

Breeding of Bivoltine Silkworm Hybrids DP0308 and DP0314 in Sri Lanka : A Simplified but Practical Approach

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ABSTRACTS

Mainly due to limited genetic resources available and also urgent need for hybrids suitable to local conditions, conventional theories and regular methods for bivoltine silkworm breeding are not easily applicable in such a tropical country as Sri Lanka which is recently planning to scale-up the silk industry. A simplified but practical methodology was introduced to overcome such constraints. Through application of such modified informal breeding methods, two hybrids named DP0308 and DP0314 were selected for cocoon production. Details of the altered method of germplasm improvement in the silkworm are presented, along with combining ability and heritability estimates for six parental pure lines in a half-diallel cross.

Key words : *Bombyx mori*, Combining ability, Heritability, Silkworm breeding

INTRODUCTION

In an effort to improve cocoon productivity and silk quality, countries in tropical climatic zone have been trying to introduce bivoltine silkworm varieties, replacing their indigenous multivoltines. For the mulberry silkworm, *Bombyx mori*, flow of genetic materials between countries is rather restricted as trade-secret due primarily to obsolete political reasons. Meanwhile, in the countries in which cocoon production became no longer important to their national economy priority to properly maintain germplasm resources gets low in administration and then naturally less operational attention is given. Consequently, either intentional or accidental loss of otherwise valuable genetic resources is unavoidable. However, in sericulturally developing countries economically important genes or gene combinations can be retrieved at best through inbreeding manipulation of imported hybrids, using them as initial crosses for generation of new gene combinations. In such countries, a breeder has so limited time requirement for development of new hybrids that he/she has virtually no time to spend in

formally following textbook principles and techniques of silkworm breeding (ESCAP, 1993). Science of germplasm improvement inevitably includes, in some part, "art" of breeding (Falconer, 1960), inevitably due to so many unknown factors involved in the long course of final selection of superior varieties. Therefore, a second choice of simplified approach comes in handy to replace time-consuming regular and formal procedures of breeding. Through this new method a breeder intends basically to gain time at some random loss of genetic variation of a source population or to be more dependent on "art" part than on "science" part of breeding.

Bivoltine silkworm varieties were first introduced into Sri Lanka by a catholic nuns' non-governmental organization during the 1950's and thereafter by UNDP/FAO sericulture development projects mostly from the sericulturally developed countries; China, India, Italy, Japan, Korea, Taiwan and Thailand. Long-term storage of bivoltine silkworm eggs is not usually practiced and most of silkworm stock varieties are maintained through continuous rearing cycles of four to five generations a year. As a result,

genetic materials go naturally through more rapid inbreeding than in the country of origin. The germplasm bank of the mulberry silkworm in Sri Lanka was further reinforced by the recent UNDP/FAO project, adding 12 more inbred lines which were newly isolated from imported hybrid varieties. A systematic breeding program has been well established in Sri Lanka and in due progress with repeated cycles of serial rearings for half-diallel crosses, simplified screenings, and local adaptability tests (LAT). Through analysis of performance data from the first filial generation, such basic parameters as combining ability and heritability (Sprague and Tatum, 1942) were previously estimated with different sets of parental pure lines in the first cycle of diallel programs (DP) (Lea and Alwis, 1995). In the section of "MATERIALS AND METHODS" of this report, I introduce a simplified, hence rapid, but still effective and practical procedure of silkworm breeding for sericulturally developing countries, along with combining ability and heritability estimates.

MATERIALS AND METHODS

1. Strategy and overall procedures

Emphasis was given in reducing time or generation numbers to meet urgent need for suitable hybrids at the new area of sericulture, accepting some risk of accidental loss of otherwise valuable source of variation. Only two most important characters or cocoon shell percentage and cocoon yields were considered in final selection of superior hybrids. Meticulous theoretical probe for superior gene combination was avoided and such statistical analysis as analysis of variance and comparison of mean

values were also canceled. Instead, F₁'s in hand were simply screened for better performing in ranking in each of five generations; two selection trials and 3 local adaptability tests. Rank-index (R-index) points or the cumulative total number of weighted-ranking points were used for screening better hybrids in each of the first two selection cycles and Occurrence-index (O-index) or the cumulative total number of un-weighted occurrence points for selection of superior hybrids during the last three local adaptability tests (LAT).

2. Half-diallel cross and rearing

Six parental inbred lines, including four newly isolated inbreds from recently imported bivoltine hybrids, were chosen for promising average performance, as designated SN28, SN29, SN43, SN44, SN45 and SN46. Prefix S stands for Sri Lanka and N for Nillambe. In a half-diallel cross with parents, 15 F₁'s and 6 parental genotypes (Table 1) were obtained at Silkworm Breeding and Grainage Station (SBGS), Silk and Allied Products Development Authority (SAPDA), and were used as an initial source population. Standard methods and procedures were taken for handling and treatment for eggs, larvae, pupae and moths, in 2 replications for each of 6 parents and 15 F₁'s. Two-hundred individual larvae were counted on the first day of the fourth instar, and were reared at SGBS, SAPDA, Nillambe, Kandy, Sri Lanka during the months of May and June 1995. Quantitative measurements were made on economically important characters; cocoon size (CPL, number of cocoon per liter), cocoon shell weight (CSW, cg), single cocoon weight (SCW, cg), cocoon shell percentage (CSP, %), pupation rate (PUP, %) and cocoon yields/10000 larvae (CoY, kg).

Table 1. Mating design in a half-diallel cross with six parental pure lines and designation of the experimental hybrids

Female \ Male	SN28	SN29	SN43	SN44	SN45	SN46
SN28	SN28	DP0301	DP0302	DP0303	DP0304	DP0305
SN29	×	SN29	DP0306	DP0307	DP0308	DP0309
SN43	×	×	SN43	DP0310	DP0311	DP0312
SN44	×	×	×	SN44	DP0313	DP0314
SN45	×	×	×	×	SN45	DP0315
SN46	×	×	×	×	×	SN46

Standard methods and techniques were observed for collection and analyses of field data, as described in the reference (ESCAP, 1993).

3. Rearing cycles and the rank-index-screening method

Following the first rearing cycle (brushing date; 15 May 1995) of making a half-diallel cross between 6 parental lines, 8 hybrids were chosen on the basis of cumulative merit points at the end of second cycle (brushing date; 7 July 1995) of rearing of 15 hybrids; where in each of three criterial economic characters of single cocoon weight, cocoon shell percentage and cocoon yields, 15 rank-index (R-index) points weighted progressively were given to the highest ranking hybrid, 14 points to the next highest, and so on and finally 1 point to the least and last one in a descending order. The ranking points in each of the three characters are added up to obtain cumulative R-index points. In the third rearing cycle (5 September 1995), another 2 genotypes with lowest R-index are selected out and the rest six hybrids were subjected to subsequent three rearing cycles (brushing dates; 1 November, 25 December 1995 and 5 March 1996) of local adaptability tests (LAT).

4. Local adaptability tests and occurrence-index-screening method

Three rearing cycles of adaptability tests were carried out in 5 different places, each representing various climatic conditions of major cocoon production areas of the country. As screening criteria for the best performing hybrids in LAT only two characters of raw silk percentage and cocoon yields were taken into account for further simplicity. Running experiments for raw silk quality and moth characters were not at the moment possible in Sri Lanka. Out of seven hybrids including a control hybrid, one occurrence-index (O-index) point unweighted was given for each case of occurrence to each of three highest performing hybrids in each locality in each of three rearing cycles. In final evaluation of the hybrids, cumulative O-index points were used to determine the order of superiority, adding up the occurrence points together for both cocoon shell percentage and cocoon yields from all

five localities in all three LAT trials.

5. Statistical analysis

The BASIC program by Magari and Kang (1994) was exclusively employed on a personal computer for statistical analysis of the observed field data. The program was originally developed for Griffing's (1954) diallel analyses and kindly provided to me by the authors. For the analysis of variance in Table 3, genetic component of total phenotypic variation was calculated by the formula (ESCAP, 1993) of

$$\sigma_G^2 = \frac{(\sigma_E^2 + r\sigma_G^2) - \sigma_E^2}{r}$$

and the heritability in broad sense was calculated according to the formula of

$$h_B^2 = \frac{\sigma_G^2}{(\sigma_E^2 + \sigma_G^2)}$$

Other statistical interpretations were made according to the concept of Dixon and Massey (1969).

RESULTS AND DISCUSSIONS

1. Performance of parents and their hybrids

Average performance of parental pure lines and their F1's in the second rearing cycle is presented in Table 2. As expressed by the number of individual cocoons in one liter volume, cocoon size ranged from the largest 74/l of SN45 to the smallest 107/l of SN44 for the parental inbred lines. For the hybrids it ranged from the largest 72/l of SN43×SN44 to the smallest 95/l of SN28×SN45. Overall mean value for cocoon size was 83/l. Cocoon shell weight ranged from 36 to 50 cg for pure lines and from 38 to 45 cg for hybrids, overall mean 42 cg/cocoon. The heaviest single cocoon weight 201 cg/cocoon was observed from the parental line SN43 and the lightest 165 cg for SN29, while 203 cg/cocoon for the hybrid SN28×SN44, 180 cg for SN43×SN45, and overall mean value of 192 cg/cocoon. Cocoon shell percentage for parental lines was highest 25.0% in SN46 and lowest 20.5 in SN43, and for hybrids highest 22.5% in SN28×SN46 and lowest 20.7 in SN28×SN43 and SN28×SN45, with overall mean of 21.8%.

Table 2. Performance of parental pure lines and their F₁'s in a half-diallel cross

Parent/F ₁	CPL	CSW	SCW	CSP	PUP	CoY ^a
SN28 × SN28	82	42	191	21.8	76	17.5
SN29	82	42	202	20.8	80	20.4
SN43	78	42	202	20.7	84	23.1
SN44	76	43	203	20.8	84	19.9
SN45	95	38	184	20.7	81	22.9
SN46	81	45	197	22.5	84	22.3
SN29 × SN29	79	36	165	21.6	90	20.5
SN43	77	40	191	20.8	92	20.3
SN44	78	42	190	22.2	82	20.3
SN45	94	45	202	22.1	87	20.1
SN46	85	44	198	22.1	87	20.1
SN43 × SN43	76	41	201	20.5	62	19.3
SN44	72	39	184	21.0	90	20.5
SN45	94	38	180	21.2	83	21.4
SN46	77	42	198	21.1	80	22.3
SN44 × SN44	107	40	180	22.7	66	18.8
SN45	83	42	188	22.0	84	20.5
SN46	78	44	201	22.0	82	21.0
SN45 × SN45	74	45	187	24.1	90	19.4
SN46	93	43	194	22.0	79	21.3
SN46 × SN46	86	50	198	25.0	63	17.9
Overall mean	82.9	41.8	191.9	21.8	81.0	20.4
LSD/.05	5.2	1.7	6.6	0.5	8.5	0.8
LSD/.01	7.6	2.5	9.7	0.7	12.5	1.2
CV(%)	3.6	2.4	2.0	1.3	6.1	2.3

^aCPL, number of cocoon per liter; CSW, cocoon shell weight (cg); SCW, single cocoon weight (cg); CSP, cocoon shell percentage (%); PUP, pupation rate (%); CoY, cocoon yields (kg)/10,000 larvae

Among 8 parental lines, highest pupation rate (90%) was observed in SN29 and SN45, the lowest (63%) in SN46. Out of 15 hybrid genotypes, lower pupation rate 79% was observed in the hybrid SN45 × SN46, and higher 92% for SN29 × SN43, with overall mean of 81%. Fresh cocoon yields per 10000 larvae ranged

Table 3. Analysis of variance for major economic characters and combining ability of parental pure lines, and heritability estimates as expressed in their F₁'s in a half-diallel cross

Source	df	Mean squares					
		CPL	CSW	SCW	CSP	PUP	CoY ^a
Total	71						
Genotype	35	151.3	17.1	189.4	2.5	142.5	4.3
GCA	7	103.9	36.7	207.7	6.0	181.9	1.3
SCA	28	167.1	10.6	183.3	1.4	129.3	5.3
Block	1						
Error	35	9.0	1.0	14.7	7.9	24.3	0.2
h^2_B (%)		89.9	89.3	85.6	93.9	70.8	90.3

^aCPL, number of cocoon per liter; CSW, cocoon shell weight (cg); SCW, single cocoon weight (cg); CSP, cocoon shell percentage (%); PUP, pupation rate (%); CoY, cocoon yields (kg)/10,000 larvae

from 17.5 to 20.5 kg for parents and from 19.9 to 23.1 kg for hybrids, with overall mean yields of 20.4 kg.

2. Combining ability and heritability estimates

Results of analysis of variance for the quantitative characters and combining abilities are contained in Table 3, along with heritability estimates. Differences between means of 21 genotypes and also between values of their combining ability were highly significant for all the characters examined. The result indicated that both additive and nonadditive gene actions contribute significantly to genetic component of existing variation between parental stocks, therefore, the population is expected to respond

reasonably to both "mass" and also "pedigree" selection methods (Falconer, 1960). Exploiting the variability of such population, inbreeding for several generation is necessary before crossing to create source population for selection of superior hybrids, because gene frequency responsible to express economic characters are still segregating and some more of hidden interaction between contributing loci may be released in the later generation.

Values of GCA and SCA estimated are shown in Table 4 for both parents and F1 hybrids. The pure line SN45 demonstrated the highest GCA and SN43 the lowest with $LSD/.05=1.83$ for cocoon size. For cocoon shell weight, GCA values ranged from -1.4

Table 4. Estimated values of general (GCA) and specific combining ability (SCA)

Parent/F1	CPL	CSW	SCW	CSP	PUP	CoY ^a
GCA						
SN28	-0.7	-0.2	3.0	-0.4	-0.4	0.0
SN29	-1.1	-1.1	-3.9	-0.2	5.0	-0.1
SN43	-4.0	-1.4	1.6	-0.8	-2.1	0.4
SN44	2.3	-0.4	-2.6	0.1	-2.0	-0.4
SN45	3.1	0.1	-2.8	0.5	3.2	0.2
SN46	0.4	2.9	4.8	0.9	-3.7	-4.6
SCA						
SN28 × SN29	0.8	1.0	10.5	-0.4	-5.5	0.0
SN43	-0.7	1.3	5.0	0.2	5.0	2.2
SN44	-8.6	1.3	10.2	-0.7	5.4	-0.2
SN45	9.2	-3.7	-8.1	-1.1	-2.8	2.2
SN46	-1.6	0.0	-2.7	0.2	7.1	1.9
SN29 × SN43	-1.4	0.7	1.4	0.0	7.7	-0.4
SN44	-6.2	1.7	4.1	0.4	-2.5	0.4
SN45	8.6	3.7	16.8	0.0	-2.6	-0.5
SN46	2.8	0.4	5.2	-0.4	4.7	-0.2
SN43 × SN44	-9.2	-1.5	-7.4	0.0	12.6	0.1
SN45	12.0	-2.5	-10.7	-0.2	0.4	0.4
SN46	-2.8	-1.8	-0.3	-0.7	4.8	1.5
SN44 × SN45	-5.8	0.0	1.0	-0.4	1.3	0.3
SN46	-8.1	-0.3	6.4	-0.8	6.2	1.0
SN45 × SN46	6.1	-2.3	-0.4	-1.1	-1.5	0.7
G(i - j) : LSD/.05	1.83	0.60	2.33	0.17	3.01	0.29
LSD/.01	2.68	0.88	3.43	0.25	4.41	0.42
S(ij - ik) : LSD/.05	4.84	1.59	6.19	0.45	7.96	0.76
LSD/.01	7.10	2.33	9.07	0.66	11.66	1.12
S(ij - kl) : LSD/.05	4.48	1.47	5.73	0.42	7.37	0.71
LSD/.01	6.57	2.15	8.40	0.61	10.80	1.04

^aCPL, number of cocoon per liter; CSW, cocoon shell weight (cg); SCW, single cocoon weight (cg); CSP, cocoon shell percentage (%); PUP, pupation rate (%); CoY, cocoon yields (kg)/10,000 larvae

to 2.4 with $LSD/.05=0.60$. For single cocoon weight and cocoon shell percentage, GCA effects varied between -3.9 and 4.8 , and -0.8 and 0.9 , respectively. The pure line SN29 found to be a good general combiner for pupation rate and SN43 for cocoon yields.

Prominent nonadditive genetic effect between responsible genes for smaller cocoon size was shown in a cross combination SN43×SN45 with SCA value of 12.0, while interaction effect for bigger size in SN28×SN44 with -8.6 . The highest SCA of 3.7 in cocoon shell weight was recognized in the cross between SN28 and SN45 and the lowest -3.7 in the hybrid SN29×SN45. The hybrid SN29×SN45 revealed the highest SCA effect of 16.8 for single cocoon weight, while SN28×SN29 the lowest -10.5 . The SCA values ranged from -1.1 to 0.4 and from -5.5 to 12.6 for cocoon shell percentage and pupation rate, respectively. Cocoon yields as expressed kilograms per 10000 silkworm larvae varied between SCA -0.5 for a hybrid SN29×SN45 and 2.2 for SN28×SN43 and SN28×SN45 with $LSD/.05=0.76$. Hybrids with extreme SCA values could be further exploited in future silkworm breeding programs for possible further release of the nonadditive gene action for that specific character under investigation.

Heritable part of total phenotypic variation or breeding value of a genotype for an economic character is quantitatively measured by heritability estimates at a certain period of breeding history with the specific genetic backgrounds under a given environmental condition. In any research programs for germplasm improvement, creation of a source population with reasonable amount of releasable variation is one of the most critical steps of the whole breeding process. In Sri Lanka where inbreeding is rapid and seasonal and yearly fluctuations of quantitative characters are rather high, it is expected that heritability estimates of any character tend to be low, mainly due to greater extent of environmental variations and in turn significant amount of experimental errors.

Heritability in broad sense ($h^2_B, \%$) was estimated as presented in Table 3. The estimated amount of phenotypic expression due to genetic effect was higher for each character than it was expected under the condition of rapid inbreeding process with a small population size in Sri Lanka. This observation

is in agreement to the significant differences of additive gene action in GCA between parental pure lines. In conclusion, the genetic population under investigation still contains reasonable amount of variation upon which superior hybrid combinations could be sorted out through selection efforts. The heritability estimates ranged from 70.8% for pupation rate to 93.9% for cocoon shell percentage.

3. Local adaptability test and selection of leading hybrids DP0308 and DP0314

At the end of the second rearing cycle, out of a total of 15, eight hybrids with higher R-index points were selected for, according to the weighted R-index points as described in the previous section. By the end of the third cycle another two were selected out again and remaining six hybrids and a control hybrid CT16 were tested for local adaptability. The results of three rearing cycles for local adaptability tests are shown in Table 5. Due to limited manpower and resources, in evaluation of a pure line or a hybrid variety it was not possible to extend selection criteria to other characters of eggs, moths or raw silk or silk. In most of sericulturally developing countries, a breeder often has to succumb scientific knowledge and technical know-how to general situations and specific conditions prevailing in the area. Consequently only two most important and critical characters (cocoon shell ratio and cocoon yields) were taken into account as selection criteria for simplicity and also for practicality during the three rearing cycles of local adaptability tests (LAT-1, brushed on 1 November 1995; LAT-2, brushed on 25 December 1995 and LAT-3, brushed on 5 March 1996). Five localities represent major cocoon production areas where also research stations for breeding and silkworm egg production or branch stations are located. ; Nillambe, Galaha, Horana, Monaragala and Balawinna. In each of three times of LAT's, the best performing three hybrids were marked with equal index point of one each (unweighted), and cumulative points from all three LAT's were added up for both characters to obtain "occurrence-index points(O-index)" in Table 6. The hybrid SN29×SN45 or DP0308 marked the highest total O-index points of 17, followed by the hybrid SN44×SN46 or DP0314 with O-index 16. As a result, DP0308 and DP0314 were

Table 5. Hybrid performance from the local adaptability tests (LAT) in five locations

Hybrids	Nillambe		Galaha		Horana		Monaragala		Balawinna	
	^a CSP	CoY	CSP	CoY	CSP	CoY	CSP	CoY	CSP	CoY
LAT-1; brushed 1 November 1995										
DP0302	20.4	21.2	21.5	19.5	nd	nd	20.2	12.1	20.0	19.6
DP0305	22.4	21.4	22.9	19.8	"	"	21.1	17.9	22.8	20.8
DP0308	23.2	21.2	24.5	19.0	"	"	21.5	16.6	22.0	18.9
DP0312	21.7	nd ^c	23.1	18.8	"	"	20.1	16.6	22.0	20.5
DP0314	22.4	22.3	23.8	19.0	"	"	21.7	16.5	22.3	19.5
DP0315	22.3	23.5	23.1	18.9	"	"	22.8	16.2	22.4	19.1
CT16 ^b	21.3	21.5	23.0	19.2	"	"	20.5	17.5	19.9	20.8
LAT-2; brushed 25 December 1995										
DP0302	20.7	17.4	22.4	21.2	19.8	16.4	nd	nd	21.5	17.2
DP0305	23.4	17.6	23.8	21.0	20.1	16.4	"	"	23.6	16.9
DP0308	23.5	12.3	24.9	18.5	22.8	14.1	"	"	25.0	14.5
DP0312	22.0	16.2	23.2	20.0	21.7	15.9	"	"	24.0	15.2
DP0314	22.8	14.8	23.9	16.7	22.8	14.4	"	"	24.6	16.4
DP0315	22.7	12.3	24.5	18.7	22.5	17.8	"	"	23.7	16.4
CT16 ^b	22.4	15.6	23.2	20.0	22.5	14.9	"	"	22.5	16.2
LAT-3; brushed 5 March 1996										
DP0302	19.7	13.3	21.5	17.5	20.3	15.2	22.3	16.0	21.5	13.6
DP0305	21.9	13.1	23.4	15.5	21.7	14.0	22.9	16.8	22.1	16.0
DP0308	22.8	15.6	23.0	17.6	20.6	15.0	23.3	17.2	24.3	16.2
DP0312	21.4	16.3	21.9	16.7	21.4	13.6	23.0	15.6	22.2	14.8
DP0314	22.0	15.6	22.9	16.5	20.7	14.7	23.0	16.7	23.2	14.7
DP0315	21.6	nd	23.1	16.5	21.7	14.4	23.6	15.9	20.2	16.4
CT16 ^b	20.8	16.8	21.1	17.9	19.4	15.3	21.9	16.8	19.9	17.8

^aCSP, cocoon shell percentage(%); CoY, cocoon yields(kg)/10,000 larvae; ^bControl hybrid; ^cnd, no data

Table 6. Occurrence-index points of six hybrids from the three rearing cycles of local adaptability tests

Hybrids	LAT-1		LAT-2		LAT-3		Total		O-index ^c
	CSP ^a	CoY	CSP	CoY	CSP	CoY	CSP	CoY	
DP0302	0	1	0	4	0	2	0	5	5
DP0305	2	3	1	4	3	1	6	7	13
DP0308	3	1	4	0	4	5	11	6	17
DP0312	1	1	2	3	3	1	6	5	11
DP0314	4	1	4	1	4	2	12	4	16
DP0315	4	1	2	2	3	1	9	4	13
CT16 ^b	0	4	1	1	0	5	1	10	11

^aCSP, cocoon shell percentage(%); CoY, cocoon yields(kg)/10,000 larvae; ^bControl hybrid; ^cOccurrence-index

selected to be candidates for further multiplication and distribution to the farmers for cocoon production. This modified method of simplified version of normal breeding methodology was very much successful in practical application in Sri Lanka in two previous selection programs of DP01 (1993) and DP02 (1994), completely replacing several imported hybrid varieties

in Sri Lanka.

摘 要

二化性品種을 이용한 잠사업을 새로 도입하거나 확장하려는 열대지역의 스리랑카와 같은 나라에서는, 보유하고 있는 遺傳子源이 빈약하고 또한 그 지역에

어느 정도 적응한 우수 교잡종이 단기간 내에 시급히 필요하므로, 학술적 이론에 근거한 정규적인 육종체계나 선발방법을 적용할 時間的 資原的 여유가 없는 경우가 일반적이다. 이러한 어려움을 극복해 보려는 시도로써 單純化 되었지만 현지의 제여건으로 볼 때 그래도 實用性이 있다고 여겨지는 일종의 變則的 育種方法을 도입하여 그 결과로 이화성 교잡종 DP 0308과 DP0314를 육성하였다. 여섯 종의 純系를 이용한 半二面交雜으로 조성된 유전적 變異源을 육종 소재로 시작한 변형된 육성과정을 제시하고 그 과정에서 얻어진 각 순계의 交配組合能力과 이 특정 遺傳集團의 실용형질에 대한 遺傳力을 추정하였다.

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