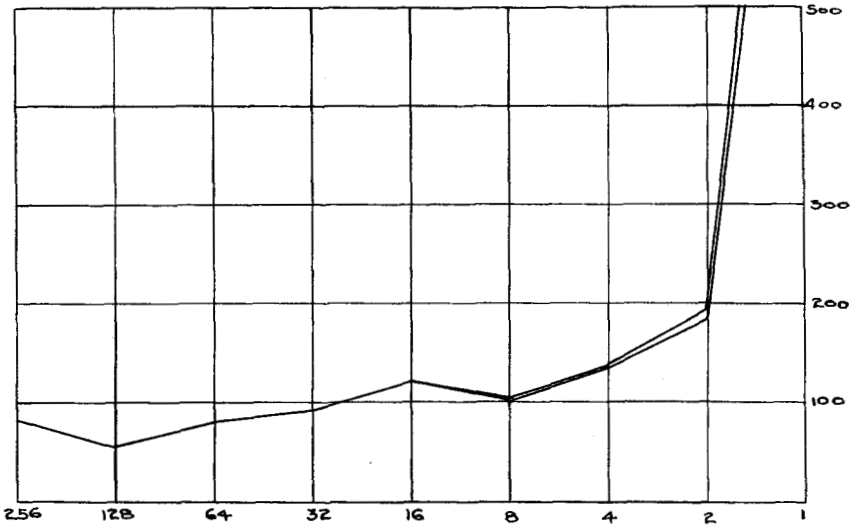

FIGURE 2.—Graph of dark-crown mutation rate from table 5. Size of mutation areas plotted as abscissae, mutation rates as ordinates.

values recorded for the larger areas are a little too large due to the inclusion of mutation areas which were not completely included in the seed-bearing part of the ear on which they occurred.

As the recurrent mutations involved in variegation are perhaps not comparable to other gene mutations, it cannot be inferred from these data

that other gene mutations will show a similar increasing frequency during ontogeny. In this connection, it is well to remember that in the conspicuous example of recurrent change in the bar gene of *Drosophila melanogaster*, STURTEVANT has shown that the changes are due to unequal crossing over in the immediate vicinity of the bar gene (STURTEVANT 1925). This is in marked contrast to the mutations recorded in this paper which occurred in somatic tissue long before the maturation divisions.

SUMMARY

Somatic mutations to red in variegated maize pericarp were classified as to the area affected. The near-self (heritable) and dark-crown (non-heritable) series of mutations were recorded separately.

The dark-crown mutations were about 3.8 times as frequent as the near-self mutations affecting equal areas.

Both the near-self and dark-crown series show an increasing rate of mutation during the period of ontogeny covered by these studies.

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