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RESEARCH

Comparison of Phenotypic Value Changes in Pure Lines of *Bombyx mori* (Lepidoptera: Bombycidae) During Consecutive Generations Following Initial Selection on Cocoon Weight

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ABSTRACT. The experiments reported here were conducted to investigate the effect of selection on three quantitative traits, namely cocoon weight, cocoon shell weight, and cocoon shell percentage, during four generations by rearing six pure breeds of domesticated silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae) of Chinese and Japanese origin compared with random unselected groups as controls. All stages of rearing and data recording were performed over four rearing periods, with generations 1–3 during successive spring seasons and generation 4 during the autumn season in year 3. Each pure line contained two groups of selected and random (control) groups. Comparisons included the effect of selection methods, pure line, and generation on the phenotypic values. We found strong main effects of pure line, generation, sex, and group and support for nearly all interactions between these main effects for all three response traits. The results indicated that cocoon weight and cocoon shell weight in the selected group were higher than in the control or nonselected group. Both selected and nonselected groups had the lowest cocoon weight, cocoon shell weight, and cocoon shell percentage in the fourth generation when environmental conditions during the autumn season were less favorable than spring. The cocoon weight and cocoon shell weight averages were higher for nonselected groups in the second and third generations, and for the selected group in the first generation due to the direct effect of selection.

Key Words: *Bombyx mori*, breeding value, genetics, cocoon, individual

Production of raw silk and cocoons by the domesticated silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae) is affected by different factors such as the genetic potential of commercial varieties, egg quality, pests and disease (Aruga 1994), mulberry quality, climate, rearing and management methods, and silk drying and reeling (Sen et al. 1999). A primary aim of silkworm breeding is the improvement of the trait average via an increase of the genetic potential of the pure lines used for hybridization and crossbreeding. With respect to current conditions of sericulture, an increase of the productive potential of commercial hybrids (improvement of cocoon weight and cocoon shell weight averages and maintenance of pure line resistance) is the main objective to increase the profits of egg producers, cocoon producers, and silk yarn manufacturers and to save the silk industry from extinction. Breeding can play a valuable role in achieving these objectives. Therefore, an accurate analysis of the current sericulture system should be conducted in each country together with a formulation of global objectives and policies based on future farming and industrial prospects (Mita et al. 2004). Development of improved breeds is one of the most effective approaches to improve the profitability of a sericultural system and reduce costs (Lu et al. 2001).

The performance and response of six *B. mori* hybrids to phenotypic selection of superior parents were reported previously (Mirhosseini et al. 2006). These studies showed that hybrids had a significant response to parental selection, and their responses to selection were significantly different. In another report (Seidavi et al. 2008), researchers studied the phenotypic responses of different varieties to parental phenotypic selection based on cocoon weight. They reported selection can change the average of total cocoon weight per batch at levels of 50, 25, and 5 g in Chinese, Japanese, and hybrid varieties, respectively.

Cocoon weight, which includes the last instar larval skin, pupa, and silk cocoon shell, is an important trait in sericulture. Because this trait has moderate heritability and is actually easy to quantify researchers select original populations based on this trait. At the present time, there is little information about the effect of individual selection based on cocoon weight on phenotypic values of commercial pure lines. Therefore, the experiments reported here were conducted to investigate the change in phenotypic value of economic traits including cocoon weight, cocoon shell weight, and cocoon shell percentage under individual selection based on cocoon weight for six commercial pure lines, which are used for silk production in Iran.

Materials and Methods

Six bivoltine strains of *B. mori* conserved as commercial pure lines in Iran were used. Three pure lines were of Japanese origin (31, 103, and 107) and three pure lines were of Chinese origin (32, 104, and 110). All six pure lines are preserved in the Iran Silkworm Research Center. Analysis of all life history stages including larval rearing, feeding, cocoon production, egg preparation and conservation, hatching, and related ancillary activities such as microscopic survey for pebrine, investigation of embryonic development, recording, and collecting data were conducted in the Iran Silkworm Research Center under standard conditions. The quantitative characteristics studied included cocoon weight (g), cocoon shell weight (g), and cocoon shell percentage (%). Cocoon weight and cocoon shell weight were recorded using a precision A&D GF-300 digital balance (A&D Weighing Design and Manufacture, San Jose, CA).

Data were recorded during four consecutive generations or rearing duration. Generations 1–3 were reared in the spring season of three consecutive years, and generation 4 was in the autumn season of year 3. First, a base population was established for each pure line from 3P (parent × parent × parent) populations. Pure lines with such high inbreeding are used as parental groups for commercial egg production.

Each pure line contained two groups designated as selected or random groups. The selected group for each pure line contained 40 male and 40 female cocoons which had superior cocoon weights in the population for each sex. Data for these selected individuals were recorded based on three individual traits, including cocoon weight, cocoon shell weight, and cocoon shell percentage, and then adults were mated together randomly. The randomized or control group was constructed in each pure line using 40 male and 40 female cocoons which were assigned randomly without any selection and had cocoon weights equal to the population average. Three individual traits including cocoon weight, cocoon shell weight, and cocoon shell percentage were also recorded for these randomized individuals, which were then mated together randomly. This gave a total of 40 egg batches in each pure line for each of the selected and randomized groups. In all, there were 960 records in the base population for each trait (cocoon weight, cocoon shell weight, and cocoon shell percentage). Rearing and egg production in succeeding generations for selected and control groups were followed in separate paths.

At the first generation (or 3P population from 40 batches for each group and pure line as indicated above), eight egg batches were selected which had superior hatchability and fecundity. Consequently, each group in each pure line consisted of eight full-sib families. At the end of the rearing duration and cocoon production and after determining the sex of each individual, 25 male and 25 female cocoons were recorded in each family based on cocoon weight, cocoon shell weight, and cocoon shell percentage to construct a pedigree. The first generation data included 4,800 records for each trait and 800 records for each pure line. Finally, 40 male and 40 female cocoons were selected randomly among the eight families from each pure line and selected versus unselected group and mated randomly for egg production for the next generation.

Various stages of rearing and producing raw data in the second (2P) and third (3P) generations were conducted in the same manner as in the first generation. The total number of data files included 15,360 records for each of the three studied traits (2,560 records in each pure line). It should be noted that individual selection was applied only in the base population, and all matings were conducted randomly in succeeding generations.

We analyzed the data on cocoon weight, cocoon shell weight, and cocoon shell percentage using four-way analysis of variance (ANOVA). For each response variable, we assessed the impact of pure line, generation, group, sex, and all of the two-, three-, and four-way interactions. All analyses were conducted in JMP 9.0 (SAS Systems, Raleigh, NC).

Results and Discussion

The ANOVA of the phenotypic values for cocoon characteristics is listed in Table 1. The main effects of pure line, generation, sex, and group on cocoon weight, cocoon shell weight, and cocoon shell percentage were significant ($P < 0.01$). The interactions of the pure line × generation, pure line × group (except for cocoon shell percentage), generation × group, pure line × generation × group, pure line × sex, generation × sex, pure line × generation × sex, group × sex (except for cocoon shell percentage), pure line × group × sex (except for cocoon weight and cocoon shell weight), generation × group × sex (except for cocoon shell percentage), and the pure line × generation × group × sex were significant on the cocoon trait characteristics ($P < 0.01$).

The means of cocoon weight, cocoon shell weight, and cocoon shell percentage of nonselected and selected groups are presented and compared in Figs. 1–3. The results of breeding values and indicated cocoon weight and cocoon shell weight (Figs. 1 and 2) were higher in the selected group than the control or nonselected group in all generations except for the autumn rearing period (fourth generation) when both selected and nonselected groups had the lowest values for all of these parameters, including high productive lines 31 and 103, likely due to unfavorable environmental conditions when leaf quality is generally low. The cocoon weight and cocoon shell weight averages were higher for selected groups in the first generation (1.569–1.860 g of cocoon weight and 0.327–0.449 g of cocoon shell weight) and for nonselected groups in the second generation (1.455–1.719 g of cocoon weight and 0.300–0.384 g of cocoon shell weight) and third generation (1.457–1.796 g of cocoon weight and 0.297–0.403 g of cocoon shell weight), presumably due to the direct effects of selection. A previous study applied similar selection indexes in three different pure lines and showed that phenotypic averages in hybrids for economical traits including cocoon weight, cocoon shell weight, and cocoon shell percentage in selected groups were significantly higher than in nonselected groups (Ghanipoor et al. 2007).

Investigation of the results obtained for cocoon shell percentage (Fig. 3) showed that this trait also had the highest average in the first generation in both selected and nonselected populations (21.0–24.7% for selected populations and 22.1–25.3% for nonselected populations). Additionally, individual selection based on the cocoon weight had no negative effects on phenotypic values for cocoon shell percentage (e.g., 22.3 and 22.5% for selected and nonselected pure line 31 in the second generation). One explanation is a likely low genetic correlation (near zero) between cocoon weight and cocoon shell percentage as reported previously (Shabdini et al. 2011). This may be related to the fact that

Table 1. Results of ANOVA statistical analysis of the main effects of pure line, generation, group, and sex on cocoon weight, cocoon shell weight, and cocoon shell percentage

Response variable		Cocoon weight			Cocoon shell weight			Cocoon shell %		
Variation source	DF	SS	F	P	SS	F	P	SS	F	P
Pure line	5	77	811	<0.001	8.93	1385	<0.001	5,457	409	<0.001
Generation	3	209	3,655	<0.001	11.34	2,931	<0.001	3,000	375	<0.001
Group	1	37	1,936	<0.001	1.63	1,263	<0.001	54	20	<0.001
Sex	1	290	15,203	<0.001	0.58	450	<0.001	36,103	13,534	<0.001
Pure line × generation	15	33	115	<0.001	2.14	111	<0.001	915	23	<0.001
Pure line × group	5	1.30	14	<0.001	0.10	16	<0.001	19	1.39	0.224
Generation × group	3	24	426	<0.001	1.34	345	<0.001	143	18	<0.001
Pure line × generation × group	15	2.77	9.69	<0.001	0.22	11	<0.001	428	11	<0.001
Pure line × sex	5	2.75	29	<0.001	0.10	16	<0.001	225	17	<0.001
Generation × sex	3	7.03	123	<0.001	0.06	16	<0.001	237	30	<0.001
Pure line × generation × sex	15	2.44	8.51	<0.001	0.13	6.94	<0.001	311	7.77	<0.001
Group × sex	1	1.12	58	<0.001	0.01	10	0.001	0.86	0.32	0.57
Pure line × group × sex	5	0.20	2.13	0.059	0.01	1.51	0.184	30	2.25	0.047
Generation × group × sex	3	1.21	21	<0.001	0.02	4.66	0.003	21	2.63	0.049
Pure line × generation × group × sex	15	0.75	2.63	0.001	0.04	2.16	0.006	116	2.90	<0.001
Error	14,961	286			19			39,910		

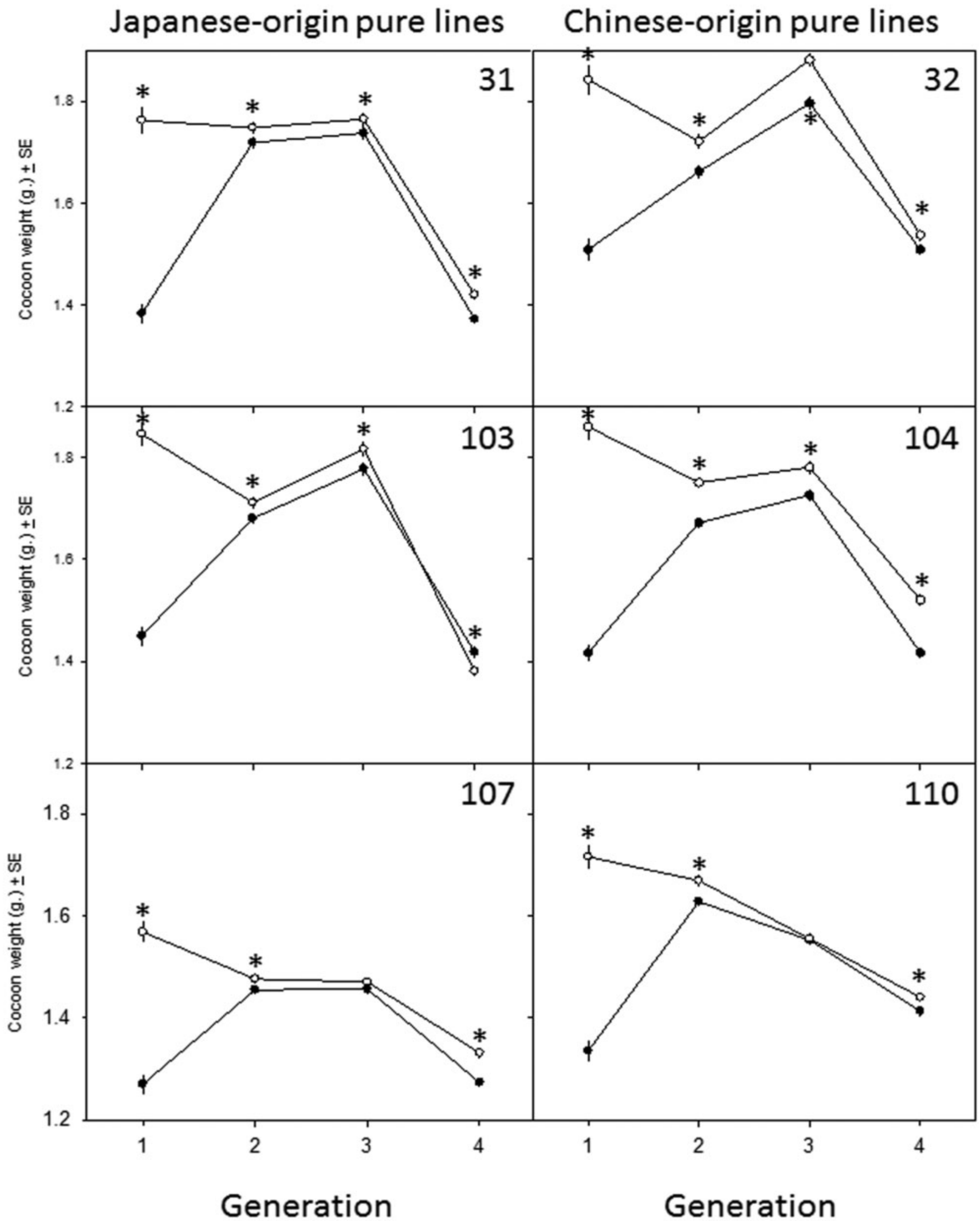

Fig. 1. Average cocoon weight (g) in six pure commercial lines (strains) for nonselected (filled circles) and selected (open circles) groups at four successive generations. Within each line × generation combination, treatments with an asterisk are significantly different ($P < 0.05$).

cocoon weight includes both the insect body (pupa) and cocoon shell, so that selection at any generation is acting on both characters. Similar conclusions regarding the genetics of quantitative traits have been published by other researchers for *B. mori* (Kumaresan et al. 2000, Nagaraju 2002, Nagaraju and Goldsmith 2002) and other silkworm species (Reddy et al. 2010a,b). On the other hand, in three strains (31, 103, and 107), the average values of cocoon shell percentage were significantly higher during the autumn rearing in the nonselected than in the selected groups (Fig. 3), suggesting that complex environmental factors may influence the relative yield of silk per insect, despite the fact that the average cocoon weight in these cases was lower (Fig. 1).

It was previously reported that the individual selection system based on cocoon weight reduced heterosis and other genetic parameters for resistance traits (Moorthy et al. 2007). In contrast, another study demonstrated that phenotypic selection of parents based on cocoon weight significantly increased the hybridization parameters of productive traits (Mirhosseini et al. 2006). In addition, yet a third study using selected pure lines found that selection based on cocoon weight significantly decreased heterosis of double cocoon weight in the selected group compared with control group, whereas selection decreased the value for general combining ability (Das 2001). These apparently contradictory results suggest that higher order interactions may significantly affect the phenotypic expression of economically important characters. This highlights the difficulty of predicting the impact of selection and the importance of testing each commercial strain for the stability of the economic traits of interest or the potential effects of inbreeding depression.

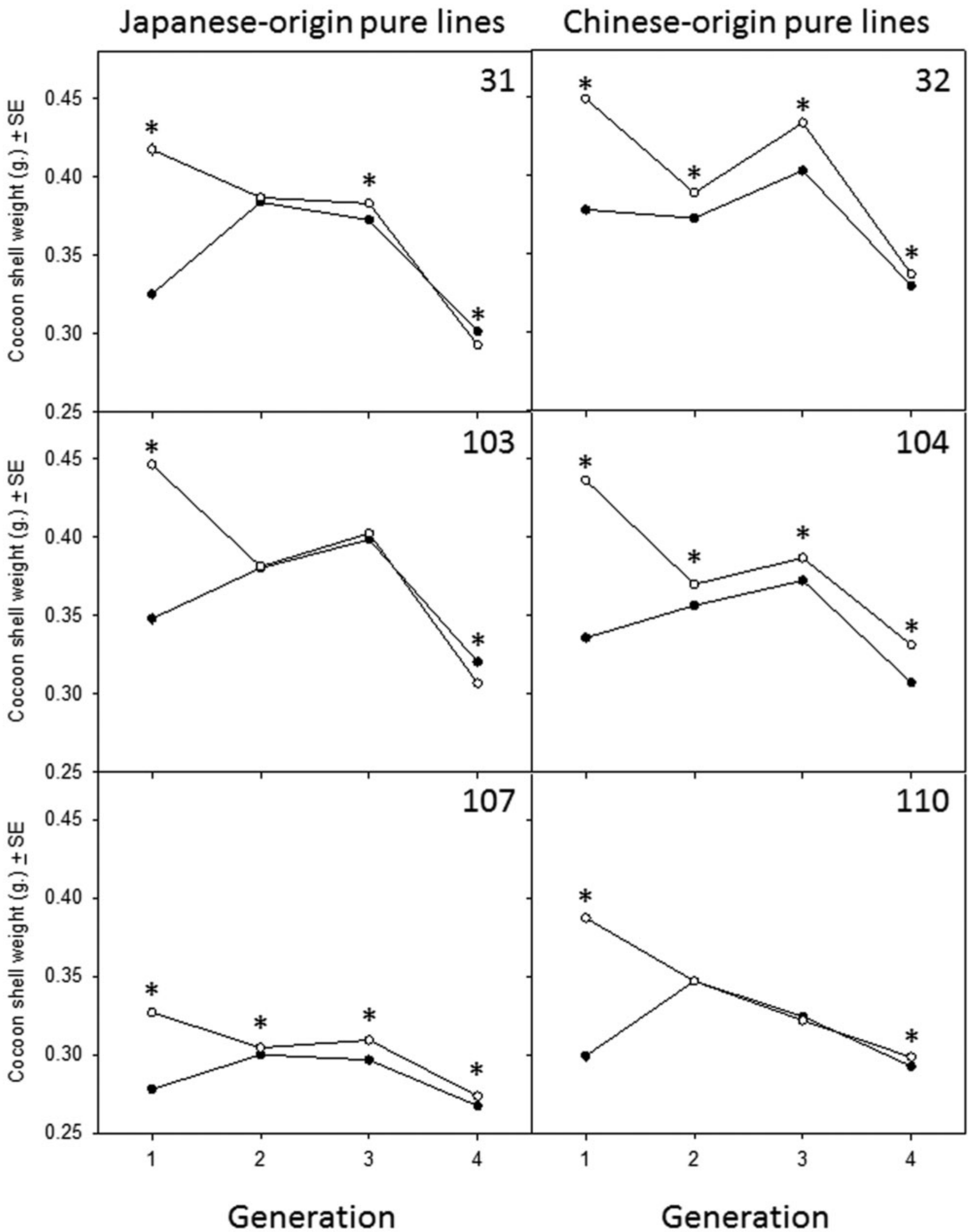

Fig. 2. Average cocoon shell weight (g) in six pure commercial lines (strains) for nonselected (filled circles) and selected (open circles) groups at four successive generations. Within each line × generation combination, treatments with an asterisk are significantly different ($P < 0.05$).

Experiments conducted to measure the effects of various selection systems on the economic characteristics of hybrid broods found that selection based on quantitative traits in 3P broods did not significantly decrease disease resistance or reproductive characteristics (Seidavi et al. 2007). Nevertheless, there are some reports of positive heterosis for reproductive and resistance characteristics (Mirhosseini et al. 2005). However, despite reports of a negative correlation between productive and reproductive traits with resistance traits in some pure lines of *B. mori*, it can be stated that selection based on cocoon traits will not have a significant negative effect on resistance and reproductive traits, and can even increase the production average. Furthermore, a study of the effects of individual selection on disease resistance in *B. mori* found a positive correlation with production characteristics (Seidavi et al. 2007).

The unselected versus selected cocoon trait means among the different pure lines (strains) are presented and compared graphically in Figs. 1–3. As noted previously, the different ranking of pure lines showed significant interactions between pure line, generation, and group. Other researchers have reported that selection based on productive and reproductive traits (Chattopadhyay et al. 2001, Mirhosseini et al. 2007) can improve economical traits during consecutive generations. Another study that investigated the effect of individual selection

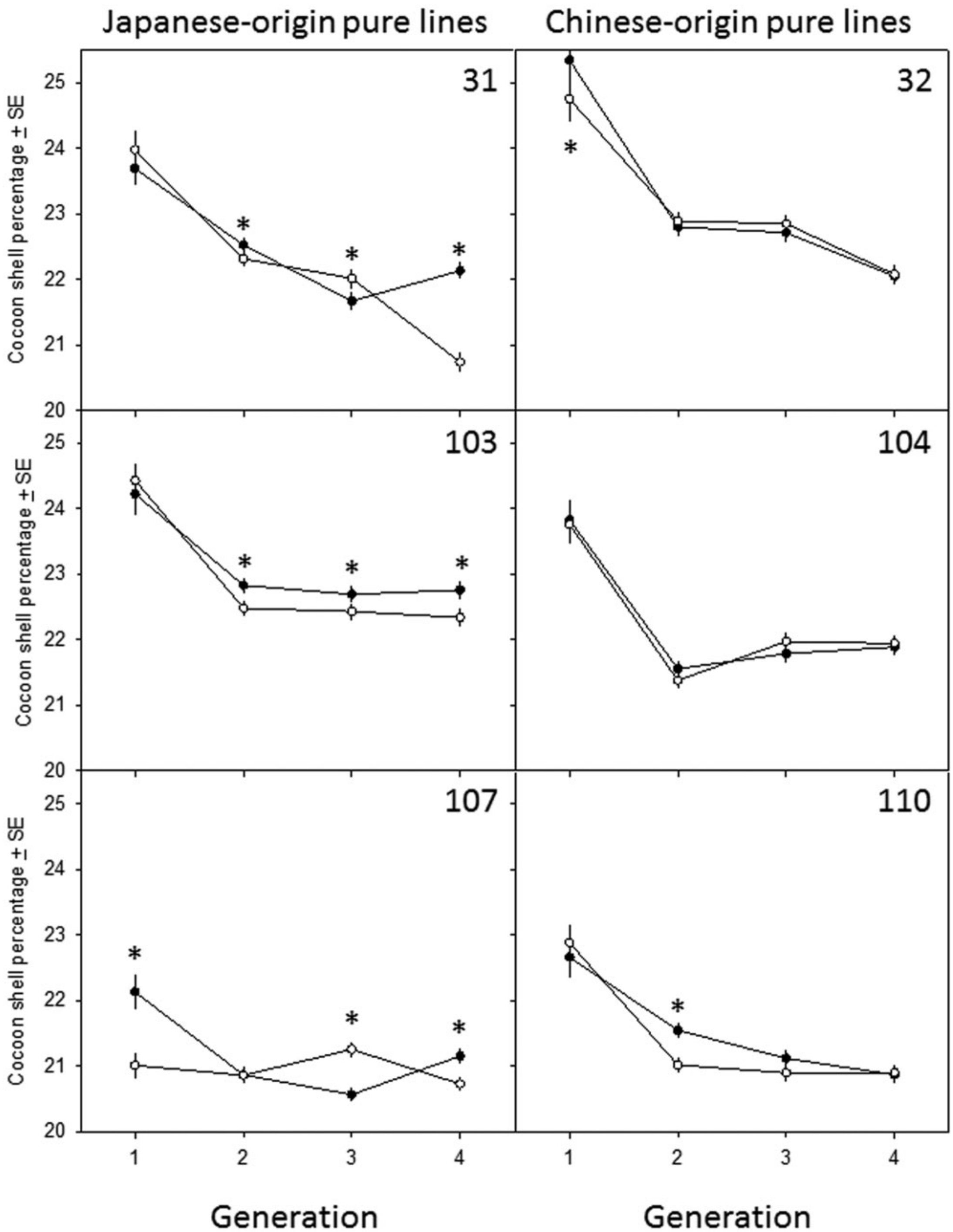

Fig. 3. Average cocoon shell percentage in six pure commercial lines for nonselected (filled circles) and selected (open circles) groups at four successive generations. Within each line × generation combination, treatments with an asterisk are significantly different ($P < 0.05$).

based on cocoon weight of a 31 × 32 hybrid found significantly higher performance in the selected group than in the control group (Seidavi et al. 2008). Individual selection based on cocoon weight also increased other performance traits such as total cocoon weight and cocoon number in the tropical tussar silkworm, *Antheraea mylitta* (Manohar et al. 2009).

The overall performance of traditional pure lines such as 110 and 107 was lower than in new pure lines including 31, 32, 103, and 104. These older pure lines should be gradually eliminated from the commercial *B. mori* egg production cycle. Meanwhile, in offspring derived from selection programs, additive and nonadditive effects (the interaction between additive genes or epistasis) were shown to have positive effects on phenotypic improvement. However, the genetic stability of the selected pure lines was not high, and hence if there is not continuous selection in similar breeding programs, their genetic structure will transform quickly.

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