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Genetically Identical Copies of the Mulberry Silkworm

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Summary. Genetically identical copies of the silkworm female obtained via ameiotic parthenogenesis are insignificantly depressed by artificial reproduction, and notwithstanding their isogenous pattern, they show the same variability in quantitative characters as in the heterogenous forms.

By means of androgenesis, genetically identical copies of strictly homozygous males were obtained; the latter were produced via meiotic parthenogenesis. The androgenetic clones of these males were transformed by backcrosses into homozygous bisexual lines. Viability and cocoon weight in these lines were higher than those in androgenetic clones of similar genotype, but they were far from the norm due to the depressing effect of semilethals left in them in a homozygous state. An extremely low phenotypical variability of quantitative characters is observed in the isogenous hybrids F_1 obtained from crossing two genetically dissimilar strictly homozygous individuals. These hybrids make excellent material for phenogenetical studies. The female and male isogenous clones have an increased combining ability which is acquired in the course of their selection for high disposition towards complete parthenogenesis. For practical purposes it is proposed to take a female and a male which when mated produce a high-quality family and clone them separately, the first via parthenogenesis, the latter via androgenesis. After mating these reproduced bisexual clones, it will be possible to obtain in successive generations a vast number of families repeating the prominent productivity of the initial family.

Key words: Meiosis – Parthenogenesis – Androgenesis – Silkworm – *Bombyx mori* – Phenotypic variability

Introduction

Production of genetically identical copies of animals is one of the most interesting and complicated problems of experimental biology and agriculture. There are two ways of studying this problem. The first involves stimulation of oocyte development with a transplanted diploid nucleus of a somatic cell taken from another organism (Gurdon 1962). The second infers such artificial reproduction which would permit a complete transfer of the genotype of one of the parents to the offsprings (cloning).

Cloning of the silkworm (*Bombyx mori* L.) has attracted the attention of researchers for nearly fifty years. The development of artificial methods of ameiotic and meiotic parthenogenesis and androgenesis has made it possible to obtain genetically identical copies of mother and father, as well as genetically similar siblings which do not repeat the genotype of their parents. These studies have made such progress that the possible applications of silkworm cloning in industrial practice are now under study.

The authors hope that the methods of silkworm cloning under development and the information concerning biological and economic properties of silkworm clones will be of interest to biologists studying similar problems on other laboratory and agricultural animals.

The Cloning of Silkworm Females

At the end of the 30's, B. L. Astaurov (1940) was the first to elaborate a reliable method for activating silkworm eggs towards ameiotic parthenogenesis. The unfertilized silkworm eggs were kept in water heated to 46 °C for 18 min. The activated eggs underwent one equational division. Due to the absence of reduction division two genetically identical diploid nuclei were formed, one of which was involved in further development up to the hatching of larvae. These larvae were, in the history of science, the first artificially obtained genetically identical copies of their mothers.

Parthenogenetic clones with a small per cent of hatched larvae (30–40%) were characterized by de-

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creased viability at post-embryonic stages. The larvae developed irregularly and there were many deformed ones among them. The weight of their cocoons also varied greatly. A direct correlation was observed between the rate of hatching of parthenogenetic larvae and their post-embryonic viability. In later experiments the parthenogenetic clones bred by Astaurov with circa 90% of hatched larvae had rather high post-embryonic viability. Under favourable ecological conditions the latter was as great as that in bisexual lines. In spite of this fact, the mean weight of the cocoon of parthenoclone, according to our data, was 82.6% that of genetically identical material obtained by sexual reproduction. The weight of cocoons in parthenoclones was less variable, but still the variability in the cocoon weight was high. Under unfavourable ecological conditions the viability of parthenoclones was somewhat lower than that of the bisexual duplicates. The most distinct depressing effect of parthenogenetic development was revealed in a strictly homozygous isogenous line. In one experiment, 68.9% of bisexually reproduced individuals survived and among parthenogenetically obtained individuals the survival rate was 20.0%. The cocoon weight of the first ones was 1.45 g, of the latter – 1.05 g (Strunnikov and Stepanova 1980) (Table 1).

These drawbacks of the parthenoclones made their direct application for practical purposes more complicated. In addition the cocoons of silkworm females had 20% less silk than those of males, and therefore their industrial rearing would be unprofitable.

The Cloning of Silkworm Homozygous Males

Astaurov (1940) suggested the possibility of obtaining genetically identical copies of silkworm males. He believed that if, in the future, it turned out to be possible to breed artificially strictly homozygous parthenogenetic males, it would be also possible to reproduce each of them via androgenesis and to transform the androgenetic clone thus obtained into a bisexual line by means of backcrosses. At the time this idea seemed fantastic. However, in later experiments, an adequate procedure of inducing meiotic parthenogenesis with low and high temperatures, CO_2 and electric shock was elaborated. These factors in low doses activated the silkworm eggs, and the reduction and equational divisions proceeded as usual. The formed haploid pronucleus, encountering no male pronucleus, divided into two sister nuclei which after fusion formed a diploid nucleus subsequently involved in egg development. As a result, male larvae hatched at a rate of 5–6%. Their sex formula was ZZ (WW were nonviable); they were homozygous for all genes (Terskaya and Strunnikov 1974, 1975). Somewhat earlier than these experiment the androgenetic procedure had already been improved, γ-irradiation of noninseminated eggs at a dose rate of 80 kR, and subsequent heating of the eggs for 85 ± 30 min after spermatozoon penetration at 38 °C for 200 min made it possible to obtain 10–15% hatching of only androgenetic male larvae (Strunnikov 1960), a percentage much higher than that found in previous works. At the same time Gurova (1960) demonstrated the possibility of carrying on androgenetic clones for several generations.

In 1974, using these methods, an androgenetic progeny was for the first time obtained from homozygous males. Since each homozygous parthenogenetic male produced genetically identical sperm, the androgenetic offsprings, despite the fact that the zygote was formed by the fusion of the haploid nuclei from two spermatozoa, again consisted of strictly homozygous males similar to each other and to their father (Terskaya and Strunnikov 1975). These were the first copies of fathers obtained experimentally. Using similar cytogenetic methods, Markert (1977) obtained homozygous parthenogenetic mouse females of a meiotic type. These females, like the meiotic males of the silkworm, inherited one-half of the parents' genotype (semicloning). Markert believes that by using the same

Table 1. Viability and cocoon weight of offsprings produced by using different means of reproducing genetically identical individuals of the silkworm

Clones and the means of their reproduction	Sex	Viability (%)		Cocoon weight (g)	
		Embryonic	Postembryonic	♀	♂
Original ameiotic 'partheniclone 29' (heterozygous control)	♀♀	81,35	74.9	1.65	–
Androgenetic clone (F_6 and F_7) of the meiotic male 4 (homozygous)	♂♂	9.77	45.5	–	1.13
Backcrosses (F_7 and F_8) of males from the androgenetic clone 4 (homozygous)	♀♂	69.40	68.9	1.45	1.27
Ameiotic parthenogenetic offspring of the females of backcross 7 (homozygous)	♀♀	1.80	20.00	1.05	–

artificial mode of reproduction of these females new parthenogenetic offsprings can be obtained which will be complete genetical copies of the homozygous mother.

Extensive and detailed experiments on breeding male copies have been carried out on 'parthenoclone 29' which shows a strong tendency towards parthenogenesis (Strunnikov and Stepanova 1980). Several parthenogenetic strictly homozygous males were obtained from this clone. Two of these males gave rise to many generations of androgenetic clones no. 4 and no. 32, each of them in total comprising tens of thousands of offsprings. The rate of complete androgenesis in homozygous males, e.g. of the clone no. 4, varied between 5.2 and 17.6% (x = 10.6%) per generation; viability at the postembryonic stages ranges from 13 to 63.7% (x = 41.24%). Variations in the viability parameters depend on ecological conditions rather than on the number of androgenetic generations, as is the case with androgenetic reproduction of common, non-homozygous males. In the latter case a progressive decrease of viability is determined by a rise in the frequency of homozygous lethals and semilethals from generation to generation in androgenetic clones. The genotype of the parthenogenetic males is homozygous with a set balance of useful and harmful genes. This genotype is transmitted without a change to the androgenetic offsprings of all generations.

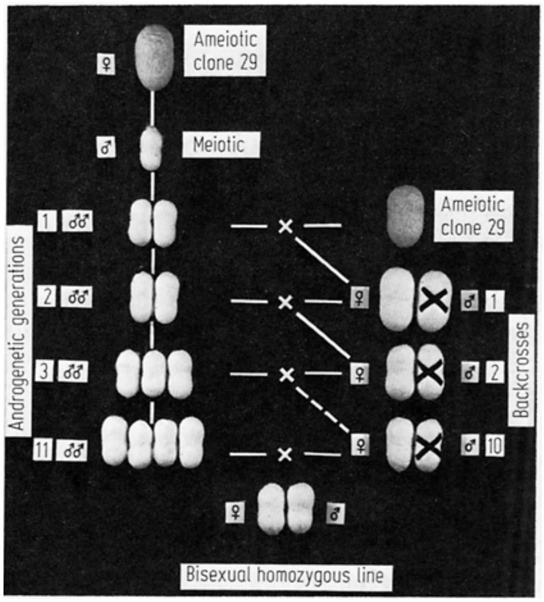


Fig. 1. The scheme of production: androgenesis of genotypically identical copies of strictly homozygous parthenogenetic males of the silkworm *Bombyx mori* L. and transformation of androgenetic clones into a bisexual homozygous line. Discarded male cocoons are indicated with a cross

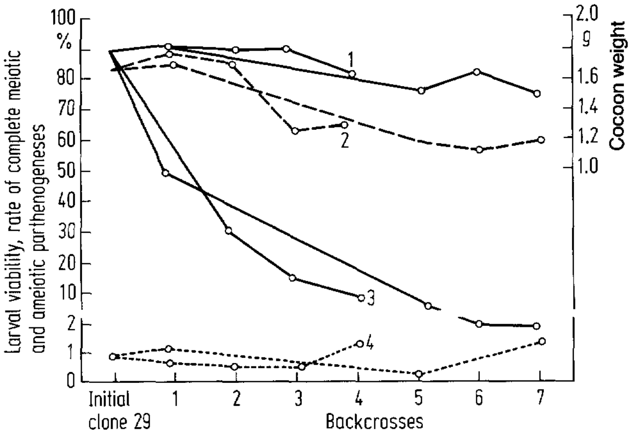


Fig. 2. Change of the average cocoon weight (*1*), larval viability (*2*), rate of complete ameiotic (*3*) and meiotic (*4*) parthenogenesis. Shorter dashes ($F_{b1} - F_{b4}$) = line 32; longer dashes (F_{b1}, $F_{b5} - F_{b7}$) = line 4

In spite of a comparatively high viability of androgenetic clones, their mass production is technically difficult. Therefore, by means of a series of backcrosses the androgenetic isogenic clones were transformed into a bisexual line. The males of this line are genetically fully identical to the initial homozygous male and to all males of the androgenetic clone. The females are also identical in all autosomes, but instead of having one Z-chromosome, they have a W-chromosome which is, fortunately, heterochromatic and its effect is therefore limited to sex-controlled characters. An early substitution of the mother chromosomes by the father-donor ones resulted from the absence of crossing-over in the silkworm females. From the above mentioned androgenetic clones no. 4 and no. 32, two bisexual lines of the same number were bred by means of backcrosses. Line no. 4 was bred up to F_{b10} in 1978 and line no. 32 to F_{b5}. Even in F_{b4}, the cocoons were very similar to the cocoon of the initial male, and in subsequent generations their shape changed little (Fig. 1). In the following backcross generations the cocoon weight, viability and predisposition of the females towards meiotic and ameiotic parthenogenesis were analysed. The parameters of all characters named gradually decreased with an increase in the number of backcrosses, and consequently became an approximation of the homozygous type of the initial male. However, these parameters strongly varied according to the years and rearing seasons. To obtain comparable data we prepared silkworm eggs of F_{b1}–F_{b4} of no. 32, and F_{b1} F_{b5}–F_{b7} of no. 4 and reared the larvae during one season under similar conditions (Fig. 2). It can be seen that the cocoon weight gradually decreases, beginning from F_{b2}; the viability of larvae and eggs of the backcross generations decreased somewhat more, but also only gradually. The rate of complete ameiotic parthenogenesis abruptly and steadily decreased up to F_{b6}, where the decrease comprised 2%. These parameters remained at this level in the F_{b7} and F_{b8} (not presented in Fig. 2), while in initial clone no. 29 and in F_{b1}, they were 89.3 and 51.3% respectively. The rate of complete meiotic parthenogenesis varied in the generations of two lines between 0.12 and 1.27%. No tendency towards a decrease in the hatching per cent of parthenogenetic males in further backcross generations was observed. Taking into account that meiotic parthenogenesis proceeds successfully only in half of the activated eggs in which a pronucleus with Z-chromosome was formed, the actual rate of parthenogenesis is doubled and reaches 0.24–2.54%. The almost similar hatching rate of larvae produced via meiotic (2.54%) and ameiotic (1.98%) parthenogenesis from the eggs of F_{b7} females are explained by the fact that there is no difference between their genotypes any more.

In our more extensive studies of 1979 F_{b7} of the line no. 4 and corresponding androgenetic clone no. 4 at the level of the 6th and 7th generations were reared under the same conditions. Then in summer F_{b8}, the androgenetic clone of the 8th generation, was reared. The averaged data for two rearing seasons are presented in Table 1. According to the cocoon weight and viability at embryonic and postembryonic stages the first place was occupied by parthenogenetic clone 29 (control); the second – by F_{b7} and F_{b8}; the third – by the androgenetic clone.

Complete similarity between the genotypes of androgenetic clones and backcrosses and somewhat better viability of the latter allows a conclusion to be drawn that androgenetic development is accompanied by a depression. Although this depression is less than during ameiotic and meiotic parthenogenesis it is still large enough.

Unfortunately, the bisexual strictly homozygous lines are also largely depressed by the homozygous semilethals. The domesticated silkworm is to such an extent saturated with semilethals that among many tens of thousands of the parthenogenetic males obtained there was none with economically valuable properties near the level of those in standard industrial varieties.

Later the androgenetic clones were brought to the 11th and backcrosses to the 14th generation. However, their parameters were extremely stable and therefore of no interest.

The Production of Mulberry Silkworm Isogenic Bisexual Siblings

The availability of completely homozygous individuals made it possible by crossing them to obtain genetically identical siblings of both sexes, which did not naturally repeat the genotype of either of their parents. The siblings obtained after the crossing of genetically distant forms are of special interest. These hybrids, unlike the isogenic female parthenoclones and isogenic androgenetic clones of homozygous males, were depressed neither by artificial reproduction, nor by semilethals passing into the homozygous state in the cloned males. Therefore they showed powerful heterosis, whose combination with the isogenic pattern led to a surprising, never before observed uniformity not only in qualitative, but also in quantitative characters of the siblings (Fig. 3). The variation coefficient for the cocoon weight in isogenic siblings equaled 3.59%, while in the isogenic parthenoclone and in the standard heterogenous hybrid it reached 7.4%–8.6%. Due to differential loss of the silk thread at the onset of cocoon spinning the variability cannot be lowered any more. However, it is so small that for the first time in the silkworm there is no earlier observed transgression between males and females for cocoon weight. A high level of uniformity of the material is of great practical and methodological value. Expectations for efficient employment of isogenic parthenoclones not justified in different experiments due to high variability of quantitative characters, became real now after the production of isogenic bisexual siblings. Uniformity of the experimental material makes it possible to carry out accurate experiments with a small number of larvae in each repetition. The isogenic siblings will be most valuable for ontogenetic studies, since the effect on the phenotype of varying environmental factors will be no longer masked by a decrease of homeostasis as a result of artificial reproduction and action of homozygous semilethals.

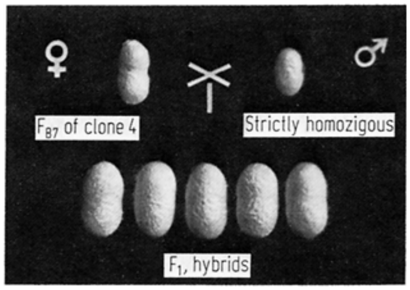
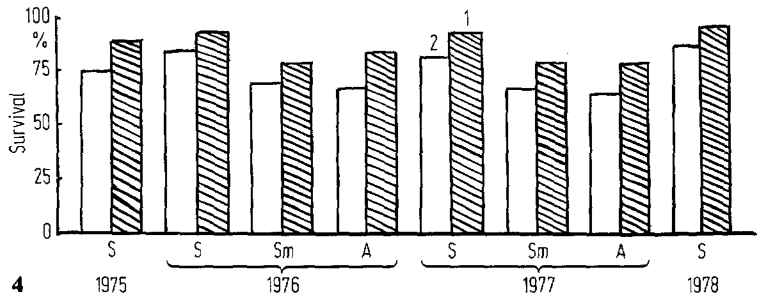


Fig. 3. The scheme of production of isogenic siblings of silkworm by crossing strictly homozygous parents

The Reproductive Properties of Genetically Identical Copies When Passing to Bisexual Reproduction

The sexually reproduced females, as mentioned above, surpass in viability and cocoon weight the females of the same genotype reproduced via parthenogenesis. However, in both cases the offsprings obtained after mating with the same males, proved to be similar, at least by the cocoon weight (Astaurov 1940).

These studies were carried out on parthenoclones with a weak predisposition towards complete par-



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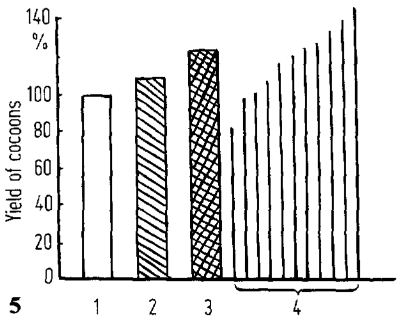

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Figs. 4 and 5. 4 Viability of F_1 hybrids of the silkworm 'Parthenoclone 29'×'Japan 115' (*1*) compared to the viability of control hybrids assigned for industrial use and showing the highest heterosis, 'China 108'×'Japan 115' (*2*). S = spring, Sm = summer, A = autumn. **5** Productivity of parental forms of silkworm and their hybrids. 1 = average for 'Parthenoclone 29' and 'Japan 115'; 2 = F_1 'Parthenoclone 29'×'Japan 115'; 3,4 = F_1 Japan 115'×♂ parthenogenetic from 'Parthenoclone 29'; 3 = mixture of families; 4 = single families

thenogenesis. It was of interest to investigate the reproductive potencies of parthenoclones with strong predisposition towards parthenogenesis achieved as a result of long-term selection. Many genetically diverse varieties were used to form the genotypes of these clones (Astaurov 1973). Therefore there is no line genetically similar to the parthenoclone, and consequently it is impossible to compare the reproductive potencies of standard and parthenogenetic experimental material of similar genotype in the course of normal development. In view of this another experiment was run differently. The hybrid China 108×Japan 115 is featured by the most powerful heterosis among all the hybrid combinations we managed to obtain. In this hybrid combination the variety China 108 was substituted for the genetically complex Parthenoclone 29 of Astaurov's selection. The control hybrid F_1 of China 108×Japan 115 and the hybrid under investigation Parthenoclone 29 × Japan 115 were simultaneously reared nine times during 1975–1980 under deliberately suboptimal ecological conditions. In all the cases viability of the hybrid Parthenoclone 29×Japan 115 was higher than in the control (Fig. 4) by 10% ($p = 0.001$).

In another experiment postembryonic viability of the F_1 hybrids from crossing genetically different parthenoclones with the variety China 108 was in all the hybrid combinations 92.4%, while it equaled 92.0% in the F_1 of Parthenoclone 29×China 108 in the above experiment. Thus the high combining ability of these hybrids appears not at random but in the course of their selection for high predisposition towards complete parthenogenesis. This conclusion was later on supported by other numerous investigations performed on many parthenoclones.

The fact that highly depressed strictly homozygous males show more powerful heterosis in hybrids than their outwardly normal parthenogenetic mothers is even of greater theoretical and practical interest. In 1974–78 11 independent experiments were carried out with the aim of comparing cocoon yield per equal number of reared larvae of two hybrids: Parthenoclone 29×Japan 115 and Japan 115×homozygous male of Parthenoclone 29 (Fig. 5). The cocoon yield depends on viability of the larvae and the cocoon weight. In 9 experiments the hybrids from crosses with the parthogenetic males exceeded in cocoon weight those from crosses with parthenogenetic females, in two experiments the yield was equal. The excess of the yield in all the experiments reached 7% ($p = 0.001$), on the average.

However it is not yet the end of heterosis escalation. Up to now we have operated with mean values obtained from the mixture of many hybrid families. But the level of heterosis varied markedly in the families themselves. The yield of some of them was 40% greater as compared with the mean yield of their parents (Fig. 5). In other experiments we observed still more astounding heterosis.

The increased combining ability of parthenogenetic individuals, of males especially, was not unexpected, and what is more, it was predicted by us in terms of our concept of adaptive heterosis (Strunnikov et al. 1972; Strunnikov 1974). These ideas arose under the impression of an experiment in which one of the silkworm varieties bred by us was subdivided into two lines. The first line was specially selected for the manifestation of heterosis when crossed with another variety designated as A. The second line was not selected for heterosis, but a recessive semilethal gene in the homozygous state was introduced into it. It decreased the viability of the second line to 72%. When the first and second lines were crossed with the variety A, the hybrid F_1 of the cross with the second line showed more powerful heterosis in all the repeated experiments, than the hybrid of the cross with the first line, although, as mentioned above, the latter was specially selected for heterosis.

The direct experiments have shown that in this case heterosis was not caused by monogenic heterosis. It is noteworthy that homozygotes for the semilethal gene isolated in the second generation again manifested low, barely threshold viability (ca. 30% vs. 72%).

A working hypothesis was advanced from the data obtained: in response to the transition of a semilethal into the homozygous state a well-coordinated complex of dominant genes of "viability" or genes-modifiers in the homozygous state was formed in the outbred line in a number of successive generations of selection. This complex suppresses to a great extent the harmful effect of the non-allelic homozygous semilethal. In F_1 hybrids the semilethal does not act due to its transition into the heterozygous state, and the compensation complex of genes is still preserved, although in a single dose, but in sufficiently coordinated state. Being unbalanced by the semilethal action it turns out to be in excess and thus intensifies the metabolic processes in F_1 above the standard. This leads to an increase of the viability level and to a powerful development of vital characters, i.e. to heterosis. In other generations of hybrids the coordination of genes of the compensation complex is disturbed due to chromosome recombination, and heterosis is not so vigorously manifested as in F_1 hybrids.

In terms of this hypothesis, the increased combining ability of parthenoclones is explained. As already stated, many eggs proceed to parthenogenesis and develop into formed embryos. However, the rate of hatching of completely formed larvae is only 3–10%. The larvae are not able to gnaw through the egg-shell, thus being suppressed by an unnatural mode of reproduction. Therefore an increased incidence of hatched parthenogenetic larvae is observed in highly viable varieties, in F_1 hybrids especially. Vice versa, inbreeding immediately brings about a strong decrease of the rate of complete parthenogenetic development. For instance, hatching of parthenogenetic larvae of the meiotic type from the eggs of a bisexual strictly homozygous line does not exceed several per cent, although the rate of its initial hatching in a heterozygous clone is about 90%. Thus high predisposition towards complete parthenogenesis is determined mainly by high viability of the experimental material. In view of the above it becomes evident that breeding of parthenogenetic clones with 90–95% of hatched larvae and high viability at the postembryonic stage is the result of long-term and intensive selection mainly for the increase of total viability of the selected material which enables an overcoming of the inhibiting effect of an unnatural mode of reproduction. It seems that thereby the compensation gene complexes are formed, as was the case with our line with the semilethal gene in the homozygous state. When parthenogenetic reproduction is substituted for the sexual one, due to crosses with males, the inhibiting effect of artificial reproduction is removed, and the compensation complex of viability genes is transferred to F_1 hybrids, although in a single but well-coordinated dose. Then the redundancy of these genes is manifested in the form of heterosis (Strunnikov 1980).

In the course of meiotic parthenogenesis the embryos were depressed not only by an unnatural mode of reproduction, but by semilethals transfered into the homozygous state as well. The males were literally between life and death. Only those could survive in which the genes of viability predominated over the destructive genes. There were few such zygotes; therefore the viability of meiotically developing oocytes and hatched larvae was extremely low. Only those males survived in which the effect of dominant genes-compensators turned stronger than that of semilethals. In this case we again come across the occurrence of the gene compensation complex, partly formed in response to the transition of semilethals into homozygous state, as was the case in the above experiment. Only in this case this process was accomplished not in the course of successive generations of selection, but throughout one generation. In spite of this, efficient selection for increased concentration of viability genes is achieved. It is accounted for by the fact that a sufficient amount of these genes was present in the Parthenoclone 29, because it was obtained as a result of long-term selection for the predisposition towards parthenogenesis. It was also due to the fact that all these genes in males were in the homozygous state. The latter is extremely important for obtaining powerful heterosis in hybrids.

The increase of combining ability in parthenogenetic males at the expense of the gene compensation complex was proved experimentally. The results of these experiments are already published (Strunnikov 1976).

It was observed that reproductive cells of the individuals depressed by different factors produce somewhat defective offsprings. It is not excluded that in the offsprings of strongly depressed strictly homozygous parthenogenetic males the combining ability is not fully realized. As mentioned above, the androgenetic clones, and especially the bisexual homozygous lines obtained by backcrosses from androgenetic clones, were less depressed, as compared to the initial males of the same genotype. It is technically impossible to obtain relative qualitative estimations of hybrid offsprings obtained from crossing genetically identical females with the initial parthenogenetic male, its androgenetic clone and its bisexual line. Here we managed only to produce F_1 hybrids from crosses of Japan 115 females with the

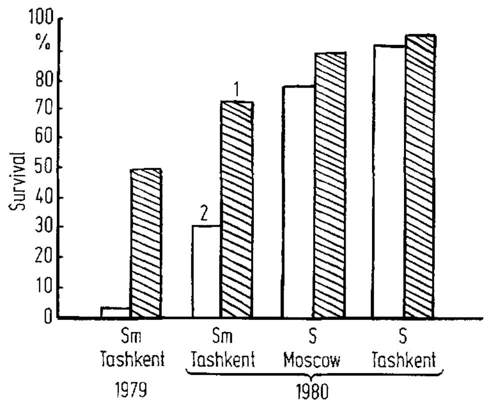

Fig. 6. Viability of F_1 hybrid 'Japan 115' × bisexual strictly homozygous line 4: *1* as compared with viability of F_1 hybrid 'China 108' × 'Japan 115'; *2* S = spring, Sm = summer

males of bisexual homozygous line no. 4. The latter originated from a homozygous male bred from Parthenoclone 29 widely used in our experiments; as in one of the above experiments industrial hybrid Japan 115 × China 108 having a high combining ability was taken as a control. The hybrids under comparison were reared under the same conditions: twice under hard ecological conditions of hot summer in Central Asia and twice under better conditions of spring rearing. Viability of the hybrids obtained from crossing of the homozygous clone was higher than that of the control hybrid in all the experiments, especially during summer rearings (Fig. 6).

Although these experimental results cannot be considered as a strict evidence of better reproductive potencies of the bisexual homozygous line, as compared to the initial parthenogenetic lines, they speak in favour of extremely high combining ability of the homozygous forms hybridized now as a bisexual homozygous line.

On the Practical Use of Silkworm Cloning

From the results described above it seems unquestionable that the genetically identical copies of both males and females are unsuitable for direct industrial use. Still, these data enable us to outline a real program for their application, the main idea of the program being that for industrial aim one should take the hybrids produced by crossing bisexual clones rather than the clones of single individuals, however productive. Our scheme involves breeding of several strictly homozygous lines, each originating from a homozygous parthenogenetic male. As was shown above, the F_1 hybrids from the crosses between genetically different lines consist entirely of genetically identical twins. The phenotypical uniformity of all their characters would be extremely valuable for practical purposes. On the other hand, the same genotypical identity of a great number of individuals might also prove to be a great drawback. It is known that isogenic plants when grown on an industrial scale often fall victims to pathogenic microorganisms, once the latter have adapted themselves to a given plant genotype. This might also become a real danger in the industrial rearing of the isogenic hybrid. By frequently changing the zone of hybrid breeding it might be possible to prevent the danger, but special experiments will be needed. Besides, the choice of the most productive hybrid combination F_1 would be a complicated task since according to this scheme a great number of homozygous lines should be bred at first. Of these only the best ones are to be chosen for hybridization. This would be technically rather difficult.

To start with we offer the following scheme which is in many respects rational and technically more accessible. First, by crossing females of a breed with a tendency towards parthenogenesis with homozygous parthenogenetic males of another breed the hybrid half-batches are produced. Half of the eggs before laying are removed from females and activated for ameiotic parthenogenesis. From every male androgenetic offsprings are produced. The development of parthenogenetic and androgenetic eggs is delayed by low temperature. At the same time the larvae from normal half-batches are reared under the same conditions to select from them, according to the cocoons produced, the most productive family. The high productivity is the result of high heterosis and high silk content in the cocoons. The parthenogenetic half-batch of the mother and the androgenetic offsprings of the father are reproduced respectively parthenogenetically and androgenetically. The androgenetic clone is then converted into bisexual line by means of backcrosses, as shown in Fig. 7. By crossing separately reproduced cloned parents it is possible to produce in unlimited numbers the families mainly similar in productivity to the first original or selected family.

Because of mothers' heterozygosity and meiosis of their sex cells the members of the reproduced families are genetically heterogeneous. Yet, the crosses between the isogenic homozygous males and isogenic, but to some extent heterozygous, females do ensure a high enough uniformity of F_1 hybrids, exceeding that of usual crossbreds.

If the economic properties of genetical copies were high enough to make them suitable for the direct practical use, the described scheme would be nevertheless a considerable advantage. It allows one to reduce by a factor of 500–600 the numbers of genetical copies to be produced by technically difficult artificial means. In this scheme the eggs intended for industrial use are

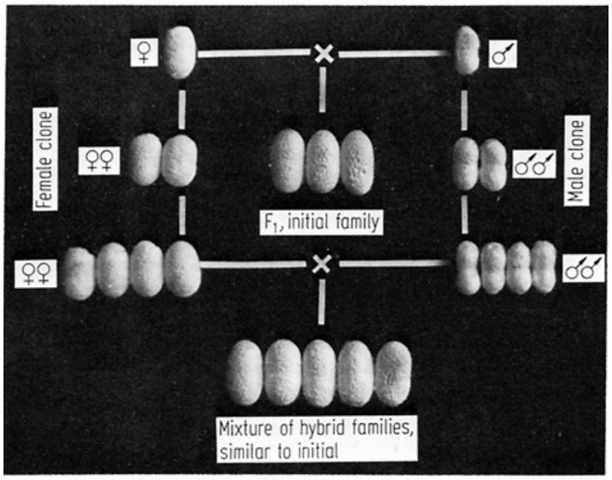

Fig. 7. The scheme of separate reproduction of two highly heterotic parents without changing their genotypes. The reproduced clones produced as large numbers of F_1 hybrids having the same powerful heterosis and high silk productivity as did the first hybrid family

produced by routine crosses between bisexual clones, and each family produced in this way consists of 500–600 offsprings. Thus, the cloning becomes technically accessible in industry.

The use of parthenogenetic males and females having an increased combining ability in selection makes it much easier to select for reproduction the families with powerful heterosis. On the other hand, the work with ameiotic parthenoclones involves certain difficulties. The genetical constancy of parthenoclones, though a valuable property in the material ready for the industrial use, is a serious drawback in the course of selectional work, since the material lacks the genetical variability necessary for selection. And to produce a parthenoclone combining all the valuable characters is extremely difficult if not impossible. Every parthenoclone needs a certain improvement. Though the offsprings produced in the crosses between the females of parthenoclones and males of bisexual line are heterogeneous, they show a much lower tendency towards parthenogenesis. With the discovery of the method of inducing meiotic parthenogenesis it became possible to overcome these difficulties. This can be achieved in two ways. The first way involves the production, by the method of meiotic parthenogenesis, from the constant female parthenoclone of homozygous males with a potential tendency towards ameiotic parthenogenesis. These males are crossed with females of their own or some other clone. Because of meiosis in females the heterogeneous bisexual offspring are produced, of which the necessary forms are selected and fixed by ameiotic parthenogenesis (Strunnikov 1977).

The second way is based on the above described peculiarity of meiotic parthenogenesis in tetraploids. Unfertilized eggs of tetraploids after being activated for meiotic parthenogenesis undergo two maturation divisions. The diploid pronucleus is formed which gets involved in further development of the eggs. Clearly the produced revertants show usual Mendelian segregation. Therefore, it is only necessary to transform the diploid ameiotic parthenoclone into tetraploid form and then to revert it to a diploid state. The diploid revertants will show the necessary genetic variability. It will only remain to select among the "fan" of newly emerged genotypes the necessary forms (Strunnikov et al. 1976, 1980). The two ways described are now widely used in selection. A new procedure has been developed for the activation of unfertilized silkworm eggs in the female body by negative temperatures. After cooling the females lay parthenogenetically developing eggs, which eliminates the need for the laborious procedure of eggs removal from the ovarioles (Strunnikova 1979).

The advanced scheme of the breeding work with the silkworm is now under testing in industry. Naturally, the introduction of the scheme demands new efforts, mainly organizational ones, but these will surely be more than compensated by large profits.

Besides the repeating of powerful heterosis in the countless families produced by crossing the clones of two parents in subsequent generations, the offered scheme will at the same time ensure the highly stable silk productivity of the hybrid. It will also make unnecessary laborious and costly breeding work in the research institutions and silkworm breeding stations, as the starting material for hybridization (parental clones) will be constant and no selection will be needed to keep it at a certain productivity level. Also, extremely valuable in the industrial practice are the entirely female parthenoclones: no laborious sex discrimination procedure will be needed.

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