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Nature of heterosis and combining ability in the silkworm

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Summary. The isogenic, highly heterotic parthenoclone 29, originating from a hybrid silkworm female, was transformed via unisexual reproduction (meiotic and ameiotic parthenogenesis) into four genotypical variants differing in well-known various levels of heterozygosity and combinations of useful and harmful genes. A comparison of these changes with the heterosis level made it possible to discover that both heterosity for adaptively neutral genes (overdominance hypothesis) and the number of allelic pairs, each of them being heterozygous for a favourable, completely dominant gene (dominance hypothesis) play no decisive role in the intensity of heterosis. The level of heterosis is largely determined by the relationship between the effects of useful and harmful genes, the first falling into the category of semidominant, cumulatively acting genes which control viability. Their favourable, joint well-coordinated effects, unlike those of genes which control quantitative characters, increase in relation to the number of genes in a geometric rather than an arithmetic progression. The interaction between semilethal genes is subjected to the same regularity. The high combining ability of parthenoclone 29 variants is determined by the number and homozygosity of the useful genes.

Key words: Heterosis – Combining ability – Parthenogenesis – Silkworm

Introduction

According to generally acknowledged concepts the main causes of heterosis in F_1 hybrids are the extinction of the action of harmful recessive genes, the favourable combination of nonallelic, totally dominant genes separately inherited from both parents (dominance hypothesis), and the favourable effects of some alleles in the heterozygous state (overdominance hypothesis).

The results of earlier experiments (Strunnikov 1974, 1983) have shown that, in addition to the above-mentioned causes of heterosis, an important role is played by the additive action of non-allelic and allelic dominant genes responsible for viability. Proceeding from this evidence we advanced a hypothesis of heterosis. In individuals of a population which have undergone selection for viability under unfavourable genetic and certain non-genetic conditions, well-coordinated compensating gene complexes (CGC) are formed which extinguish the indicated harmful effects. CGC consists of dominant and semi-dominant genes, mainly in the homozygous state. It was shown experimentally that semilethals abruptly decreasing viability served as a rather efficient depressing factor. After increasing the viability of these lines up to the norm, and subsequently being crossed with non-selected lines, the F_1 hybrids received one dose of sufficiently coordinated genes of the complex coming from the line under selection. At the same time the depressing action of the semilethal was eliminated due to its transition to the heterozygous state. The excessive number of favourable genes not being balanced by the semilethal brought about an increase in viability and intensive development of the characters which had been depressed in the parental line.

In the mulberry silkworm (Strunnikov 1974) and *Drosophila melanogaster* (Maresin et al. 1985) the lines selected for heterosis were crossed with other lines and produced hybrids whose heterosis was 15 and 20% higher, respectively, than in the genetically similar

hybrids which differed only in the absence of semilethals in their parental forms not selected for viability.

In the beginning of the 70's, immediately after advancing the hypothesis of heterosis, we inferred that silkworm parthenoclones of the ameiotic type, whose eggs developed with an unreduced nucleus, should have high combining ability (CA) (Strunnikov 1982). This prognosis was based on the decreased viability of parthenogenetic eggs. Practically all the activated eggs commenced parthenogenetic development but only 5–6% of them reached the hatching of larvae stage. Completely formed larvae were not hatched from other eggs since they could not gnaw through the shell. In viable lines, and in F_1 hybrids in particular, the percentage of hatched parthenogenetic larvae increased: in especially selected parthenoclones it reached 90–95%.

We assumed that when selecting for disposition towards parthenogenesis (DP), a complex of favourable dominant genes which compensate the negative effect of parthenogenesis is also formed. The experimental results convincingly supported the validity of this supposition. Heterosis in the hybrids from crosses between 11 different parthenoclones and the line 'Japan 115' (J-115) was not lower, and sometimes even higher, than that found in the best industrial hybrids obtained from crosses of genetically remote lines.

The advanced hypothesis of heterosis adequately explained many peculiarities of heterosis (Strunnikov 1974), however, it required direct experimental evidence.

The purpose of the present investigations is to reveal the relative role of each of the four causes of heterosis. It goes without saying that an ideal method for solving such a complicated task would be the transformation of a single highly heterosis genotype into different strictly controlled genotypical variants with varying degrees of both heterozygosity and ratios between useful and harmful genes. The comparison of genetic changes in each variant displaying a new heterosis level relative to that of the initial genotype would permit a distinguishing of the role of each heterosis factor. The evaluation of CA quality in various genotypical variants would help elucidate the nature of CA development.

Convergent crosses, which made it possible to change the level of heterozygosity, have already been used for such investigations. These experiments concluded that there was no parallelism between heterosity and heterozygosity (Richey 1927). However, the employment of crossing and selection in these experiments did not permit an unambigous conclusion to be drawn.

Truly accurate studies have only been possible with the breeding of isogenic parthenogenetic clones of the silkworm and the development of various methods of their unisexual reproduction. This enabled us to obtain variants of the same genotype, paternal hereditary information being excluded.

Material and methods

Female parthenoclone 29 (PC-29), bred by B. L. Astaurov (1978), was used for the experiment. It originated from a complex hybrid female comprising hereditary information of several genetically dissimilar lines. As a result, the parthenoclone produced proved to be heterozygous for many convenient gene markers. Because of the inherited characters controlled by these genes, it was easy to establish the genotypes of PC-29 variants. The production of about 90% parthenogenetic larvae points to a high viability of this clone, which is similar to that of best industrial hybrids. Using not one genotypical variant but a great number of genetically identical individuals essentially simplified and increased the accuracy of the experiment, since the material could then be calculated by simple methods of variation statistics.

Changes in the highly heterotic genotype of PC-29 were related to the level of heterozygosity of various types of genes and to the degree of extinction of harmful recessive genes: i.e. those very properties which are believed to determine heterosis. The required variants of the PC-29 genotype were obtained via several, sometimes combined, means of reproduction: normal, parthenogenetic of ameiotic and meiotic type, and polyploidization, which is reported in detail in the following section.

Results and discussion

The first control variant of PC-29 was produced via ameiotic parthenogenesis induced by heating unfertilized eggs at 46 °C for 18 min. By means of such reproduction, the PC-29 genotype was maintained in an unchanged form for 14 generations. PC-29 was used in our experiments as control in order to compare its genotypical variants with it. Heterozygosity, average mass of one cocoon, and viability of PC-29 were taken as 100%, and parameters of other genotypical variants were estimated with respect to this level (Fig. 1).

The second variant of PC-29 was produced as a result of subsequent polyploidization and depolyploidization of PC-29 which brought about a successive change of ploidy: $2n \rightarrow 4n \rightarrow 2n$. For this purpose the diploid PC-29 was transformed into an autotetraploid ($Aa \rightarrow AAaa$) (Ruban 1983). Then the autotetraploid unfertilized eggs were activated by cooling (–11 °C, 30 min) to meiotic parthenogenesis which started on the basis of a diploid pronucleus. During the change of 4n into 2n, each heterozygote pair of alleles, as shown by our experiments (Strunnikov et al. 1980), underwent theoretically predicted changes: $AAaa$ into $1AA:4Aa:1aa$.

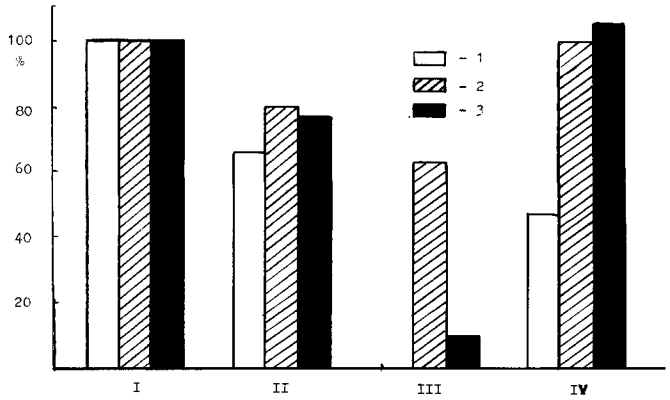

Fig. 1. Indices of heterozygosity (*1*); mean mass of one cocoon (*2*), viability (*3*) of PC-29 (*I*) and its genetical variants (*II–IV*) in the mulberry silkworm

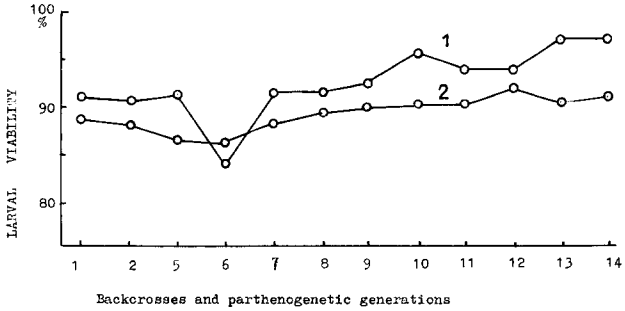

Fig. 2. Viability of backcross generations (*1*) and PC-29 (*2*)

Thus, depolyploidization of the PC-29 autotetraploid resulted in the initiation of diploid females with innumerably variable genotypes. The level of heterozygosity increased up to 66% on the average as compared to the ploidy of the initial diploid PC-29 (taken as 100%).

At the same time the mean mass of one cocoon and viability decreased as much as 80% and 77%, respectively.

The third variant of PC-29 was produced via meiotic parthenogenesis induced by cooling at −11 °C for 30 min. An important feature of this form of parthenogenesis was that all the parthenogenetic offsprings were solely males homozygous for all loci ($Aa \rightarrow AA : aa$) (Terskaya and Strunnikov 1975).

Due to the large number of recessive lethals and semilethals transformed into a homozygous state, parthenogenetic males had extremely low viabilities: of the artifically activated eggs, only about 0.5% males survived; on the average about 10% of the number which hatched as larvae. The mean mass of one cocoon decreased almost in half. The number of unextinguished harmful recessive genes was in this case close to the limiting one, after which the whole population would perish.

Analysing the first three genotypical variants of PC-29 one can see a classical example of a relationship between mass of one cocoon viability and the degree of heterozygosity. As similar relationships have been found in the literature it seemed that our findings were valid. The next variant of PC-29 changed these concepts fundamentally, however.

This fourth variant of PC-29 was produced by backcrossing homozygous parthenogenetic males with initial PC-29 females.

The backcrosses were performed as follows: homozygous parthogenetic males were obtained from PC-29 females; these were subsequently crossed with simultaneously reared PC-29 females, producing the first bisexual generation (F_{b1}). In the following generation parthenogenetic males were again bred from the reproduced PC-29 females, and then the first were crossed with females F_{b1}, producing F_{b2}, etc.

The experimental results surpass all possible concepts. Notwithstanding the mode of reproduction, which in principle corresponds to self-fertilization, the mean mass of one cocoon in 14 backcrosses (on the average) proved to be at the level of the initial PC-29, and viability was even higher than in the initial clone (104%).

Taking into consideration the singularity and importance of these data we investigated the viability of 14 successive F_bs (Fig. 2). In the first 6 backcrosses it proved to be similar to that of PC-29, but in F_{b7}–F_{b13} viability of backcrosses generations averaged 92.7%, compared to 88.4% in PC-29 ($P=0.01$). In four experiments one of the best known heterosis hybrids PC-29 × J-115 (control) was reared together with backcross generations F_{b1}, F_{b2}, F_{b5}, F_{b8}. Viability of backcross generations reached 91.3% against 93.7% in the control. The difference was unreliable. Thus viability of later backcross generations exceeded that of PC-29 and was practically at one level with the best bisexual hybrid, although this type of reproduction, as mentioned above, can be attributed to automixis.

When starting to analyse this phenomenon, it should be noted first of all that peculiar genetic processes occurred in the course of the backcrosses. The initial heterozygote *Aa* of PC-29, consisting of both adaptively neutral alleles, produced two equally viable classes of parthenogenetic males: *AA* and *aa*. The offspring from their crosses with the initial females of PC-29 (*Aa*) had the genotypes $1AA : 2Aa : 1aa$. It is easy to calculate that in the following backcross generations this genotype ratio will be steadily repeated: i.e. heterozygosity will be constantly maintained at 50%.

On the contrary, adaptively non-equivalent alleles of one gene pair behaved otherwise: the adaptively useful ones were accumulated in the backcrosses in a homozygous state, while the harmful ones were eliminated because of the death of their carriers. Consequently, the total number of heterozygous pairs from adaptively neutral and useful alleles should be below 50% in comparison to PC-29.

This evidence points to the absence of a positive correlation between heterosity and the level of heterozygosity and substantially reduces the role of the overdominance hypothesis. The dependence of viability on the heterozygous condition of some rare alleles has been reported by a number of researchers but such a relationship is probably not universal. As seen in our experiments, this relationship seems to be deceptive. The data on backcrosses show that it is not heterozygosity of all the genes that is most important in the rise of viability, but heterozygosity of recessive lethals and semilethals. The robust viability of highly homozygous species due to automixis, and the high viability of our homozygous lines with removed semilethals, negates the fatality of the harmful action of homozygosity on adaptively useful and neutral genes. On the contrary, a homozygous state of adaptively valuable genes is useful for the manifestation of high viability.

It should be noted here that in standard experiments one cannot distinguish between the effects on viability produced by heterosity of semilethals on the one hand and that of adaptively useful and neutral genes on the other.

Now we have only to analyse the importance of extinguishing recessive semilethals in the manifestation of heterosis.

In the backcross line the number of extinguished semilethals having normal alleles was lower than that found in PC-29. It was reported by us earlier that completely homozygous parthenogenetic males, though being to a great extent freed from lethals and semilethals, were rather strongly depressed by partly complete semilethals with a weak effect in the homozygous state. Among the many thousands of parthenogenetic males obtained no individuals with a cocoon mass close to the norm were observed. Such a phenomenon cannot only be explained by unfavourable physiological effects resulting from meiotic parthenogenesis because: 1. all the bisexual lines obtained whose individuals were genetic copies of the initial male had lower viabilities and smaller cocoons, similar to their homozygous prototypes, in spite of the fact that these lines were reproduced in a standard way (Strunnikov et al. 1983); 2. depolyploidized females also obtained via meiotic parthenogenesis also had larger cocoons than the parthenogenetic males, since these females were homozygous only by 25% compared to the initial parthenoclone (Ruban 1983). The first backcross of parthenogenetic males, homozygous, for instance, for a number of semilethals, with PC-29 females indispensably heterozygous for the same semilethals resulted in the appearance of backcross zygotes. One half of them were heterozygous, another one homozygous for each of semilethals (PP: ♀ + /l × ♂ l/l → F_1 + /l, l/l). Since a number of the parthenogenetic males of the following generation may also turn homozygous for the same semilethals, this homozygosity will remain in subsequent generations. These findings indicate that high viability in backcross generations is not stipulated by a larger number of extinguished semilethals in the initial PC-29.

In accordance with D. Johe's hypothesis it is believed that one of the main causes of heterosis is an increased number of allelic pairs in hybrids F_1 as compared to each of their parents. These allelic pairs were heterozygous for favourable, completely dominant genes which had been separately inherited by the hybrids from both their parents. PC-29 seems to have many such heterozygous pairs. However, the sum of hetero- and homozygotes for useful dominant genes (*Aa* and *AA*, for instance) will be always smaller in backcross generations than the number of heterozygotes for these genes in PC-29. As already mentioned, in backcrosses, some recessive harmful alleles passed into the homozygous state. Consequently our investigations do not support the dominance hypothesis in its strictest form.

Thus, in our study, all those genetic factors which are believed to induce heterosis did not promote the maintenance of heterosis in backcross generations although heterosis was still preserved in them at the level of the initial high-heterosis PC-29. This surprising phenomenon can be explained only by the fact that the genes responsible for viability were semidominant and that their action in the homozygous state was stronger than in the heterozygous one. The possibility of such an additive action of two allelic genes was considered but in the final analysis rejected because a number of the F_2 offsprings should have the maximal set of dominant genes in the homozygous state while the latter should exhibit the same heterosis as in the hybrid F_1. Yet no such phenomenon was observed. It seems to be due to the fact that in F_2 semilethals passed into the homozygous state with the same frequency as the dominant genes, therefore, there was no heterosis effect.

In our backcross generations the effects of the semilethals were abruptly reduced due to their preceeding elimination; subsequently powerful positive effects of homozygosity of semidominant and dominant useful genes were manifested with singular intensity.

An abrupt shift in the viability level induced by changes of the positive/negative gene ratio observed in

our experiment indicates that the expression of each category of these genes did not correspond to the simple arithmetic sum of their effects, as is the case with polymeric inheritance of quantitative characters. This effect increased in a somewhat geometric progression with a yet unknown denominator. During the change of useful/harmful genes ratio which resulted not only from the elimination and extinction of the first ones, but from the accumulation of the second ones as well, viability should also increase in the geometric progression but with an even greater denominator. From this viewpoint one can better understand the expression of viability and heterosis and see that it is wrong to calculate heterosis intensity only by means of the elementary or arithmetic addition of dominant genes effects. At the same time the new approach to the complex interaction of useful genes opens wider possibilities for heterosis control.

The following experimental series dealt with CA in PC-29 and its two variants: backcrosses (designated I in Fig. 2) and homozygous parthenogenetic males (II in the same figure). The yield of hybrid cocoons produced by crossing PC-29 and each of its variants with J-115 served as CA criterion. The hybrids compared were reared under similar conditions for 7–8 rearing seasons. There were no less than 4 repetitions with 150–200 larvae in each experimental variant. CA of PC-29 proved to be very high, therefore the viability of the hybrid produced from crossing PC-29 with J-115 taken as a control was higher ($P=0.001$) than that of PC-29 (Fig. 2).

The yield of F_1 hybrids produced from crossing J-115 with backcrosses of the first four generations not made sufficiently homozygous for useful genes, did not exceed that of the control hybrid. But as shown in Fig. 2, the yield of the hybrids from backcrosses more homozygous for these genes was in the following seven generations 5% higher ($P=0.001$) than in the control hybrid. The F_1 hybrids of J-115 × PC-29 homozygous males in eight independent experiments carried out in different years and rearing seasons proved to be 7% ($P=0.001$) more productive than the control hybrid. There were no special experiments performed to state the reliable difference in the yield of hybrids originating from two variants of PC-29. This difference was so insignificant (2%) that a great number of experiments would be required to prove its reliability.

The data obtained point to the inverse relationship between CA level and heterozygosity. It was more pronounced in hybrids produced from crosses between J-115 and PC-29 and homozygous males of this clone. Analysis of the genetic difference between these hybrids and their parents throws additional light on the nature of CA and its development. Fig. 3 gives a detailed pattern of all genetic events which take place when

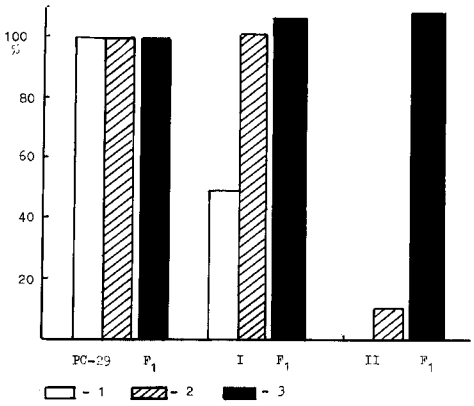

Fig. 3. Indices of heterozygosity (*1*), viability (*2*), of PC-29 and of its two genetical variants (*I* and *II*) against PC-29 (taken as 100%) and of the yield of hybrids (*3*) resulting from crossing each of the three variants mentioned with J-115. Hybrid F_1 of PC-29 × J-115 is considered to be 100%

producing hybrids from crosses of J-115 with PC-29 in one case and with homozygous parthenogenetic males produced from the same PC-29 in another.

The ameiotic females of PC-29 were obtained by heating unfertilized eggs at 46 °C for 18 min and parthenogenetic males by cooling the same eggs at −11 °C for 30 min.

The cooled oocytes of PC-29 ameiotic females (see the left and right parts of Fig. 3) gave gametes genetically similar to those produced one generation later from similar oocytes but stimulated towards meiosis by the sperm (see the central part of Fig. 3). The significant feature of this experiment was that the gametes obtained as a result of cooling before fusing with J-115 gametes passed an unusual diplophase as homozygous males of meiotic origin. Only after this, i.e. one generation later, were the gametes of these males and standard gametes of ameiotic females simultaneously fused with J-115 gametes during fertilization.

Shown on the left of Fig. 3 is a tentative variant with assumed 100% viability of gametes and the homozygous parthenogenetic males formed by them. Since the gametes of each male were identical to the one from which this male had been originated, it is clear that the set of initial gametes should be equal to those of homozygous males. At the same time the same gametes of homozygous males ought to be similar to those obtained via meiosis after egg insemination. Consequently, the cytogenetic mechanism of meiotic parthenogenesis alone could not bring about changes in the genetic structure of gametes.

Since the initial gametes of parthenogenetic males and females in their turn fused with similar J-115 gametes, in the course of fertilization the produced hybrids ♀ PC-29 × ♂ J-115 and ♀ J-115 × ♂ homozygous from PC-29 in the tentative variant must also have

similar genotypes and hence display the same heterosis intensity. Actually the hybrid ♀J-115 × ♂ homozygous from PC-29 turned out to have more intensive heterosis than the control hybrid ♀PC-29 × ♂J-115 (see the right part of Fig. 3).

Consequently the gametes of homozygous males participating in the formation of the hybrid had better CA than the set of initial gametes produced by the PC-29 females after meiosis stimulated both by cooling and insemination. Since the gametes of each homozygous male were identical to the initial gamete which gave rise to this male, it is clear that the genotypes with high CA were not initiated de novo in the course of meiotic parthenogenesis. They were present in the total set of the initial gametes and were only differentiated due to the elimination of zygotes formed by the gametes with low CA. Therefore, investigation into the genetic difference between the gametes which produced viable and nonviable zygotes should reveal the processes which brought about formation of the genotypes with high CA.

The real experimental variant (right part of Fig. 3) differs from the tentative one (with 100% viability) by the fact that in reality very strict selection proceeded. As a result only 0.5% of the males, relative to the total number of eggs which started meiotic parthenogenetic development, grew to fertility. Other zygotes perished at different developmental stages due to the effect of lethals and semilethals which had passed into the homozygous state. All the lethals and a large portion of the semilethals were eliminated during only one generation. Thus, the hybrid ♀J-115 × ♂ homozygous from PC-29, unlike the control hybrid, was completely devoid of heterozygotes for its father's lethals and had a smaller number of heterozygotes for semilethals. In spite of this fact it had a higher heterosis than the hybrid F_1 PC-29 × J-115. Consequently, heterozygosity for harmful genes in the hybrids, at least in the experiment presented, did not play a decisive role in the expressed heterosis. Accepting this, changes in the useful genes' concentration induced by the homozygosity of harmful genes in the parthenogenetic diplophase seem to be responsible for gamete differentiation. Lethals cannot be classified as harmful genes which launch genetic mutations because with the lethals being independently distributed in meiosis and having zero adaptive value, the elimination of gametes burdened with such lethals would not initiate the difference between the eliminated and preserved genotypes. Quite another genetic situation was created by the transition of semilethals into the homozygous state. Undoubtedly, semilethals with the most harmful effect were eliminated during the homozygous diplophase. However, the concentration and efficiency of preserved semilethals in survived meiotic males was so high that all homozygous males without exception grew, developed very slowly, and produced 2–3 times lighter cocoons than the normal ones.

It is quite evident that under such hard genetic conditions, when each homozygous male was literally at the verge of dying, selection for useful genes which compensated the harmful effect of non-allelic homozygous semilethals should play a significant role. Only those males in which the effect of dominant and homozygous genes-compensators was stronger than that of the semilethals survived.

What events take place in the course of meiotic parthenogenesis? It has already been mentioned that in hybrid females of PC-29 not all the useful genes passed into the homozygous state during selection for predisposition towards parthenogenesis. The recessive lethals and semilethals abundant in the parthenoclone genotype were mostly in the heterozygous state, otherwise the females would not express such powerful heterosis.

If the female is heterozygous for some lethal recessive gene (l/+), then during meiotic parthenogenesis, the genotype of the cleavage nucleus is l/l and +/+. It goes without saying that the embryos originating from the first genotype nucleus die. And in the case where the recessive gene is semilethal, the fate of males homozygous for this gene, as mentioned already, totally depends on the composition of withstanding non-allelic useful genes. Let us assume that a female of PC-29 was heterozygous for 4 genes and that the alleles of each of them produced a somewhat non-equivalent effect on the viability of the organism. Let us designate the best genes by capital letters and the alleles producing the weaker effect by the small ones. The female genotype should look as follows: *Aa, Bb, Cc, Dd.* The extreme variants of parthenogenetic male genotype are *AA, BB, CC, DD . . . aa, bb, cc, dd.*

The intermediate genotypes have a descending number of useful genes.

It is evident that the parthenogenetic males with a lesser number of useful gene pairs have a lower possibility to survive if there occurs some semilethal gene in the homozygous state in their genotype. Thus, in the surviving parthenogenetic males, unlike the initial parthenoclone, all, or the majority, of useful genes will be in the homozygous state. It is enough for the parthenogenetic males to exhibit in their hybrids a higher heterosis than their mothers did, since the latter out of two adaptively non-equivalent genes *Aa* transmit the best gene *A* to only a half of the hybrid offspring and the homozygous male *AA* – to the whole progeny. Consequently, in the latter case the hybrid obtains the whole complex of useful genes, although in one dose. The presence of this complex and the extinguishing of semilethals in hybrids provide certain conditions for the expression of heterosis.

Thus again, as in our earlier experiments (Strunnikov 1974) we come across the initiation of the formation of CGC as a response to the transition of semilethals into the homozygous state. Only now this process proceeds not gradually during the course of successive generations but during one generation only. In spite of this fact, a strong effect of selection for higher concentrations of gene-compensators was achieved, which may be, evidently, explained by the following: first, in the initial PC-29 a sufficiant number of these genes was present because this clone had been bred as a result of long selection for predisposition towards parthenogenesis; second, the strong effect of selection was determined by a high concentration of semilethals in the gametes of the initial population, which caused extremely high selection. These factors are quite important and they should be always kept in mind when planning such investigations.

It is apparent from the two experiments described above that the complex of useful genes served mainly as the basis for CA. But at the same time an ambigous pattern of CA is clearly manifested. If CA is considered in terms of its ability to produce more vigorous offsprings at any, not necessarily genetically, distant crossings, then CA will be determined by the ratio between useful and harmful genes (lethals, semilethals). The latter may turn up in the second parent and therefore pass in the F_1 hybrids into the homozygous state. During the hybridization of genetically remote forms, which is usually accompanied by almost complete extinction of harmful genes, CA is almost entirely determined by CGC. CGC of parents with low viability may be extremely valuable, but it is not expressed due to the depressing effects of harmful genes. The nature of this phenomenon is such that in the F_1 hybrids after the suppression of all the harmful genes, the complex of useful genes acts with all its vigour since it is no longer required for the struggle against harmful genes.

These findings lead to a somewhat paradoxical conclusion: CGC lies at the basis of heterosis and CA, but they are expressed differently in hybrids and parental forms. CGC which did not manifest itself in parents displayed a powerful heterosis in F_1 hybrids, and vice versa, CGC which provided a powerful heterosis in the F_1 hybrids lost their combining value, giving mediocre hybrids in the course of further hybridization. These contradictory phenomena can be explained by the above experiments. The point is that in the hybrids F_1 the complex of useful genes providing heterosis can at the same time have a good CA only when the homozygosity of its genes is high enough. If there is not complete homozygosity, the reduction division results in the formation of gametes with various ratios between useful and harmful genes. Therefore, the gametes with poor CA will also be present and form zygotes with low viability. The portion of the useful genes which the F_1 hybrid obtains from its parent seems to be in the heterozygous state and therefore their CA abruptly decreases.

As additional evidence the results of one more experiment are presented.

We managed to develop a method for producing triploids resulting from fusion of a non-reduced female nucleus (2n) with a haploid male nucleus. Such triploids were obtained by heating eggs of PC-29 females inseminated by the sperm of males of other varieties. These triploids received a valuable genotype of PC-29 (two genomes) with two unchanged CGC which conditioned powerful heterosis in this clone. The autotetraploid of PC-29 was also available. When crossed with males of the lines used in the previous experiment the reduction equation with a formation of female diploid gametes of different genotypes proceeded in the inseminated eggs. When two pairs of alleles (*AAaa*) were present in the autotetraploid genotype, their gametes involved three classes $1AA:4Aa:1aa$, while in the first experiment all the diploid nuclei without reduction had *Aa*. If the allele *A* is more valuable adaptively than the allele *a*, it is natural that *aa* individuals have lower CA. The results of four separate experiments presented in Fig. 4 fully proved these concepts. In all experiments the average viability

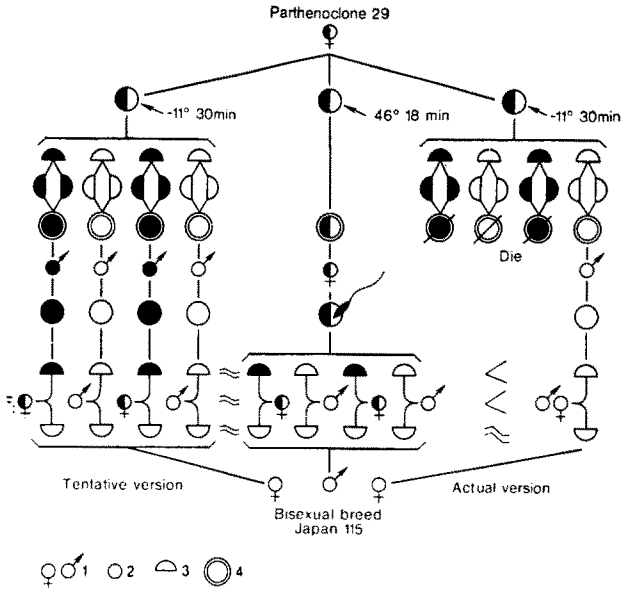

Fig. 4. Cytogenetic processes in the course of bisexual and parthenogenetic reproduction of meiotic and ameiotic types explaining the initiation of increased combining ability in totally homozygous parthenogenetic males. *1* individuals; *2* oocytes and spermatocytes; *3* gametes (depending on allele – *dark* or *white semi-circles*); *4* zygotes. Heterozygotes for the pair of alleles – *half dark circles*

of trihybrids arising from crosses of the autotetraploid line was 69.8%, the mean mass of one cocoon −1.36 g. The trihybrids obtained by inseminating eggs with an unreduced female nucleus had 80.2% and 1.55 g ($P=0.001$), respectively.

This difference results from the fact that in all triploid zygotes with the unchanged female nucleus the same valuable complex of useful genes was present: from the heterotic mother. At the same time some of the triploid females with a changed female nucleus had two maternal genomes, which accidentally had a low number of useful genes. Therefore, the viability of their triploid carriers was lower. Thus in these experiments it was possible to use the genotype of the hybrid PC-29 which was both heterotic and heterozygous at the same time, as the genetic basis of high CA. In the course of usual bisexual reproduction it is impossible.

In all the above experiments F_1 hybrids were investigated. They were produced from crossing J-115 not selected for heterosis with the second parent selected for high CA by means of one of two methods developed by us. Consequently, the nature of total and specific CA was not analysed whereas its study would greatly contribute to the further understanding and control of heterosis. Having no reliable evidence one can only suggest, proceeding from our hypothesis, that with the hybridization of genetically remote parents CA will entirely depend upon the presence and integration of useful genes in CGC. A powerful compensation complex with the genes in the homozygous state will be transferred to the hybrids, although in one dose only and in a coordinated state. It seems that in this case the parental form with such CGC will have a common (similar) CA.

The effect of both strong and not so strong CGC will be increased if during the course of hybridization the second parent adds allelic and non-allelic useful genes acting in one direction with the CGC of the first form to the genotype of the F_1 hybrid. In this case specific CA will be expressed. There are many possible combinations of such useful genes and consequently it is impossible to give unambigous characteristics of specific CA. The importance of the combination of useful genes can be judged by powerful heterosis in the hybrid obtained from crossing two parents with powerful CGC formed against the background of two depressing factors.

We refer to the tentatively named line B, homozygous for the semilethal gene w_4, and PC-29, both having good CA. These forms were first crossed with one another, and the second time – with the industrial line SANIISH-30, not selected for CA. The parental forms and their hybrids were reared under very hard ecological conditions of a hot Tashkent summer. The industrial hybrid J-115 × K-108 was taken as a control.

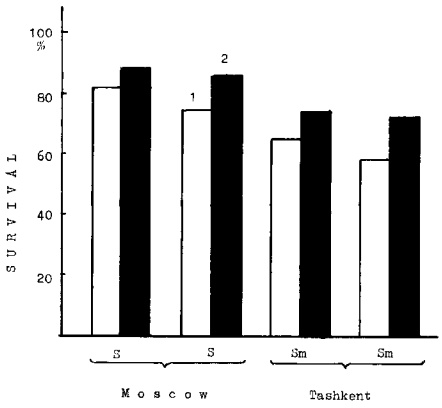

Fig. 5. Viability of silkworm triploids of meiotic (*1*) and ameiotic (*2*) origin. S = spring, Sm = summer

Table 1. Viability of hybrids produced by different crossings of two forms selected for CA

Hybrids	No. of larvae in 4 repeats	Viability (%)
PC-29 × Line B (W_4/W_4)	600	91.8 ± 2.0
Line B (W_4/W_4) × SANIISH 30	600	81.0 ± 2.6
PC-29 × SANIISH 30	600	82.7 ± 3.5
J-115 × K-108 (control)	600	73.5 ± 1.25

As shown in Table 1, the hybrid F_1 produced by crossing two parental forms selected for CA ranked first according to viability. Second place was held by two hybrids bred from one selected for the CA parent; in third place was the industrial hybrid whose both parents had not been selected for CA. The last places were held by the parental forms.

These findings open new possibilities for raising heterosis by crossing two parents separately selected for CA. This trend, insufficiently investigated as yet, seems to have many applications both in theory and in practise.

Proceeding from all these findings adaptive heterosis in hybrids can be considered as one of the higher stages of viability expression. In terms of genetics the latter does not differ from its poorer expression in the offsprings, which result from the crossing of two parents, the genetical difference between them being sometimes less than during hybridization. Thus, heterosis and inbred expression are only extreme variants of the same populational-genetic process. The basis of the extreme, as well as of the intermediate variants of the viability provision, is constituted by the achievement of limiting required predominance of these effect of useful genes over that of underdepressed harmful recessives in the homozygous state. A provision for this predominance is required by continuous mutagenesis which

constantly supplies harmful recessive mutations. In nature the reduction of their harmful action on viability is realized in three ways.

1. Elimination of harmful recessives which is performed by automixis, hemozygosity, and by complete homozygosity of one of the sexes resulting from cyclic meiotic parthenogenesis.
2. Depression of the effect of harmful recessives by their transfer into the heterozygous state which is provided by crossfertilization and ameiotic parthenogenesis giving no homozygous recessive offsprings.
3. Formation of useful genes with coordinated action, which compensate the effect of semilethals not suppressed by normal alleles.

The second and third ways are of more interest to us since they are of primary importance in crossfertilizing species to which the mulberry silkworm belongs. In these species there are usually a wide range of genotypes of varying viability and CA, each of them fluctuating around some mean genotype. In certain cases, depending on environmental conditions and interrelations with other species, the frequency of the perishing individuals, mostly having adaptively worse genotypes, also varies, but in the course of successive generations it remains near some mean value. Those individuals will survive which have genotypes with moderate, good, and outstanding viability. In favourable years, characterized by the higher survival of individuals, the population is threatened by some decrease of the genotype mean value, which is naturally not favourable for the given population. We believe that in such cases, especially in less numerous isolates, the role of harmful, semilethal mutations increases. Some of them pass into the homozygous state and stimulate selection, resulting in the formation of CGC. Thus, even under good ecological conditions, harmful semilethals are responsible for supporting the reserve of useful genes which can come into action in any moment difficult for the species. And then only the individuals with highly viable genotypes will survive.

Such a role for the semilethals in nature seems more probable in the case when the harmful effect of these semilethals is not strong and consequently it can be easily compensated for by the corresponding gene complex. In terms of the advanced hypothesis of heterosis, it is now possible to explain the causality of the main manifestations of heterosis which could not be done exhaustively proceeding only from the hypotheses of dominance, overdominance and genetic balance.

Among the phenomena being deciphered are the following:

1. The preservation of CGC in only F_1s and its destruction (shown by us experimentally) which was accompanied by a decrease in combination value, as well as the transition of a number of the recessive genes into the homozygous state beginning from F_2, explain the powerful manifestation of heterosis in F_1 and its gradual extinguishing in successive generations.
2. Since CGC are formed in parental forms in response to the depressed characters vitally important for the organism, it becomes clear why in F_1, heterosis for these very characters is mainly expressed.

Thus a causal relationship exists between depression and heterosis since the origin of highly heterosis forms is conditioned by the depression in preceeding generations.

3. Acceleration of metabolic processes in hybrids is explained by the fact that in the initial or parental forms the depressing effect of semilethals manifests itself in the inhibition of these processes.

CGC increases the rates of these processes in the parental forms, bringing them nearer to the norm. They increase above the norm in F_1 after the depressing effect of the semilethals has been removed.

4. It becomes clear in terms of causal dependence of heterosis on inbred depression, why the most depressed inbred lines show more powerful heterosis than the less depressed ones. Apparently the strongly depressed inbred lines contained powerful semilethals whose action in this line was opposed only by respectively powerful CGC formed as a result of selection.

Exceptions from this rule can be also explained. Thus, for example, low CA of strongly depressed inbred lines can be explained by the insufficient gene reserve for the formation of efficient CGC. On the contrary, high CA of slightly depressed lines can result from the formation of powerful CGC, almost completely compensating the effect of semilethals.

5. The known cases of more powerful manifestation of heterosis in the homogametic sex (cattle, hens, turkeys, *Drosophila*) than in the heterogametic one are explained by the fact that offsprings of two opposite sexes get non-equal CGC from their parents. The Y-chromosome is almost empty genetically; its partner, the X-chromosome, carries the larger number of vital genes, some of which seem to be present in CGC. These very genes are not transferred from the heterogametic parent of the genotype XY to its offsprings because only the Y-chromosome is inherited from this parent. The homozygous sex gets all the chromosomes in one dose, and consequently the CGC is preserved. The greatest difference between sexes in the expression of heterosis can be expected in the karyotype with a small number of chromosomes when the absence of one of the genetically active chromosomes affects CGC integrity.

6. There are known examples of heterosis resulting from heterozygosity only for one allelic pair (monogenic heterosis). This concept has been proven in some

investigations but in other cases monogenic heterosis can be explained from the standpoint of unbalanced effect of CGC. It is supported by a well-known phenomenon: manifestation of the most powerful heterosis by the heterozygotes whose recessive allele in the homozygous state produces strong depression. This suggests two possible processes of CA increase in the semilethal gene carriers: 1. When the line homozygous for a recessive gene is bred, CGC inevitably appears. Its influence on the heterosis level is especially strong in F_1, and it will be, probably, preserved in the following generations, in particularly these species with a small number of chromosomes. 2. The semilethal appears in the chromosome carrying one or several vital genes whose action is stronger than that of their alleles located in the opposite chromosome. This primarily makes possible the survival of the individual homozygous for the semilethal. Further, by providing close linkage between the semilethal and the vital genes it gives a selective advantage in the heterozygote as compared to the individual homozygous for the opposite chromosome not carrying hypervital genes.

In conclusion I would like to mention a very important aspect of the proposed hypothesis of heterosis. Unlike many other hypotheses it introduces new efficient means of breeding types with high CA. This will be the subject of our next communication.

References

Astaurov BL (1978) Selection for predisposition towards thermal parthenogenesis and production of silkworm parthenoclones improved for this character (in Russian). Genetika 9:93–106

Maresin VM, Stepanova NL, Strunnikov VA (1985) Effect of selection of *Drosophila melanogaster* for combination ability at the background of the action of dominant semilethal (in Russian). Dokl Akad Nauk SSSR 281:1455–1458

Richey FD (1927) The convergent improvement of selfed lines of corn. Am Nat 61:430–449

Ruban VTs (1983) Experimental production of autotetraploids in mulberry silkworm (in Russian). Genetika 19: 115–120

Ruban VTs (1983) Genetic correction of silkworm ameiotic parthenoclones by means of successive polyploidization and depolyploidization (in Russian). Genetika 19:108–114

Strunnikov VA (1974) Initiation of the compensating gene complex is one of causes of heterosis (in Russian). Zh Obshch Biol 35:666–677

Strunnikov VA, Lezhenko SS, Stepanova NL (1982) Genetically identical copies of the mulberry silkworm. Theor Appl Genet 63:307–315

Strunnikov VA (1983) New hypothesis of heterosis: its scientific and practical significance (in Russian). Vestn Skh Nauki (Moscow) 1 (316):34–40

Strunnikov VA, Stepanova NL, Terskaya ER, Ruban VTs (1980) Depolyploidization of silkworm tetraploids (in Russian) Genetika 16:1096–1108

Strunnikov VA, Lezhenko SS, Stepanova NL (1983) Cloning of mulberry silkworm (in Russian). Genetika 19:82–94

Terskaya ER, Strunnikov VA (1975) Artificial meiotic parthenogenesis in mulberry silkworm (in Russian). Genetika 11:54–67