NEGATIVE HETEROSIS AND DECREASED EFFECTIVENESS OF ALLELES IN HETEROZYGOTES[1]

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INTEREST in the causes of heterosis has been renewed again with emphasis on the possibility of its being due to heterozygosity of single loci. Accordingly, any cases of heterozygotes which show effects outside of the range of phenotypes of the homozygotes deserve attention.

One series of such cases was reported recently (STERN, MACKNIGHT, and KODANI 1946). It concerns the cubitus interruptus locus in *Drosophila melanogaster*. For this locus DUBININ and SIDOROV (1934) had shown that the normal allele ci^+ which is dominant over the mutant allele *ci*, looses its dominance in many instances when a chromosome rearrangement has occured in its neighborhood. In consequence, heterozygotes of the constitution $R(+)/ci$, where $R(+)$ designates a "position allele" caused by a rearrangement near the ci^+ allele, possess a phenotype of interrupted veins. Different rearrangements produce genetically different $R(+)$ alleles which may be distinguished by the various mean degrees of vein interruptions produced in the heterozygotes $R(+)/ci$. Out of a series of 17 rearrangements labeled in order of discovery from $R^1(+)$ to $R^{21}(+)$ (of these 21 rearrangements several were not suitable for analysis) four—$R^2(+)$, $R^3(+)$, $R^{12}(+)$, and $R^{15}(+)$—were found to exhibit remarkable properties.

The simplest case is that of $R^{15}(+)$ which may be summarized as follows:

homozygotes $R^{15}(+)/R^{15}(+)$—normal
homozygotes ci/ci—moderate degree of deficient venation
heterozygotes $R^{15}(+)/ci$—extreme degree of deficient venation

Thus these heterozygotes show a "negative heterosis," being more deficient in venation than either of the homozygotes of their component alleles.

For $R^2(+)$ the facts are:

both homozygotes $R^2(+)/R^2(+)$ and ci/ci—moderate degree of deficient venation

heterozygotes $R^2(+)/ci$—extreme degree of deficient venation

Like $R^{15}(+)/ci$, the heterozygotes $R^2(+)/ci$ show negative heterosis.

Similar extreme interruptions of veins are found in $R^3(+)/ci$ and $R^{12}(+)/ci$ heterozygotes but no direct comparison with the translocation homozygotes is possible since $R^3(+)/R^3(+)$ and $R^{12}(+)/R^{12}(+)$ are inviable.

The extreme phenotypes of the four kinds of heterozygotes are significant in

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a further respect. Hemizygotes for each of these position alleles proved to possess *normal* phenotype. The fact that the "addition" of the recessive allele *ci* to the hemizygous $R(+)$, thus resulting in the heterozygote $R(+)/ci$ causes *deficient* venation may be described as a decrease in the effectiveness of the genic action of the $R(+)$ allele due to the presence of the *ci* allele. Considered in this way, it had remained undecided whether this decrease goes in one direction only—effect of $R(+)$ influenced by *ci*—or whether it is reciprocal, involving the effects of both $R(+)$ and *ci*. If the latter were true the effect of the heterozygote might conceivably be found to be lower, and resulting in more deficient venation than the effects of either one of the constituent hemizygous alleles $R(+)$ and *ci*.

This problem has been attacked on a more favorable material, by using the same $R(+)$ alleles referred to above and, instead of the mutant *ci* allele, the normal iso-allele $+^3$ (STERN and SCHAEFER 1943b). This allele, like the more frequently used normal allele $+^C$ as found in the Canton-S stock, causes wild type venation (at 26°C) both in homozygous or hemizygous constitution. Unlike $+^C$, heterozygotes of $+^3$ with *ci* give a large proportion of individuals with interrupted veins. The allele $+^3$ promised to be a sensitive tool for the discovery of *ci*-like effects of $R(+)$ alleles.

The experiment consisted of crosses between $R(+)$ and $+^3$ in order to study the heterozygotes $R(+)/+^3$. The normal allele involved in the rearrangements was $+^C$ of the Canton-S stock. As a control a cross was made between $+^C/+^C$ and $+^3/+^3$. It yielded 230 $+^C/+^3$ heterozygotes, all normal. *In contrast the heterozygotes* $R(+)/+^3$ *showed varying degrees of deficient venation* (table 1, lines 1–4). For technical reasons the data consist of the F_1 of crosses $R(+)/ey^2 \times +^3$. Two different genotypes, apart from duplication-deficiency types which survive in certain cases, are represented in the F_1, namely $R(+)/+^3$ and $+^2ey^2/+^3ey^+$ ($+^2$ is another wild type iso-allele of *ci*). The latter constitution results in normal phenotypes, as tested in a control cross involving 800 individuals, so that all individuals with deficient venation plus an unknown number of normal overlaps constitute the $R(+)/+^3$ heterozygotes.

In order to relate these results to the problem of heterosis it becomes necessary to distinguish the classical "heterosis by comparison with the homozygotes" from the rarely studied "heterosis by comparison with the hemizygotes." Since homozygotes for $R^{15}(+)$ as well as homozygotes for $+^3$ are normal, while the heterozygotes $R^{15}(+)/+^3$ are deficient in venation, these latter constitute a case of negative heterosis by comparison with the homozygotes, due to heterozygosity at the *ci* locus. In addition, the deficient $R^{15}(+)/+^3$ also represent a case of negative heterosis by comparison with the hemizygotes since both constituent hemizygotes, $R^{15}(+)$ and $+^3$, are normal, or nearly so. Negative heterosis by comparison with the hemizygotes is likewise shown by the venation-deficient heterozygotes, $R^2(+)/+^3$, $R^3(+)/+^3$, and $R^{12}(+)/+^3$, since again all constituent hemizygotes are normal or nearly so. However, $R^2(+)/+^3$ does not represent a case of negative heterosis by comparison with the homozygotes since $R^2(+)/R^2(+)$ are more deficient in venation than the heterozygotes.

The experiments were broadened by obtaining heterozygotes for $+^3$ and four other $R(+)$ alleles, $R^{11}(+)$, $R^5(+)$, $R^8(+)$, and $R^{21}(+)$ (table 1, lines 5–8). These alleles in heterozygotes with *ci* cause only lower mean degrees of deficient venation, within the range of normal and that produced by *ci/ci*. Parallel to the slighter deficient effect of these $R(+)/ci$ heterozygotes as compared with the earlier discussed greatly abnormal $R(+)/ci$ heterozygotes, the heterozygotes $R^{11}(+)/+^3$, $R^5(+)/+^3$, $R^8(+)/+^3$, and $R^{21}(+)/+^3$ as a group cause less deficient venation than the earlier named group of $R(+)/+^3$. Indeed, $R^{11}(+)/+^3$ gave all but one individual which were completely normal, in agreement with the fact that the $R^{11}(+)/ci$ heterozygotes also were less deficient than any other of the 19 $R(+)/ci$ constitutions studied. There exist exceptions to the parallelism in seriation of $R(+)/+^3$ and $R(+)/ci$ phenotypes. They are partly to be expected from secondary genetic complications (see the discussion of $R^3(+)$ and $R^{15}(+)$ in STERN, MACKNIGHT, and KODANI 1946), and partly

TABLE 1

F_1 of 2 $R(+)/ey^2 \times 1+^3/+^3$ 26°C

(Class 0 signifies normal venation, classes 1–4 increasing degrees of deficient venation, STERN, MACKNIGHT, and KODANI 1946)

		♀♀					♂♂				
$R(+)$	Allele	0	1	2	3	4	0	1	2	3	4
$R^{12}(+)$		311	11	56	43	12	258	5	47	59	14
$R^2(+)$		307	8	44	72	7	290	7	35	77	6
$R^{15}(+)$		219	42	56	21	—	212	25	68	31	2
$R^3(+)$		225	27	59	22	1	177	19	60	26	—
$R^{11}(+)$		269	1	—	—	—	295	—	—	—	—
$R^5(+)$		231	31	86	33	—	250	22	67	14	—
$R^8(+)$		174	28	67	43	3	157	28	54	27	3
$R^{21}(+)$		524	85	127	39	1	508	69	132	60	1

due to statistical variations. For the purpose of the present report the three genotypes $R^5(+)/+^3$, $R^8(+)/+^3$, and $R^{21}(+)/+^3$ add further cases in which the heterozygotes between $R(+)$ alleles and $+^3$ show deficient venation. Since the hemizygotes of the alleles $R^3(+)$, $R^{12}(+)$, $R^{15}(+)$, and $R^2(+)$ as well as the hemizygotes of three other $R(+)$ alleles studied by DUBININ and SIDOROV are normal in venation it may be assumed that the same is true for the hemizygotes of $R^5(+)$, $R^8(+)$, and $R^{21}(+)$, which in heterozygotes proved to be more closely normal than the former named alleles. For the same reason and in agreement with the normal phenotype of $R^{15}(+)/R^{15}(+)$, and of the two other $R(+)$ homozygotes studied by DUBININ and SIDOROV, it may be assumed that the homozygotes of $R^5(+)$, $R^8(+)$ and $R^{21}(+)$, if viable, will cause normal phenotypes. The deficient venations caused by the heterozygous constitutions $R^5(+)/+^3$, $R^8(+)/+^3$, and $R^{21}(+)/+^3$ may therefore be regarded as further

cases of negative heterosis, by comparison with both the homo- and hemizygotes, due to heterozygosity at the *ci* locus.

It is clear that there is a decrease in effectiveness of *both* the $+^3$ and the $R(+)$ alleles since the heterozygotes are more deficient in venation than either $+^3$ or $R(+)$ hemizygotes. Such decrease in phenotypic effectiveness is in contrast to the observations that increases in gene dosage from one mutant *ci* to three *ci* alleles lead to increasingly less deficient phenotypes, as do increases in dosage (at 14°C) from one $+^C$ or one $+^3$ to two $+^C$ or two $+^3$ alleles (STERN 1943). The increasingly more closely normal venation covered in these dosage experiments extends over the same range in which the decrease in effectiveness of heterozygous combinations occurs, as reported in the present paper.

The mechanism of this decrease of effectiveness is a matter of speculation. Conceivably, a kinetic scheme may be invented according to which some or each of the different alleles exert both vein promoting and inhibiting influences, and where these opposing influences operate at different levels of saturation curves. On the other hand the decrease of allelic effectiveness in certain combinations may be the result of a mutual antagonism of the alleles involved. If this is the case, it may be inferred from a consideration of $R^{15}(+)/+^3$, that the mutual inhibition is probably not due to direct interaction of the chromosomal alleles themselves. $R^{15}(+)$ represents an insertion of an excised section of chromosome 4 into the middle of the left arm of chromosome 2 (STERN, MACKNIGHT, and KODANI 1946). The insertion carries the $R^{15}(+)$ allele and, in salivary gland nuclei, does not pair with the homologous region of the intact chromosome 4. It may be assumed that no pairing occurs in the nuclei of other cells either so that there is not sufficient proximity between the loci of $R^{15}(+)$ and of $+^3$ to permit a direct interaction. The indirect interaction may be thought of in terms of competition at later developmental stages between different products of the action of the different alleles. As an alternative it may consist of a mutual, partial inactivation of the alleles by their products or in a mutual antagonism of the products themselves which might result in their partial inactivation. Whatever the interpretation, it must finally include the fact that a decrease in effectiveness may occur in the transition from hemizygous to homozygous constitution: hemizygous $R^2(+)$ veins are normal while homozygous $R^2(+)/R^2(+)$ are deficient (STERN, MACKNIGHT, and KODANI 1946). Moreover it should be noted that a decrease in allelic effectiveness is not restricted to genotypes containing position alleles. A case similar to the interaction of $R(+)$ alleles and $+^3$ has been described earlier for the spontaneous mutant allele ci^W, and $+^3$: hemizygotes for ci^W and $+^3$ are nearly or fully normal, but the heterozygotes $ci^W/+^3$ are greatly deficient in venation (STERN and SCHAEFFER 1943a). Similar to the case of $R^2(+)$, homozygous ci^W flies are more deficient in venation than are hemizygous ci^W individuals.

As unusual as the phenomenon described in this report may appear it does not stand completely alone. The heterosis at the *ci* locus of Drosophila may be compared with the situation in Habrobracon (WHITING 1943) where hemi- and homozygotes for different sex alleles result in maleness, while heterozygotes for these alleles develop into females. In the guinea pig the coat color

caused by certain genotypes is more intense brown in *Pp* than in either *PP* or *pp* animals (WRIGHT 1947). It may be pointed out further that mycelia of *Neurospora crassa* heterocaryotic for the sex alleles *A* and *a* show a different growth from that of homocaryotic *A* and *a* cultures (BEADLE and COONRADT 1944). Finally, the interaction of the *ci* alleles offers similarities with that of sterility alleles in *Oenothera organensis* (D. LEWIS 1947) where two alleles in heterozygous diploid pollen permit tube growth in styles on which haploid pollen containing either of the constituent alleles does not grow.

SUMMARY

Hemizygotes and at least one type of homozygotes for a series of position alleles $R(+)$, at the cubitus interruptus locus of *Drosophila melanogaster*, possess normal venation, as do hemizygotes and homozygotes for the normal allele $+^3$ of this locus. Heterozygotes $R(+)/+^3$ exhibit various degrees of deficient venation. Thus decreases in effectiveness of the alleles $R(+)$ and $+^3$ occur when they are joined together in one genotype. These decreases furnish cases of negative heterosis due to heterozygosity at a single locus. It is pointed out that a distinction needs to be made between heterosis by comparison with the homozygotes and by comparison with the hemizygotes.

LITERATURE CITED

BEADLE, G. W., and VERNA L. COONRADT, 1944 Heterocaryosis in *Neurospora crassa*. Genetics **29:** 291–308.

DUBININ, N. P., and B. N. SIDOROFF, 1934 Relation between the effect of a gene and its position in the system. Amer. Nat. **68:** 377–381.

LEWIS, D., 1947 Competition and dominance of incompatibility alleles in diploid pollen. Heredity **1:** 85–108.

STERN, CURT, 1943 Genic action as studied by means of the effects of different doses and combinations of alleles. Genetics **28:** 441–475.

STERN, CURT, R. H. MACKNIGHT, and M. KODANI, 1946 The phenotypes of homozygotes and hemizygotes of position alleles and of heterozygotes between alleles in normal and translocated positions. Genetics **31:** 598–619.

STERN, CURT, and ELIZABETH WHITE SCHAEFFER, 1943a On primary attributes of alleles in *Drosophila melanogaster*. Proc. Nat. Acad. Sci. **29:** 351–361.

1943b On wild type iso-alleles in *Drosophila melanogaster*. Proc. Nat. Acad. Sci. **29:** 361–367.

WHITING, P. W. 1943 Multiple alleles in complementary sex determination of Habrobracon. Genetics **28:** 365–382.

WRIGHT, SEWALL, 1947 On the genetics of several types of silvering in the guinea pig. Genetics **32:** 115–141.