Application of genetic principles for improving silk production

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During the last three decades, silk production increase benefited, to a great extent, from the application of genetic principles in the silkworm breeding programmes. The conventional breeding methods such as progeny testing, exploitation of hybrid vigour, genotype × environment interaction coupled with utilization of silkworm stocks that carry a translocated W chromosome provided continued success. Recent developments in transgenic silkworm technology, application of DNA markers for strain characterization and construction of linkage maps, and understanding the genetics of viral resistance provide requisite tools that can expedite further silkworm improvement.

THE domesticated silkworm, *Bombyx mori* is one of the genetically well-characterized insects next only to the fruitfly, *Drosophila* and has recently emerged as a lepidopteran molecular model system[1,2]. The well-developed genetics of this species includes more than 400 well-described mutations which have been mapped to > 200 loci, comprising 28 linkage groups or chromosomes[3]. In addition, hundreds of geographical races and genetically improved strains are maintained in different countries where sericulture was/is in vogue. These races and strains differ not only in well characterized Mendelian traits but also in not so well-studied complex or quantitative traits such as body size, feeding duration, thermal tolerance, and disease resistance. The monophagous nature of silkworm initially confined its rearing only to those countries where its food plant, mulberry (*Morus* species) is cultivated for silk production on industrial scale. However, research carried out in the early 1960s on the nutritional requirements of the silkworm led to the formulation of artificial diet, which freed silkworm rearing from the use of mulberry leaves and helped to expand the study of silkworm to academic laboratories.

Genetic strategies

Cross-breeding strategies and exploitation of hybrid vigour

As with many agricultural crops and livestock, silkworm improvement through conventional breeding strategies followed farmers' requirements and needs of the markets, with reasonable degree of success. However, the breeding objectives followed in different countries where sericulture was/is being practised remained quite different and quite often contradictory for many of the traits. For example, in Japan, which achieved remarkable success in improving the qualitative and quantitative output of silk, the breeding objectives centered on obtaining higher silk productivity of superior quality. Silk productivity per larva improved remarkably over the years. Conventional hybridization and selection was and still is widely used for improving yield potential in silkworm (Figure 1 *a*, *b*). This strategy generates variability by hybridizing the elite genotype (the breeding lines having the desired traits) with other improved varieties or local varieties, followed by selection of the desirable recombinants. The breeding objectives were achieved with precise selection starting from the initial choice of parents, meticulous progeny testing and selection and matching ecological and nutritive conditions. To further increase the yield potential of silkworm, heterosis breeding – which exploits the increased vigour of the F_1 hybrid (improved growth rates, better crop stability by virtue of increased resistance to biotic and abiotic stresses) has been used in silkworm[4]. Today, most sericulturists rear silkworm hybrids in almost all the countries where sericulture is in vogue.

In fact, the two big leaps involving the use of hybrid vigour were taken with the silkworm and maize, the former coming slightly earlier than the maize. The demonstration that hybrid vigour is manifested in F_1 hybrids when two genetically distinct silkworm strains are crossed was carried out by Toyama as early as in 1906 (ref. 5). The superiority of F_1 (Figure 2 *a*) hybrids was so much apparent that by 1919, 90% of the eggs produced were hybrid borne, reaching 100% by 1928, in Japan[6]. The average weight of the cocoon shell, increased by selection from around 180 mg in 1804 to about 200 mg in 1910, was further enhanced thereafter to more than 400 mg in less than 20 years, when the F_1 hybrids were introduced in Japan[6] (Figure 3). Another important application is the use of tetra-parental hybrids involving the crossing of two F_1 hybrids, each produced by different combinations of Japanese or Chinese silkworm stocks (Figure 2 *b*).
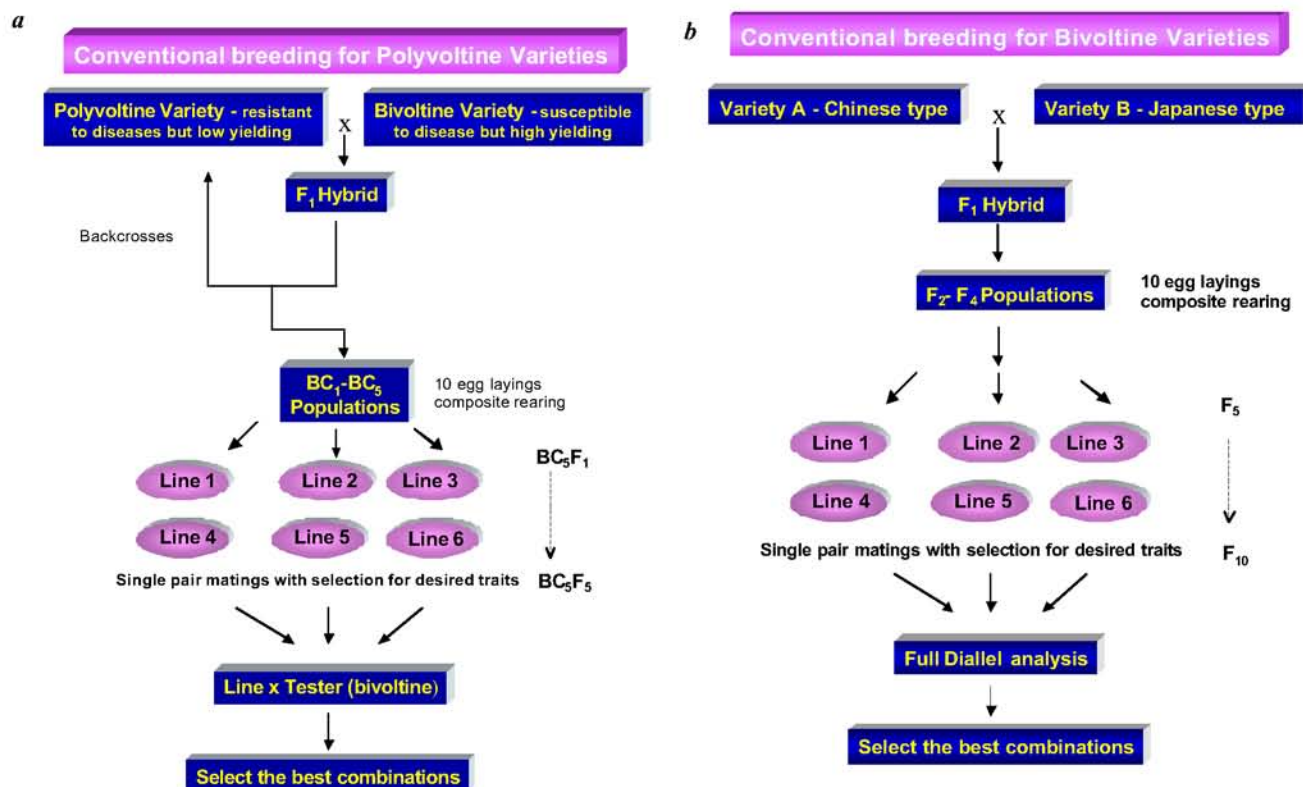
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a

b

Figure 1. Conventional silkworm breeding approaches. *a*, High-yielding polyvoltine varieties are bred typically by crossing the existing low-yielding but highly disease-resistant (resistance to viral and bacterial diseases) polyvoltine strain to high yielding but susceptible bivoltine variety. 3–4 recurrent back-crosses are made to polyvoltine parent mainly to recover non-diapause and resistance traits while exercising selection for moderate cocoon yield attributes and quality parameters. After 8–10 generations of inbreeding coupled with selection, the resultant polyvoltine inbred lines are tested for their combining ability by using a highly inbred bivoltine strain as a male tester parent. *b*, High-yielding bivoltine varieties are bred by crossing a Chinese type (with oval-shaped cocoons) silkworm strain with a Japanese type strain (with peanut-shaped cocoons). The desired traits are selected in the segregating population. Inbreeding coupled with selection is carried out for about 10 generations followed by diallel analysis and selection of suitable parents/hybrid combinations.
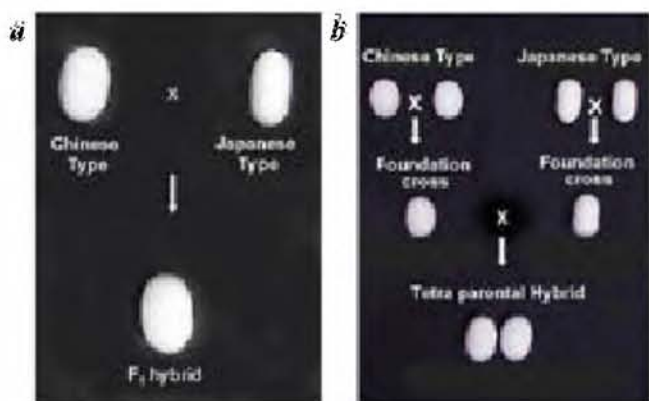

Figure 2. Single and double cross hybrids (tetra-parental hybrid) of silkworm. *a*, In single cross hybrid, a Chinese type (with oval-shaped cocoons) bivoltine strain is crossed with a Japanese type (with peanut-shaped cocoons) silkworm strain to obtain an F₁ hybrid; *b*, In tetra-parental hybrid two foundation cross hybrids are obtained first and are then crossed to obtain a double cross hybrid.

Use of translocated W-chromosome stocks for sexing animals

Sex chromosome constitution in silkworm is ZZ in males and ZW in females. Hasimoto[7] unequivocally demonstrated that the W chromosome nearly monopolizes the power of determination of femaleness through the findings that even triploids with ZZW and tetraploids with ZZZW chromosome constitution become normal females (Table 1). The conclusive proof of evidence that W chromosome determines femaleness came when the W chromosome carrying the translocated dominant gene for larval markings from second chromosome was discovered by Tazima[8] in an irradiated batch of eggs.

When the translocated dominant gene was present, the larvae carrying the markings were invariably females, while all the larvae devoid of markings were males (Fig-

ure 4 a). This discovery not only confirmed the female-determining role of the W chromosome but also led to an important sericultural application where males could be separated from females at larval stage for the purpose of making precise hybrids between two different silkworm strains. Such translocated stocks came to be known as sex-limited strains. After this initial discovery, strains with sex-limited cocoon or egg colour were generated by translocating the yellow cocoon colour gene from chromosome 2 (Figure 4 b) and the black egg colour gene from chromosome 10 to the W chromosome[9,10] (Figure 4 c). Sex-limited strains became a reality and heralded a new era in the application of fundamental genetic principles to the commercial use in sericulture. In all the sex-limited strains, whether the discrimination is at larval, egg or cocoon stage, females and males can be easily separated and little labour is required for such a task. Besides saving labour, the sex-limited strains have an added advantage. The females generally consume more leaf and produce lesser quantity of silk per unit amount of leaf than males since some portion of nutrition is allocated for egg production. In Japan, when the sericulture
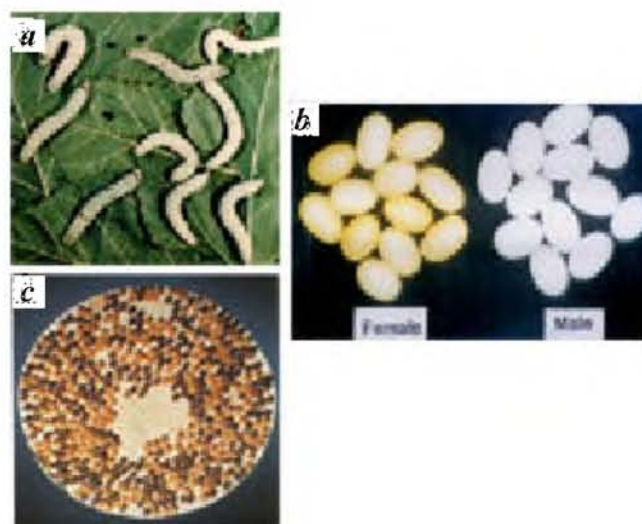
Figure 4. Silkworm strains which carry W-chromosomes translocated with autosomal genes. *a*, The strain carrying W-chromosome with a part of 2nd chromosome which harbours dominant allele ($+^p$) for larval markings only in females, males are devoid of markings; *b*, The strain carrying translocated part of 2nd chromosome which harbours dominant allele ($+^Y$) for cocoon colour only in females, male cocoons lack pigmentation; *c*, The strain carrying translocated part of 10th chromosome which contains dominant allele ($+^{w2}$) for egg serosal pigmentation, male eggs are devoid of such pigmentation.

Table 1. Sex determination in polyploids, with special reference to the ratio of sex chromosomes to autosomes

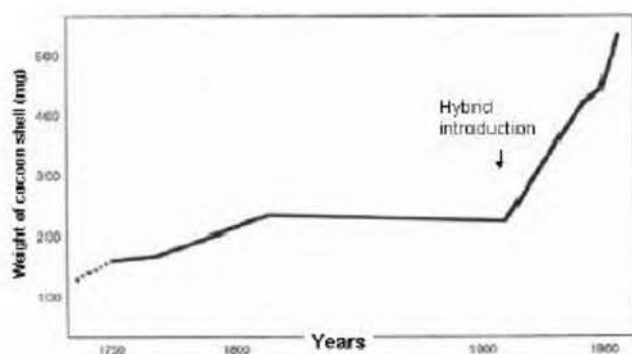| Ploidy	Number of autosomal sets	Number of Z-chromosomes	Number of W-chromosomes	Sex expression
2n	AA	ZZ	–	Male
		Z	W	Female
		Z	II.W.Z.	Female
		Z	–	Male
3n	AAA	ZZ	–	Male
		ZZZ	–	Male
		ZZ	WW	Female
		ZZ	W	Female
		Z	WW	Female
4n	AAAA	ZZZ	W	Female
		ZZ	WW	Female
		ZZZZ	–	Male
6n	AAAAAA	ZZZZ	WW	Female

Figure 3. Increase in cocoon shell weight after the introduction of hybrid silkworm rearing in Japan.

was in its glory, more than 60% of the total silk production was contributed by sex-limited breeds[11].

Genetic correlation of traits

Silkworm breeders have uncovered the fact that some characters or aspects of them affect other characters. Each of these characters has its own optimal mean value which is commensurate with the adaptive fitness of the strain. The interrelationships between characters are expressed in statistical terms, as correlations, which show how one variable changes as the other changes. Positive correlations show that as breeders change the mean of one character towards the higher side, the other also goes up with it, while in the negative as the mean value of one character goes up, the value for the other character goes down. In silkworm, most of the correlations between different characters have been worked out by selection experiments. These studies have shown that direct selection for one trait has correlation with the other quantitative characters. The correlation of some characters was found to be negative while in others it was positive[12] (Shibukawa, unpublished results, Table 2).

The correlations between various characters were clearly revealed by systematic studies which enabled breeders to exercise selection for various traits keeping a vital balance so that the growth, viability and silk yield are not affected. In a classical experiment by Miyahara[13], the silk filament length was increased from 1094 meters

Table 2. Correlation of the various characters (based on fifteen years of silkworm breeding data)

Sl. no.	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	−0.150	−0.20	−0.046	0.054	−0.126	−0.124	−0.154	−0.165	−0.155	−0.073	0.160	0.086	0.218	0.022	−0.121
2		0.00	−0.377	−0.433	−0.361	−0.343	−0.079	−0.303	−0.432	−0.477	0.175	0.054	−0.339	−0.799	−0.332
3	*		0.259	−0.249	−0.278	0.001	0.452	0.256	0.066	−0.130	−0.235	0.195	−0.178	−0.260	−0.365
4	–*			0.416	0.121	0.144	0.103	0.341	0.288	0.159	−0.277	0.215	−0.194	0.450	0.097
5	–*				0.871	0.710	−0.011	0.097	0.399	0.638	0.109	−0.319	−0.033	0.752	0.857
6	–*			***		0.852	0.084	0.184	0.472	0.771	0.140	−0.368	0.104	0.705	0.975
7	–			***	***		0.586	0.544	0.665	0.918	0.019	−0.231	0.129	0.760	0.715
8		**				***		0.781	0.547	0.554	−0.198	0.106	0.054	0.350	−0.135
9	–			–	**	***			0.672	0.691	−0.257	0.013	0.068	0.561	0.019
10	–*			**	**	***	**	***		0.750	−0.678	−0.122	0.240	0.671	0.349
11	–**			***	***	***	***	***	***		−0.028	−0.199	0.144	0.839	0.642
12									***			0.421	−0.248	−0.107	0.180
13				–	–*						–*		0.005	−0.150	−0.395
14	–													0.161	0.083
15	***		**	***	***	***	*	***	***	***					0.615
16	–	–*		***	***	***			*	***		–*		***	

0.1% $r > 0.554$ significant at 0.1% level; 1% $r > 0.449$ significant at 1% level; 5% $r > 0.349$ significant at 5% level; 10% $r > 0.296$ significant at 10% level.

1, Hatchability (per fertilized egg); 2, Duration of feeding period 5th instar; 3, Duration of feeding period larval stage; 4, Percentage of healthy pupae to the 3rd ecdysed larvae; 5, Amount of reelable cocoons produced per 10,000 rd ecdysed larvae; 6, Cocoon weight; 7, Cocoon shell weight; 8, Percentage of cocoon shell weight; 9, Raw silk percentage; 10, Length of cocoon filament; 11, Weight of cocoon filament; 12, Size of cocoon filament; 13, Reelability percentage; 14, Neatness defects point; 15, Raw silk weight per day of the 5th instar; 16, Pupal weight.
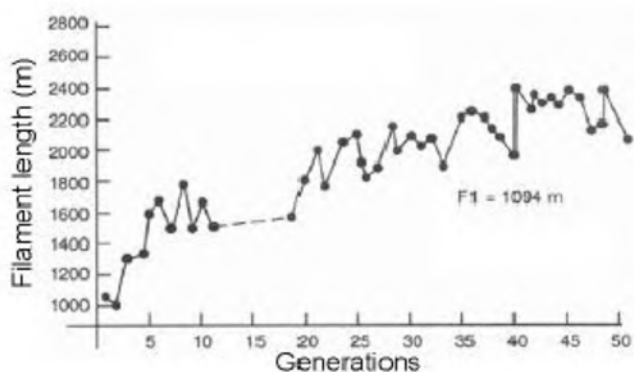

Figure 5. Selection for silk filament length in 'MK' silkworm strain.
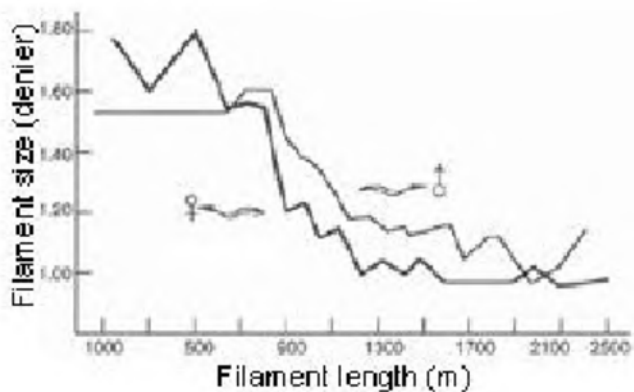

Figure 6. Correlated changes in silk filament size during selection for increased silk filament length in 'MK' silkworm strain.

to a remarkable length of 2400 meters at the fiftieth generation of selection (Figure 5). The increase in filament length appears to have manifested with a corresponding decrease in the thickness of the silk fibre (Figure 6).

Genetics of viral resistance

Silkworms are routine victims of various viral pathogens and cocoon crop losses are quite recurrent in tropical countries like India. These include cytoplamic polyhedrosis virus (CPV), nuclear polyhedrosis virus (NPV), densonucleosis virus (DNV) and infectious flacherie virus (IFV). Some silkworm strains used in tropical countries are naturally hardy and are more resistant to pathogens than the more productive temperate climate-adapted strains. Our basic knowledge about insect resistance to disease organisms mainly concerns bacterial agents; but there is a paucity of information concerning viral pathogens. Resistance to NPV, CPV and IFV has been reported to be under the control of polygenes[14,15], except for one instance of a strain harbouring a major gene for CPV resistance[16], which renders breeding for resistance difficult (see the accompanying paper by Watanabe in this issue, page 439). In contrast, a single recessive gene (*nsd-1*) controlling refractoriness to DNV-1 has been reported[17] and mapped to chromosome 21 (ref. 18). Resistance to DNV-2 has also been reported to be controlled by a major recessive gene *nsd-2* (ref. 19). Recently, molecular tags closely linked to DNV-1 resistance have been reported[20].

Transgenic silkworm approach to inculcate viral resistance

Transgenic silkworms have been recently generated using a lepidopteran transposon (*piggyBac*) to integrate the foreign genes of interest into the chromosomes of germ-line cells[21]. The transgenic silkworm system provides novel opportunities to improve strains of sericultural interest (see the accompanying article by Prudhomme and Couble in this issue, page 432). Already efforts are on in the University of Lyon, France and CDFD, Hyderabad, to inculcate viral resistance by targeting baculoviral genes (Pierre Couble, Jean Claude Prudhomme and Nagaraju, pers. commun.).

As a result of the systematic studies on selection and hybridization, high-yielding silkworm varieties were developed and sericulture vocation proved quite remunerative and cost-effective. As electronics and other industries started flourishing in Japan, the greater emphasis was laid on the reduction of manual labour in sericulture, which was very effectively achieved through introduction of many precise automated technologies in egg production, rearing, and silk reeling technologies. The success in silkworm breeding in Japan was also largely due to rearing of silkworm breeds that are native to temperate conditions. Countries like Korea, which enjoy a temperate climate, took advantage of the Japanese technologies to harness higher silk productivity.

Issues confronting Indian silkworm breeders

In India where sericulture is predominant in tropical regions, silkworm breeding research is a mixed bag of success and failure. The success and spread of silkworm rearing was mostly due to the use of F_1 silkworm hybrids of exotic temperate silkworm male parental strains and native female tropical silkworm strains. The hybrid vigour in terms of survival and silk cocoon yield was achieved to an extent of 40% over the mid-parental values. However, the quality of silk achieved was a trade off between the tropical and temperate parental strains involved in the hybridization programme. The successful introduction of F_1 hybrids of tropical female and temperate male silkworm strains was the major contributory factor for sustainable sericulture in India. (The reciprocal cross hybrid, temperate female × tropical male is not used since females of temperate strains lay diapausing eggs and there is distinct reduction in cocoon yield.) However, cocoon and silk yield and quality of silk yarn remained inferior to those of temperate silkworm hybrids. The attempts to spread temperate silkworm strains throughout the sericulture belt of India resulted in extensive crop failures, especially during hot and humid seasons and under rainfed farming conditions. Now it has become clear that the temperate strains (bivoltine varieties) can only be propa-

gated profitably during favourable seasons and under irrigated farming conditions. The breeding objectives followed to generate high yielding bivoltine varieties in
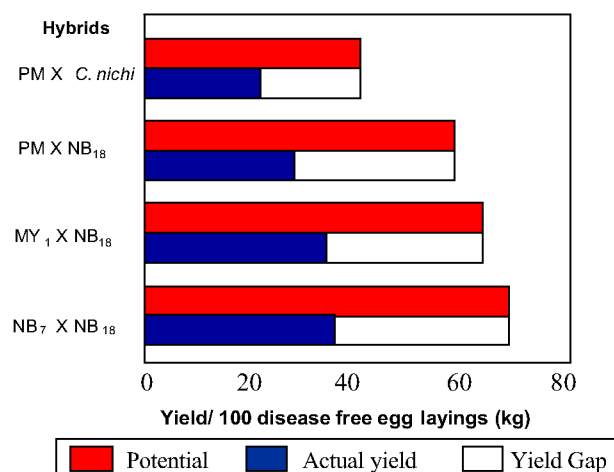

Figure 7. Yield gaps between full genetic potential and realized potential in polyvoltine × polyvoltine (PM × *C. nichi*), polyvoltine × bivoltine (PM × NB_{18} and MY_1 × NB_{18}) and bivoltine × bivoltine (NB_7 × NB_{18}) silkworm hybrids.
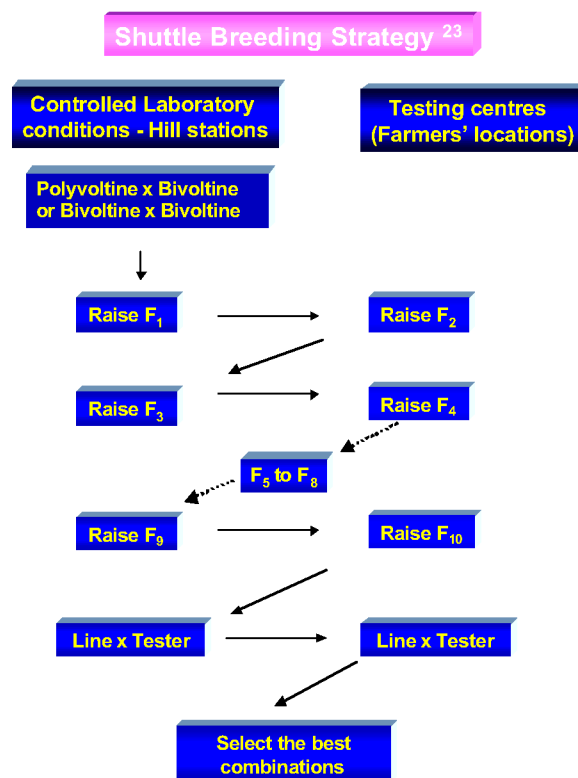

Figure 8. Shuttle breeding strategy for breeding silkworm varieties aimed at reducing yield gaps. Controlled laboratory conditions and hill stations refer to optimal conditions (in terms of environmental, nutritional and disease prevention/control requirements) whereas farmers' locations refer to sub-optimal conditions, for raising silkworm strains/ hybrids.

the breeding laboratories centered around higher body weight, higher fecundity, longer silk fibre, almost all of which had negative correlation with the survivability of silkworm. Unfortunately, such negative correlations tend to get pronounced under poor ecological and nutritive conditions. As a result, gap between genetic and realized potentials of the silkworm hybrids remained quite large[22] (Figure 7). The contributory factors for this gap need to be analysed and they seem to lie very much in the silkworm breeding process and ecological and nutritional status in which farmers raise silkworms. In the years ahead, there is a need to develop silkworm varieties with higher yield potential, more importantly with higher yield stability. In the years to come, traditional breeding methods with additional refinements will continue to be used, supplemented by tools and techniques developed by recent advances in silkworm genomics, which are offering novel opportunities to facilitate the development of new silkworm varieties. Typical breeding methods practised by Japanese breeders cannot be simply repeated under Indian conditions hoping to get the kind of success they had achieved.

Thus the important issues to be addressed are:

- There is a need to integrate the physiological, nutritional and ecological requirements of the silkworm strains under tropical conditions to the Indian silkworm breeding programmes to make them need-based.
- The need to make silkworm breeding programmes more 'scientific' given the harsh realities of 'genotype × environment' interaction.
- Shuttle breeding approach, a concept conceived and used successfully in wheat breeding programmes by Norman Borlaug[23] could be tried in silkworm breeding. This method allows selection of individuals adapted to diverse environments by breeding alternate generations of segregating populations at different locations (Figure 8).
- Adoption of well-proven genetic principles (tetraparental hybrid, introduction of translocated W-chromosome stocks for easy discrimination of sex, large scale progeny testing, screening for monogenic controlled densonucleosis virus resistance) to the silkworm improvement programmes.
- Maintenance of a broad genetic base in the early segregating populations of breeding, use of efficient selection procedures, testing and multiplication procedures, taking various genetic correlations and population size into account.
- Adoption of 'modern biotechnological tools' (such as use of DNA marker-assisted selection (MAS), selection of parental strains for cross-breeding programme based on their genetic homozygosity and genetic distance based on DNA marker evaluation, transgenic silkworm strains carrying genes for viral and protozoan

disease resistance and identification of markers for QTLs) to the silkworm breeding programmes.

Conclusion

Several important traits, e.g. increasing disease resistance or larval health, have not been handled very successfully in traditional breeding schemes so far. One reason for this may be the low heritability and lack of application of appropriate statistical tool for analysis of phenotypic data. The most important traits of sericulture, as in agriculture, are not controlled by a single gene but the concerted action of several genes (polygenic or quantitative traits) and non-hereditary factors. Dissecting such traits require substantially enhanced efforts on the part of silkworm geneticists. Recent developments in silkworm genome analysis provide tools and techniques which, coupled with conventional breeding will help silkworm geneticists and breeders to perform such a task.

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