SUMMARY

1. The growth response of six single-gene mutants in maize to gibberellic acid is reported. Four of these, *anther ear-1*, *dwarf-(5232)*, *dwarf-1*, and *dwarf-(8201)*, responded by normal growth. The fifth mutant, *dominant-dwarf*, did not respond and the sixth mutant, *dwarf-(4963)*, showed only a slight response.

2. The sensitivity of response to gibberellic acid and the requirement of a continuous supply of gibberellic acid to maintain normal growth for four of the mutants are reported.

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[5] Stocks carrying recessive genes for dwarfism were furnished by Dr. E. G. Anderson, California Institute of Technology. *Dominant-dwarf* stock was obtained from Dr. R. R. Seaney, USDA, Cornell University. It was originally found by G. H. Stringfield, U.S.D.A. Ohio Agricultural Experiment Station, Wooster, Ohio.

[6] Gibberellic acid (mixture) was obtained from Dr. James Bonner, California Institute of Technology. It was originally obtained from Dr. Reed Gray, of Merck and Company, Inc. The pure sample of gibberellic acid was obtained from Dr. Frank H. Stodola, USDA, Peoria, Illinois.

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*THE q LOCUS OF NEUROSPORA CRASSA**

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Investigations with several organisms have demonstrated that crosses of individuals carrying mutant alleles may give rise to rare nonmutant progeny. In a number of instances[1] the event which produced the exceptional offspring was shown to involve crossing over within a small chromosomal segment. In *Neurospora crassa*, Bonner[2] has described the occurrence of rare nonmutant progeny in crosses of niacin-requiring mutants, which he has designated "*q* mutants." These mutants do not form niacin-independent heterocaryons with one another, and there is evidence that all of them are blocked in the same step in niacin synthesis.[2] Apparently, no similar cases have been reported in which both a genetic study and a biochemical

analysis of the metabolic alterations in the mutants were performed. The *q* mutants therefore offered unusual advantages for a study of the emergence of non-mutant progeny. The origin of the *q* mutants used in this investigation is given in the accompanying table.

Symbol Used in This Publication	Isolation Number	Mutagen Used	Reference
q1	3416	X-ray	1
q2	1413	Ultraviolet	2
q3	39113	Ultraviolet	1
q4	Y31873	Nitrogen mustard	3

1. G. W. Beadle and E. L. Tatum, *Am. J. Botany*, **32**, 687, 1945.
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Reversion Behavior.—Investigations of the reversion rates of the *q* mutants have shown them to be exceedingly stable in the vegetative state. Conidia of *q1* stocks have shown spontaneous reversions of the order of 0.3–0.5 per 10^8 macroconidia. No spontaneous reversions have been recovered from macroconidia of the other *q* strains; in the case of *q4*, it was not even possible to induce reversions with ultraviolet or X-rays.

Linkage Behavior.—Since all four of the *q* mutants were originally induced in different stocks and outcrossed during the process of selection, the available stocks would be expected to differ in their genetic background. Therefore, an extensive analysis of the relationships of the four *q* mutants to certain linked loci was undertaken. These experiments were also designed to provide stocks of the *q* mutants carrying markers suitable for analysis of niacin-independent progeny which might occur in crosses.

The *q* mutants are located in linkage group I, and Pittenger[3] has shown that *q1* is closely linked to *lysine-3*[4] (the symbol used here is *lys*). In addition to *lys*, the linked markers *albino-2*[4] (*al*) and *osmotic* (*os*) were used.

The sequence of markers from the centromere is as follows: *al*, *lys*, *os*. Analysis of ascospores isolated at random indicates that there is approximately 15 per cent recombination between *al* and *lys* and approximately 20 per cent recombination between *lys* and *os*. These values, derived from a number of crosses, were here taken as standard for the genetic analysis of the *q* mutants.

The *q* mutants are located between *lys* and *os* and show approximately 0.5–1 per cent recombination with *lys*. Stocks of both *q1* and *q2* have been obtained that show the standard recombination values given above when crossed to niacin-independent stocks carrying various combinations of the markers *al*, *lys*, and *os*. However, in both *q3* and *q4*, crossing over in the interval from *al* to *lys* was significantly reduced; all strains of these mutants derived from a number of crosses and carrying various markers have shown this reduction, as have crosses of *q3* by *q3*, and *q4* by *q4*. Tests with *q3* suggest that reduction in recombination in the interval between *al* and *lys* is inseparable from the mutant character of *q3*. At present it is not known whether a similar situation obtains in the case of *q4*.

Mutant *q4* has a distinctive characteristic: in contrast to the other *q* mutants, 1–2 per cent of the progeny isolated from crosses of *q4* to various stocks do not grow well on any media tested. Therefore, it was not possible to characterize such isolates

with respect to the markers segregating in the cross. Thus, among approximately 1,000 ascospores isolated at random from crosses of *lys* by *q4*, no strain carrying both *q4* and *lys* was recognized, although the reciprocal recombinant was obtained.

Isolation of Niacin-independent Progeny.—In order to examine the possibility that niacin-independent colonies obtained from crosses between the *q* mutants arise as the result of crossing over, a number of strains were prepared with suitable combinations of markers. Crosses were then made between strains carrying the same or different *q* mutants. For convenience the former type of cross will be referred to as a "selfing." The crosses were set up in a reciprocal manner: a particular combination of markers was introduced by one parent in one series and by the other parent in a second series. Unfortunately, reciprocal crosses could not be performed with *q4* because lysine-requiring strains of this mutant were not recovered.

In these crosses it was necessary to determine whether the markers used affected the frequency of recovery of niacin-independent colonies. Accordingly, crosses of *q* mutants carrying markers were compared with analogous crosses of *q* mutants without markers; at least four separate stocks each of *q1*, *q2*, and *q3* and three of *q4* were used.

To obtain niacin-independent colonies, mature ascospores from these two types of crosses were collected in distilled water, heat-shocked in a sorbose-containing medium supplemented with any required growth factors except for niacin, plated in the same medium, and incubated at 30° C. The total number of ascospores plated was ascertained by direct count. Germination was determined by heat-shocking and plating ascospores in suitably supplemented medium containing niacin, and counting the germinated spores after incubation. In almost all crosses, 90 per cent or more of the ascospores germinated.

The results obtained indicated that the presence or absence of segregating markers did not significantly influence either the per cent germination or the frequency of niacin-independent colonies recovered. Crosses between any two different *q* mutants gave niacin-independent colonies, although only three such colonies were recovered from crosses of *q1* and *q3*. No niacin-independent colonies were recovered from any of the four possible selfings; at least 10^5 ascospores were examined for each selfing.

The frequency with which niacin-independent colonies were obtained is given in the second column of Table 1. It can be seen that the frequency of occurrence of such colonies in reciprocal crosses is approximately constant. It therefore appears

TABLE 1

DISTRIBUTION OF MARKERS IN NIACIN-INDEPENDENT ISOLATES FROM CROSSES BETWEEN *q* MUTANTS

Mutants Crossed	No. of Niacin-independent Isolates/10^4 Viable Ascospores	No. of Isolates with No Crossover between *lys* and *os* (*lys q* — *os*)			No. of Isolates with a Crossover between *lys* and *os* (*lys q* — *os*)			Total No. of Isolates Analyzed	Ratio of *lys*/*lys*$^+$ Recovered
		+ *os*	*lys* +	Total	*lys os*	++	Total		
q1 + *os* × *q2 lys* +	4.3–6.9	25	14	39	18	15	33	72	0.80
q2 + *os* × *q1 lys* +	4.1–7.5	8	13	21	7	17	24	45	0.84
q2 + *os* × *q3 lys* +	4.4–6.5	18	18	36	9	7	16	52	1.18
q3 + *os* × *q2 lys* +	4.9–6.3	10	4	14	5	2	7	21	0.75
q4 + *os* × *q1 lys* +	0.8–1.3	1	16	17	6	19	25	42	1.10
q4 + *os* × *q2 lys* +	2.1–2.5	2	13	15	13	3	16	31	5.20
q4 + *os* × *q3 lys* +	0.4–0.8	2	17	19	9	6	15	34	3.25

reasonable to conclude that the recovery of niacin-independent progeny results from events at the *q* locus in the process of crossing the different mutants.

Analysis of Niacin-independent Progeny.—For a study of the distribution of the segregating markers in the niacin-independent progeny, a sample of approximately 20–70 colonies from each type of marked cross was examined. Because of the possibility that some or all of the niacin-independent progeny were pseudo-wilds,[3, 5] each of the colonies isolated was crossed to wild type. In each case, asci consisting entirely of niacin-independent spores were obtained. Therefore, these asci presumably were not derived from a pseudo-wild parent or from a parent carrying a randomly segregating suppressor. Such niacin-independent asci were used for the determination of the distribution of markers.

The results obtained are presented in Table 1. The data have been grouped in two classes: one class represents the recovery of parental chromatids in which no crossovers in the interval from *lys* to *os* were detected, and the second class represents chromatids in which a crossover in this interval was found. Table 1 provides the following evidence regarding the question whether or not the occurrence of niacin-independence is related to crossing over:

a) If the *q* mutants are arranged along the chromosome in a linear manner and if niacin-independence is related to crossing over in a region between the *q* mutants, the majority of the niacin-independent isolates would be expected to occur in the crossover class. However, it is seen that in general there are approximately equal numbers of niacin-independent isolates in the crossover and in the noncrossover classes. Crosses of *q2* to *q3* appear exceptional in that niacin-independent colonies were recovered somewhat more frequently in the noncrossover class.

b) Table 1 shows that both of the two possible recombinant classes of *lys* and *os* are represented in the niacin-independent progeny from all crosses. However, if niacin-independence arises as the result of crossing over, the recovery of only one of the recombinant classes would be expected.

On the assumption that the class in which no crossovers were detected between *lys* and *os* represents recovery of an even number of crossovers of the two-strand type, the niacin-independent isolates would again be expected to carry one of the two possible arrangements of the *lys* and *os* markers. Table 1 shows that in general both arrangements are well represented, although in crosses involving *q4* one of the arrangements predominates.

c) If niacin-independent isolates arise as the result of crossing over between *q* mutants, the vast majority of these isolates should carry one of the two possible alleles of *lys* (because of the close linkage between *q* and *lys*). Furthermore, the reciprocal crosses should exhibit a reciprocal relationship with respect to the *lys* allele carried by the niacin-independent isolates. In the sixth column of Table 1 is presented the ratio of niacin-independent isolates that required *lys* to those that did not. In general this ratio approximates unity, although in crosses of *q4* by *q2* or *q3*, isolates carrying *lys* are in moderate excess.

The results discussed in *a*, *b*, and *c* above fail to provide evidence for the occurrence of niacin-independence as the result of crossing over in the usual sense. If crossing over were to account for the results obtained, the number of multiple crossovers that would be required in most cases is equal to, or exceeds, that of the single crossovers. On the other hand, the emergence of niacin-independent isolates

does not seem entirely unrelated to crossing over. Thus it is of interest that recombination between *lys* and *os* is in general twice as frequent in the niacin-independent as in the niacin-requiring isolates from any cross. A similar increase in recombination has been reported in investigations of other series of biochemically similar mutants.[6, 7]

In the various crosses given in Table 1, the marker classes obtained for any pair of *q* mutants seem characteristic and not random. This nonrandomness is particularly clear in crosses involving *q4*. Table 1 reveals that the marker combination brought into a given cross by *q4* is present in very low frequency in the niacin-independent progeny. Since the same marker combination appears in high frequency if introduced by a mutant other than *q4*, it would appear that the marker combination as such was not selected against. It seems reasonable to conclude that the distribution patterns of the markers in niacin-independent isolates depend on the individual *q* mutants and their interaction in crosses.

Possible Origin of Niacin-independence.—Since, in the crosses examined, the occurrence of niacin-independence does not appear to be caused by crossing over, other mechanisms must be considered. A similar problem was encountered by Mitchell[6] in her elegant and convincing analysis of the *pdx* mutants of *Neurospora* and by Lindegren[8] in his study of yeast mutants. Both authors have considered the possibility of a gene-to-gene conversion similar to that proposed by Winkler[9] some time ago. The appearance of niacin-independence in the present experiments may also be said to represent gene conversions in a broad sense or may be thought of as reflecting a kind of mutation.

Among possible mechanisms that could account for the present observations, one, suggested by the incisive studies of McClintock[10] on mutation in maize, seems especially worthy of consideration. She has shown that the insertion of chromosomal material into, or near to, the locus of a number of known mutants may cause a mutant phenotype to appear. In some cases the insertion of material leads also to changes in linkage relationships.[11] Removal of the inserted material may result in return to the original phenotype.

It may therefore well be that the niacin requirement of the *q* mutant is occasioned by the insertion of material into, or near, the functional area of the chromosome concerned with one step in niacin synthesis. Some support for this "insertion hypothesis" is provided by the present findings that at least two of the *q* mutants, *q3* and *q4*, affect linkage relationships in neighboring areas of the chromosome. It would appear likely that these mutants reflect structural alterations of the chromosomal material rather than point mutations. Mutants *q1* and *q2* may also be the result of such structural alterations, although they behave like point mutations within the relatively low sensitivity of the methods used. Occasional removal of the inserted chromosomal material in the course of crosses between *q* mutants could readily account for the appearance of niacin-independence.

Summary.—A genetic study of four biochemically similar niacin-requiring strains of *N. crassa* has been undertaken. The mutants involved (*q* mutants) have been characterized as to linkage relationships. Only one of the mutants gives rise to spontaneous reversions (at an infrequent rate) in vegetative culture.

Crosses between different *q* mutants yielded (rare) niacin-independent progeny, whereas "selfings" did not. Niacin-independent progeny were recovered whether or

not a crossover was detected in a marked region including the *q* locus, and regardless of the direction of any such crossovers. For these reasons, and from a consideration of the relative frequency of niacin-independence in the various marker classes obtained, it was concluded that niacin-independence does not seem to be the result of crossing over in the usual sense. Nevertheless, increased crossing over in the marked region appeared to be associated with the occurrence of niacin-independence.

The possibility has been discussed that various characteristics of the *q* mutants are occasioned by the insertion of chromosomal material at or near the region of the *q* locus.

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A CASE OF POLYPLOIDY IN DIPTERA

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The repeated discovery of polyploidy in animals which show parthenogenesis, hermaphroditism, or vegetative reproduction and the rarity of its discovery in strictly bisexual forms have in a sense verified the prediction made by Muller[1] that the chromosome mechanism in many bisexual forms would tend to prevent the establishment of polyploid strains. Although apparent exceptions to this general prediction are known,[2] still it seems clear that Muller's prediction holds to a large extent. In female-producing (thelytokous) parthenogenetic species no problem of