GENETIC MANIFESTATION OF HETEROSIS IN HYBRID BREEDS OF MULBERRY SILKWORM, *BOMBYX MORI*

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ABSTRACT

Hybrid vigour of six bivoltine silkworm breeds was assessed based on eight quantitative traits. The heterosis was estimated using multiple evaluation indices (EI), mid parent heterosis (MPH), better parent heterosis (BPH), and sub-ordinate function indices (SF). The results showed highly significant ($p<0.01$) difference in rearing performance of inbred and hybrid breeds. Hybrid JL04xCL04 showed superior performance in the fecundity (596.50±4.03 eggs per female), larval body weight (3.55±0.07 g), pupation rate (94.80±1.23%), cocoon yield (14.85±0.22 kg per 10,000 larvae) and cocoon weight (1.42±0.03 g), while hybrid C02x05M was superior in the shell weight (0.37±0.01 g) and shell ratio (26.47±0.04%). The EI values ranged between 40.96 and 61.92. All hybrids except 05POx06M resulted in greater than 50 EI values. Four hybrids showed positive MPH for the cocoon yield, cocoon weight and shell ratio, while three hybrids showed positive BPH for the cocoon yield, cocoon weight and shell ratio. The cumulative SF values ranged between 2.91 and 5.55. Based on mean EI and cumulative SF values, hybrids JL04xCL04 and 05POx06M were ranked first and sixth, respectively. The study identified hybrids JL04xCL04 and C02x05M as potential breeds for commercial rearing.

Key words: Mulberry silkworm, hybrid vigour, heterosis, multiple evaluation index, sub-ordinate function

INTRODUCTION

Silkworm breeds play an important role in sustainable cocoon yield of superior quality silk, and subsequently making sericulture a profitable enterprise. There are more than 3,000 silkworm breeds worldwide. These breeds are either univoltine, bivoltine or polyvoltine (Nagaraju, 2002; Thangavelu, *et al*., 2003). The univoltine and bivoltine breeds are superior in qualitative as well as quantitative traits as compared to polyvoltine, however, polyvoltine breeds are relatively superior in their survival and hardiness (Rao, *et al*., 2006). The silkworm breeds are classified also into two groups according to their genetic combination, *viz*. inbred or hybrid. Generally, the hybrid breeds are better yielding as compared to inbred breeds.

Japanese scientists first realized the use of hybrid vigour in sericulture in 1906 (Toyama, 1906). Later on, introduction of the hybrids at commercial level revolutionized sericulture industry with substantial increase in quality silk production (Chandrashekaraiah and Babu, 2003, Choudhary and Singh, 2007). The hybrid vigour is a result of assemblage and recombination of genetic variability, and expressed in qualitative and quantitative traits. In mulberry silkworm, 21 interlinked traits contribute to silk yield (Thiagarajan, *et al*., 1993). These traits are under complex polygenic control and influenced by environmental conditions. The multiple trait control of silk yield under polygenic influence warrants continuous exploring of genetic recombination with

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maximum exhibition of heterosis in economically important silk traits for viability and productivity.

In the changing scenario of the globe, especially for the developing countries, there is a great need to develop potential silkworm hybrids of higher quality and quantity to sustain the sericulture industry. In pursuit of this goal, Pakistan Forest Institute started silkworm-breeding programme to synthesize new hybrids and consequently developed six hybrid breeds, which required to be evaluated for hybrid vigour. Present study, therefore, was conducted to assess hybrid vigour of recently developed bivoltine silkworm hybrids to select potential breed(s) for commercial rearing.

MATERIALS AND METHODS

Six inbred bivoltine breeds, *viz*. C102, 205PO, 206PO, J101, 205MKD, 206MKD were used to synthesize hybrids C02x05PO, C02x05M, J01x06PO, 05POxJ01, 05POx06M and JL04xCL04. The experiment was conducted at the Pakistan Forest Institute, Peshawar during spring 2010 silkworm rearing season.

The disease free layings of inbred parents and hybrids laid during spring 2009 silkworm rearing season were surface sterilized using 2.0% formalin aqueous solution. The eggs were incubated in a well-disinfected incubator at 26±1°C with 80±5% relative humidity. The newly hatched larvae were brushed on finely chopped mulberry leaf and reared under hygienic conditions. The young age larvae (1^{st} to 3^{rd} instar) were reared at 25±1°C with 80±5% relative humidity. Before the first feeding of third instar, four hundred larvae were selected at random for each breed and reared in wooden trays (30x20x2.5) cm^3. The larvae were fed with mulberry variety PFI-1 five times daily. The late age larvae (4^{th} to 5^{th} instar) were reared at 23±1°C with 70±5% relative humidity. The full-grown fifth instar larvae were collected manually and mounted in plastic collapsible mountages for pupation. The cocoons were harvested on day six after cocoon formation and cut to collect pupae. Pupae were sorted out, and male and female pupae were kept separately until the moth emergence. The adult moths were coupled immediately after emergence and coupling lasted for five hours. The moths were decoupled and females were caged in conicals {5.5 (bottom)-1.5 (top) dia x 5.5} cm^2 on craft paper.

The rearing performance and heterosis were assessed based on eight quantitative traits giving equal weight to each trait. The quantitative traits estimated were the fecundity (number of eggs per female), full-grown fifth instar larval body weight (g), pupation rate (%), cocoon yield/10,000 larvae (kg/10k larvae) , single cocoon weight (g), shell weight (g), cocoon-shell ratio (%) and floss (%). The traits were calculated as follow:

$$\frac{\text{Cocoon Yield (kg)}}{\text{10,000 larvae}} = \frac{\text{WC x 10,000}}{\text{larvae retained after 2nd moult}}$$

$$\text{Pupation Rate(\%)} = \frac{(GC + 2DC)}{\text{larvae retained after 2nd moult}} \text{ x 100}$$

$$\text{Cocoon Weight(g)} = \frac{\text{W (25male} + \text{25female)C}}{50}$$

$$\text{Cocoon Shell Weight (g)} = \frac{\text{W (25 male} + \text{25 female) CS}}{50}$$

$$\text{Cocoon Shell Ratio (\%)} = \frac{\text{CSW}}{\text{CW}} \text{ x } 100$$

Where:

W = weight
C = cocoon
GC = number of good cocoons
DC = number of double cocoons
CS = cocoon shell

The data were further analyzed using evaluation index and sub-ordinate function methods, and the superiority of hybrids was ranked based on these indices.

Evaluation Index Method

Evaluation index values (EI) for eight traits of each breed were calculated following method as described by Mano, *et al*. (1993). The formula used to calculate evaluation index was:

$$\text{Evaluation Index} = \frac{(\text{A} - \text{B})}{\text{C}} \text{ x } 10 + 50$$

Where:

A = mean of particular trait of a breed
B = overall mean of particular trait of all tested breeds
C = standard deviation of a trait of all breeds
50 = constant

Hybrid Vigour Manifestation

The data generated for different traits were pooled separately for parental and hybrid breeds to estimate heterosis for each trait. The mid parent heterosis (MPH) and better parent heterosis (BPH) were calculated using formulae:

$$\text{Mid Parent Heterosis} = \frac{(\text{F1} - \text{MPV})}{\text{MPV}} \text{ x } 100$$

$$\text{Better Parent Heterosis} = \frac{(\text{F1} - \text{BPV})}{\text{BPV}} \text{ x } 100$$

Where:

F_1 = mean performance of the hybrid
MPV = mid parental value
BPV = better parental value

Sub-ordinate Function Method

Mean performance of each breed, and maximum and minimum performance across the six breeds for a trait were used to estimate sub-ordinate function values. Sub-ordinate function was measured using the formula as described by Gower (1971):

$$\text{Sub-ordinate function}(Xu) = \frac{(Xi - Xmin)}{(Xmax - Xmin)}$$

Where:

X_i = measurement of trait of tested breed
X_{min} = minimum value of the trait among all the tested breeds
X_{max} = maximum value of the trait among all the tested breeds

EXPERIMENTAL DESIGN AND ANALYSIS

The experiment was conducted in a randomized complete block design with four replications. The rearing performance of inbred and hybrid breeds was analyzed applying 1-Way analysis of variance test using Minitab version 15.1 statistical software. The difference among the breeds was tested by running Tukey's honestly significant difference (HSD) test at p=0.05.

RESULTS

The rearing performance of inbred and hybrid breeds for the fecundity, larval body weight, pupation rate, cocoon yield per 10,000 larvae, single cocoon weight, shell weight, shell ratio (SR) and floss are presented in Table 1. There was highly significant ($F_{11, 36}$ = 562.35; p<0.01) difference in fecundity. The highest fecundity was 596.50±4.03 eggs per female in hybrid JL04xCL04, while the lowest fecundity was 405.88±3.01 eggs per female in inbred 205PO. There was a significant (CV = 21.33, P = 0.05) difference among the breeds. All hybrids except 05POx06M laid greater number of eggs as compared to inbred breeds.

The full-grown larval body weight varied significantly ($F_{11, 36}$ = 4.11; p<0.01). The heaviest full-grown larva was 3.55±0.07 g in hybrid JL04xCL04, while the lightest larva was 2.97±0.06 g in inbred C102. The larval body weight of hybrid JL04xCL04 was 19.5% greater as compared to C102. Pupation rate was highly significant ($F_{11, 36}$ = 24.11; p<0.01). The highest pupation rate was 94.80±0.41% in hybrid JL04xCL04, while the lowest pupation rate was 81.95±1.18% in 05POxJ01. The pupation rate was relatively higher in hybrid breeds as compared to inbred breeds except 05POxJ01. The difference in cocoon yield was highly significant ($F_{11, 36}$ = 6.35; p<0.01) with the highest cocoon yield of 14.85±0.22 kg per 10,000 larvae in hybrid JL04xCL04. All the tested breeds both inbred and hybrid yielded greater than 13 kg cocoon per 10k larvae except J101 and 206MKD (Table 1).

There was highly significant difference in economic cocoon characters, *viz*. the cocoon weight ($F_{11, 36}$ = 3.22; p<0.01), shell weight ($F_{11, 36}$ =17.34; p<0.01) and SR ($F_{11, 36}$ =163.81; p<0.01), whereas, the floss did not differ significantly ($F_{11, 36}$ = 0.84; p>0.01).

Table 1. Rearing performance of inbred and hybrid silkworm breeds

Breed	Quantitative parameters							
	Fecundity± SE (No.)	Larval Wt± SE (g)	Pupation± SE (%)	Yield± SE (kg)	Cocoon Wt± SE (g)	Shell Wt± SE (g)	SR± SE (%)	Floss± SE (g)
C102	451.52 ±1.37g	2.97 ±0.06c	86.50 ±1.75cd	13.28 ±0.21bc	1.33 ±0.02ab	0.32 ±0.01bcd	23.48 ±0.07bc	4.36 ±0.49ns
205PO	405.88 ±3.01i	3.26 ±0.06abc	88.15 ±1.09bc	14.10 ±0.32ab	1.39 ±0.01a	0.32 ±0.01bc	22.69 ±0.20de	4.55 ±0.14
206PO	443.75 ±3.06g	3.15 ±0.06abc	93.43 ±0.41ab	13.88 ±0.12ab	1.37 ±0.02ab	0.29 ±0.01cde	20.90 ±0.15h	4.42 ±0.38
J101	430.88 ±2.32h	3.40 ±0.03ab	82.75 ±0.75de	11.98 ±0.23c	1.24 ±0.02b	0.24 ±0.01f	20.04 ±0.10i	3.93 ±0.21
205MKD	496.88 ±2.83ef	3.30 ±0.12abc	82.25 ±0.48de	13.18 ±0.51bc	1.32 ±0.05ab	0.29 ±0.01bcde	22.31 ±0.17ef	3.82 ±0.04
206MKD	510.50 ±2.78d	3.32 ±0.04abc	80.75 ±1.75e	12.95 ±0.28bc	1.30 ±0.03ab	0.28 ±0.01ef	21.19 ±0.16gh	3.87 ±0.17
C02x05PO	523.38 ±1.84c	3.32 ±0.11abc	93.40 ±1.17ab	13.15 ±0.41bc	1.31 ±0.04ab	0.31 ±0.01bcde	23.70 ±0.11b	3.92 ±0.40
C02x05M	488.25 ±2.01f	3.31 ±0.05abc	93.00 ±0.41ab	14.05 ±0.21ab	1.39 ±0.02a	0.37 ±0.01a	26.47 ±0.04a	4.10 ±0.17
J01x06PO	580.00 ±1.47b	3.36 ±0.09abc	93.50 ±1.19ab	13.03 ±0.23bc	1.31 ±0.03ab	0.28 ±0.01ef	20.87 ±0.19h	4.29 ±0.57
05POxJ01	501.50 ±1.19de	3.53 ±0.15a	81.95 ±1.18de	13.66 ±0.32ab	1.36 ±0.04ab	0.30 ±0.01bcde	21.74 ±0.09fg	4.31 ±0.24
05POx06M	444.75 ±1.43g	3.05 ±0.077bc	86.23 ±0.34cd	13.29 ±0.11bc	1.33 ±0.02ab	0.28 ±0.00def	21.26 ±0.04gh	4.44 ±0.31
JL04xCL04	596.50 ±4.03a	3.55 ±0.07a	94.80 ±1.23a	14.85 ±0.22a	1.42 ±0.03a	0.33 ±0.01b	23.01 ±0.17cd	3.70 ±0.14
CV	21.33	0.41	5.38	1.42	0.14	0.04	0.67	1.54

Yield = cocoon yield per 10,000 larvae, ns= non-significant; Figures in a column with same letter (s) are not significant at P=0.05, Tukey's HSD

The highest single cocoon weight was 1.42±0.03 g in hybrid JL04xCL04, while the lowest single cocoon weight was 1.24±0.02 g in inbred J101. On the other hand, the highest shell weight was 0.37±0.01 g in hybrid C02x05M and the lowest shell weight was 0.24±0.01 g in inbred J101. The SR results followed pattern of shell weight (Table 1). The floss values ranged between 3.70±0.14% and 4.55±0.14%, however, the difference among the breeds was not significant (CV = 1.54; p>0.05).

Multiple evaluation index (EI) values estimated for eight quantitative traits both of inbred and hybrid breeds are presented in Table 2. The mean EI values were ranged between 40.96 and 61.92. The mean EI values of all hybrids except 05POx06M were greater than 50, while EI values were less than 50 in all inbred breeds (Table 2). The highest EI value was 74.55 for SR in hybrid C02x05M followed by 72.91 for shell weight in same hybrid. The lowest EI value was 32.98 for cocoon yield in inbred J101 followed by 35.02 for larval weight in inbred C102. The hybrid JL04xCL04 showed highest EI values for six traits out of eight traits, while J101 showed the lowest EI values for four traits.

Table 2. Multiple evaluation values of different inbred and hybrid silkworm breeds

Breed	Quantitative parameters								
	Fecundity	Larval wt.	Pupation	Yield	Cocoon wt.	Shell wt.	SR	Floss	Mean
C102	43.29	35.02	47.59	48.05	50.17	50.38	57.02	46.26	47.22
205PO	35.22	48.68	50.71	55.56	53.59	50.61	52.34	42.82	48.69
206PO	41.92	43.25	58.63	54.97	51.40	46.60	41.79	45.20	47.97
J101	39.64	55.38	40.41	32.98	34.41	34.10	36.77	53.98	40.96
205MKD	51.31	50.43	39.45	46.85	48.66	49.74	50.06	51.94	48.56
206MKD	53.71	51.70	36.58	44.15	44.99	43.37	43.51	54.95	46.62
C02x05PO	55.99	51.43	60.84	46.56	45.06	54.85	58.26	54.12	53.39
C02x05M	49.78	50.97	60.05	57.00	58.79	72.91	74.55	50.87	59.36
J01x06PO	66.00	53.39	61.02	45.11	45.98	43.52	41.65	47.54	50.53
05POxJ01	52.12	61.26	38.91	52.44	55.56	50.14	46.74	47.12	50.54
05POx06M	42.09	38.53	47.12	48.14	48.94	45.48	43.92	44.82	44.88
JL04xCL04	68.92	62.47	60.85	66.20	64.33	60.40	54.23	57.97	61.92

Yield = cocoon yield per 10k larvae

The mid parent heterosis values showed variable heterotic effects on quantitative traits (Table 3). The highest MPH effect over parental performance was 35.2%, 11.4%, 10.39% and 10.84% on the fecundity, larval weight, pupation rate, cocoon yield and cocoon weight, respectively in hybrid JL04xCL04, while the highest MPH effect was 24.40% and 15.60% on the shell weight and SR, respectively in hybrid C02x05M. The hybrids C02x05M and J01x06PO expressed positive MPH effect for all the tested traits followed by JL04xCL04 which expressed MPH effect for seven traits. The hybrid 05POx06M exhibited positive MPH effect on least number of traits (pupation rate, floss).

The level of better parent heterotic effect was relatively less as compared to mid parent heterosis. Four hybrids expressed positive BPH effect in five or more traits (Table 4). The highest BPH effect over parental performance was 32.11%, 19.50% and 8.0% for the fecundity, larval weight and pupation rate, respectively in hybrid JL04xCL04. The highest better parent heterotic effect on the cocoon yield (14.01%) and cocoon weight (10.44%) was in hybrid 05POxJ01, while the highest BPH effect on the shell weight (23.99%) and SR (12.69%) was in hybrid C02x05M. The MPH effect was greater in the floss as compared to BPH except hybrid 05POxJ01.

Table 3. Mid parent heterotic effect (%) in different silkworm hybrids

Hybrid	Quantitative parameters							
	Fecundity	Larval Wt.	Pupation	Yield	Cocoon Wt.	Shell Wt.	SR	Floss
C02x05PO	22.08	6.49	7.00	-3.94	-3.26	4.63	2.63	-12.12
C02x05M	2.96	5.55	10.22	6.22	4.30	24.40	15.60	0.32
J01x06PO	32.63	2.63	6.86	0.76	1.46	3.78	1.97	2.77
05POxJ01	13.67	10.64	-3.14	4.74	5.20	9.21	-0.12	4.08
05POx06M	-2.93	-7.48	2.15	-2.60	-1.13	-1.67	-3.11	5.44
JL04xCL04	35.20	11.44	10.39	17.52	10.84	21.17	5.73	-10.69

Yield = cocoon yield per 10,000 larva

Table 4. Better parent heterotic effect (%) in different silkworm hybrids

Hybrid	Quantitative parameters							
	Fecundity	Larval Wt.	Pupation	Yield	Cocoon Wt.	Shell Wt.	SR	Floss
C02x05PO	28.95	1.78	6.01	-6.73	-4.28	4.50	4.43	-13.99
C02x05M	8.13	11.33	7.51	5.81	3.94	23.99	12.69	-5.96
J01x06PO	30.70	6.81	1.35	-6.13	-2.46	-3.41	-0.11	-2.98
05POxJ01	16.39	3.65	-0.95	14.01	10.44	20.66	8.45	9.85
05POx06M	9.58	-6.58	-2.13	-5.77	-2.53	-5.45	-6.31	-2.48
JL04xCL04	32.11	19.50	8.00	11.78	6.48	10.67	-2.02	-15.15

Yield = cocoon yield per 10,000 larva

The cumulative sub-ordination function values ranged between 2.91 and 5.55 (Table 5). The difference in cumulative SF was marginal between C02x05M and JL04xCL04; J01x06PO and 05POxJ01. The highest SF value for any single parameter was 0.99 for the shell ratio in hybrid C02x05M followed by 0.95 for the fecundity in hybrid JL04xCL04. The lowest SF value was 0.17 for SR in hybrid J01x06PO.

Table 5. Sub-ordinate function values of different silkworm hybrids

Hybrid	Quantitative parameters								
	Fecundity	Larval Wt.	Pupation	Yield	Cocoon Wt.	Shell Wt.	SR	Floss	Cum. SF
C02x05PO	0.60	0.51	0.83	0.45	0.38	0.51	0.58	0.29	4.15
C02x05M	0.43	0.50	0.81	0.70	0.68	0.90	0.99	0.36	5.37
J01x06PO	0.87	0.55	0.83	0.41	0.40	0.27	0.17	0.43	3.93
05POxJ01	0.49	0.71	0.25	0.59	0.61	0.41	0.29	0.44	3.79
05POx06M	0.22	0.25	0.47	0.48	0.47	0.31	0.22	0.49	2.91
JL04xCL04	0.95	0.73	0.83	0.91	0.80	0.63	0.48	0.21	5.55

Yield = cocoon yield per 10,000 larva

Based on EI and SF values, hybrids were ranked and presented in Table 6. The hybrid JL04xCL04 was ranked first with mean EI value of 61.92 and cumulative SF value of 5.55, while 05POx06M was ranked sixth with EI and SF values of 44.88 and 2.9, respectively. The ranking of hybrids based on EI values was same as based on cumulative SF except hybrids 05POxJ01 and J01x06PO. Based on mean EI value, hybrid 05POxJ01 was ranked fourth while according to cumulative SF values it was ranked fifth. The hybrid 05POxJ01 interchanged its position with J01x06PO (Table 6).

Table 6. Ranking of silkworm hybrids according to mean EI and cumulative SF values

Hybrid	Mean EI	Rank	Cumu. SF	Rank
JL04xCL04	61.92	1	5.55	1
C02x05M	59.36	2	5.37	2
C02x05PO	53.39	3	4.15	3
05POxJ01	50.54	4	3.79	5
J01x06PO	50.53	5	3.93	4
05POx06M	44.88	6	2.91	6

DISCUSSION

The results showed significantly greater rearing performance of hybrid breeds as compared to inbred. The hybrid JL04xCL04 showed superior performance in five quantitative traits including important traits of viability and productivity, such as, cocoon yield and cocoon weight. Hybrid C02x05M showed superiority in shell weight and shell ratio. Five hybrids obtained mean evaluation index values greater than 50. The present greater rearing performances as well as mean evaluation index values of hybrids are in conformity with Rayar (2007) and Seshagiri, *et al*. (2009). The variation in performance of different traits may be explained in terms of complicated polygenic control of traits under the influence of environmental conditions.

This study indicated heterosis (both mid and better parent) for different economically important traits. The heterotic effect was highest on economic cocoon traits, such as, cocoon yield, cocoon and shell weight, and SR. The present highest positive mid parent and better parent heterotic effect on cocoon yield, cocoon weight and SR is in corroboration with Doddaswamy, *et al*. (2009), Das, *et al*. (1994), Farooq, *et al*. (2006), Kumaresan, *et al*. (2003), who found a positive heterotic effect on economic cocoon traits of different silkworm hybrids. The number of hybrids showing mid parent heterosis for more traits was greater as compared to better parent heterosis. Five hybrids expressed positive mid parent heterosis for four traits, while three hybrids expressed positive better parent heterosis for three traits. These findings are in parallel to Talebi and Subramanya (2009) who reported positive and significant mid parent heterosis for eight hybrids, while better parent heterosis for two hybrids out of 12 hybrids. Similar findings have also been reported by Murkami (1994); Kumaresan, *et al*. (2003); Iftekher *et al*. (2005); Ahsan and Rahman (2008); Nagalakshmamma and Jyothi (2009). The results of better parent heterosis showed no single hybrid with positive heterotic effect on all tested traits. This is in agreement with Datta, *et al*. (2001). The high degree of heterosis in specific breeds for some characters in this study may be assigned to complimentary gene effect as well as genetic heterogeneity in parent breeds as has been reported earlier by Falconer (1988); Udupa and Gowda (1988).

The cumulative sub-ordination function values ranged between 2.91 and 5.55. The present sub-ordinate function values are comparable with the values reported by Ramesha, *et al*. (2009) for polyvoltinexbivoltine combinations. Based on sub-ordinate function values, hybrid JL04xCL04 was ranked first, while 05POx06M was ranked sixth. This ranking substantiated further the superiority of hybrids as indicted by multiple evaluation index values. These findings are similar as previously breeders (Gower, 1971; Babu, *et al*., 2001; Rao, *et al*., 2004; Rao, *et al*., 2006) have reported nearly same ranking of different hybrids reckoned either using multiple evaluation index method, sub-ordinate function method or both. The slight variation at lower ranks, however, justifies application of multiple evaluation index method as well as sub-ordinate function method for precise measurement of genetic manifestation of heterosis.

CONCLUSION

The results exhibited considerable heterotic effect in five out of six hybrids. The degree of heterosis, however, varies with hybrid and quantitative trait. The highest mid parent and better parent heterosis was 35.20% and 32.11%, respectively for the fecundity in hybrid JL04xCL04. The multiple evaluation and sub-ordinate function methods indicated JL04xCL04 and C02x05M the most productive hybrids. Based on these findings, it is concluded that the hybrids JL04xCL04 and C02x05M may be synthesized as promising breeds for commercial use.

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