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Breeding of Near Isogenic Lines of Silkworm (*Bombyx mori* L.)

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Four different backcrossing methods were designed and 23 near isogenic lines (NILs) of 22 linkage groups were obtained using *Hb* as recurrent parent, the mutant gene lines which held markers as donor parents. Eleven of them had been mated with the recurrent parent for 10 times, and the others for 7 ~ 8 times. The NILs of other 6 linkage groups are under way and had been backcrossed to the recurrent for 3 ~ 4 times. These NILs will act important roles in the construction of molecular linkage map and gene location and positional cloning.

Key words: Silkworm, *Bombyx mori* L., Linkage group, Near isogenic line, Gene/Mutant

Introduction

Near isogenetic lines (NILs) mean a group of lines that are genetically identical except at one or a few loci. A NIL is developed by several backcrosses (more than six generations) between the recurrent and donor (which take the aim marker) parent. In each generation, the individuals which are similar to the recurrent parent except the aim gene should be selected and are backcrossed to the recurrent parent. Self-crossing is taken out after backcrossing for more than 6 generations, and the aim gene is homozygoused. When the NIL is completed, it is genetically identical to the recurrent parent except the region close to the aim gene locus on the chromosome. Molecular markers linked to the aim gene can be identified using this NIL, and the molecular linkage map can be parallelism to the traditional mutant linkage map. Yong *et al.* (1988) Identified DNA markers linked to the *Tm-2a* gene in tomato using isogenic lines. Martin *et al.* (1991) used random markers and NILs to identify markers linked to a pseudomonas resistance gene in tomato. Abe *et al.* (1998, 2000) bred the NILs of *nsd-1* and *nsd-2*, then identified and mapped several RAPD markers linked to the *nsd-1* or *nsd-2* allele successively. Li *et al.* (2001) had constructed the near-isogenic lines of *nsd-Z* and had identified RAPD markers linked to *nsd-Z*. We designed 4 methods to breed NILs, and began to construct NILs of silkworm mutants since 1999, and have obtained 23 NILs of 22 chromosomes. The other 6 NILs of other chromosomes are under way.

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Materials and Methods

Silkworm races

Recurrent parent: *Hb*Chinese bivoltine strain, tetramolting. Its egg-color was celadon; newly hatched larvae were black; larvae were plain, white blood and robust; cocoon-color was white. It was preserved in Sericultural Research Institute, Chinese Academy of Agricultural Sciences.

Donor parents: One or two markers of each linkage group were selected as donor parents. They were: *sch* (1-21.5, sex-linked chocolate newly hatched larva), *Y* (2-25.6, Yellow blood), *Ze* (3-20.8, Zebra), *L* (4-15.3, Multilunar), *re* (5-31.7, red egg), E^{Kp} (6-21.1, Kp-supernumerary legs), *q* (7-0.0, quail), *st* (8-0.0, stony), *Ia* (9-22.1, Dominant chocolate), *w-2* (10-16.1, white egg 2), *K* (11-25.4, Knobbed), *ms* (12-5.5, multistars), *ch* (13-9.6, cholocate), *U* (14-40.5, Ursa), *bl* (15-0.0, blind), *nsd-Z* (15-0.0, nonsusceptible to DNV-Z) *cts* (16-4.6, cheek and tail spots), *bts* (17-30.1, brown head and tail spots), *mln* (18-41.5, melanism), *nb* (19-31.2, narrow breast), *oh* (20-20-0.0, hoarfrost translucent), *rb* (21-0.0, red blood), or (22-8.9, r-translucent), *tub* (23-6.9, tubby), *Sel* (24-0.0, Sepialumazine), *Nd* (25-0.0, Naked pupa), *so* (26-0.0, sooty), *Xan* (27-0.0, Xanthous) and *E-tr* (28-?, Tr-extra-legs) (Okido *et al.*1998).

Hb × *Ze*
↓
Hb × F_1
↓
Hb × BC_1 (♂ individuals expressed *Ze* were selected)
↓
Hb × BC_2 (♂ individuals expressed *Ze* were selected)
⋮
⋮
BC_x (x > 6) Selfed
↓
Homozygous *Ze*
↓
Ze NIL

Fig. 1. *Ze* as a modle to construct the NILs of dominant genes.

Hb × *re*
↓
F_1 (Selfed)
↓
Hb × F_2 (♂ individuals expressed *re* were selected)
↓
$F2BC_1$ (Selfed)
↓
Hb × BC_1F_2 (♂ individuals expressed *re* were selected)
↓
F_2BC_2 (Selfed)
↓
Hb × BC_2F_2 (♂ individuals expressed *re* were selected)
⋮
⋮
BC_xF_2 (x > 6) (Selfed)
↓
Homozygous *re*
↓
re NIL

Fig. 2. *re* as a modle to construct the NILs of recessive genes.

Methods

According to Mendels law of inheritance, four different backcrossing methods were designed to breed different NILs with different aim genes. In each generation, the individuals which expressed all of the characters of recurrent parent except the aim gene were selected to backcrossed to recurrent parent again.

Breeding of NILs of dominant genes: When the aim gene was dominant, it was very easy to select the individuals which express the aim gene in the generations of backcrossing. Take *Ze* as example, the model was shown in Fig. 1 All of the NILs of dominant genes were bred by means of this method.

Normal method of breeding NILs of recessive genes: Recessive genes would be lost if the method was the same as the dominant genes to breed NILs, because it could not express when the offspring was hybrid during the backcrossing period. So the F_1 was selfed, then 1/4 of the F_2 offspring would express the aim recessive gene, and then these individuals were backcrossed to the recurrent parent. After several circles, NILs of recessive genes could be obtained (shown in Fig. 2, taking *re* as example). The NILs of *re, q, st, w-2, bl, cts, bts, mln, oh, rb, or, tub* and *so* were constructed using this method.

A quick method of breeding NILs of recessive genes: The normal method of breeding NILs of recessive genes was very slow, so we design a quick method to breed NILs of recessive genes. In order to ensure that the recessive gene would not be lost, we used a single male moth, which carried the aim recessive gene, to mate successively with two females of different races from the BC_1 generation. In other words, the male moth was mated to a female moth of a recurrent race first, and then mated to another female moth of donor parent. The eggs from the two females were reared separately, and the offspring of backcrossing to donor parent were observed to test for the presence of the aim gene. If all of those individuals did not express the aim gene, the offspring obtained from the same male crossed to the recurrent parent was discarded. On the contrary, if 50% of the offspring obtained from the backcrossing to the donor parent express the aim gene, the offspring obtained from the same male moth crossed to the recurrent parent were reared (shown in Fig. 3, using *ch* as example). NILs of *ch* and *nsd-Z* were bred by means of this method.

A quick method of breeding *sch* NIL: *sch* was located on the sex chromosome (Z chromosome), another quick method was designed with fewer trivial details than the quick method for the recessive genes located on autosomes. If we used male *sch* parent, the males of F_1 (Z^+/Z^{sch}, did not express sch) were mated to the female of recurrent, then in the BC_1 generation, 1/2 female express *sch* (W/Z^+; W/Z^{sch}). The female individuals expressed *sch* were mated to the males of recurrent parent, half of their male offspring (BC_2) will take *sch* (Z^+/Z^{sch}; Z^+/Z^+). All the male offspring (BC_3) was selected to mated to female recurrent parent, and 1/4 female of the offspring would express *sch* ($3W/Z^+$; $1W/Z^{sch}$). After several circles, the NILs of *sch* had been obtained (shown in Fig. 4).

Results and Discussion

After 10 times of backcrossing to *Hb*, the NILs of *sch, Y,*

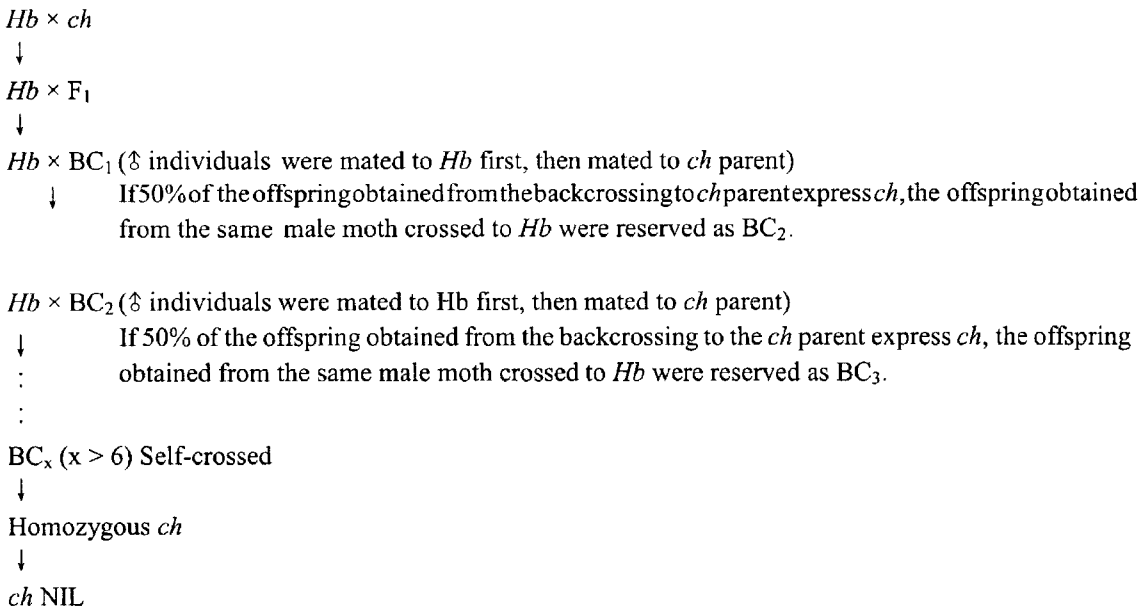

Fig. 3. *ch* as a model as a quick method of breeding NILs of recessive genes.

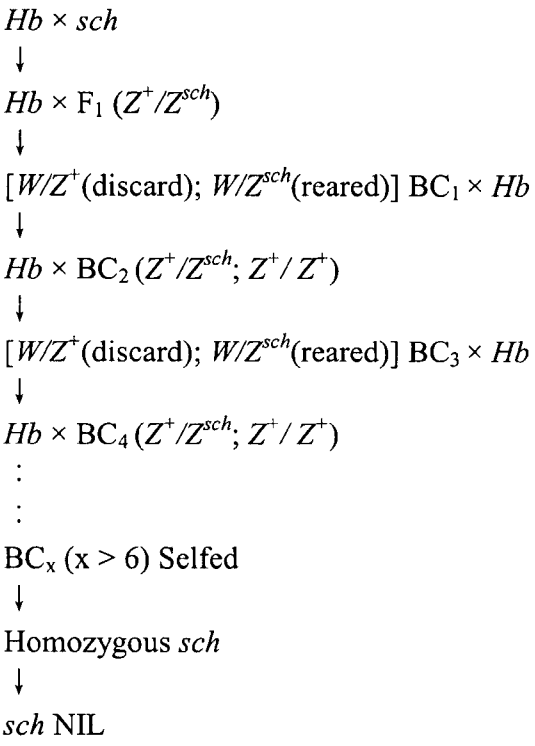

Fig. 4. Construction *sch* NIL.

Ze, L, E^{kp}, Ia, K, U, Nd, nsd-Z and *E-tr* had been obtained. The NILs of *re, q, st, w-2, ms, cts, bts, nb, oh, rb, tub* and *so* had been bred after 7 ~ 8 times of backcrossing to *Hb*. All of the individuals in these NILs expressed the same characters as *Hb* except the aim gene. The other NILs had been backcrossed for 3 ~ 4 times and planed to complete before 2004.

Because there is not exchange in female silkworm, male individuals were used to mate to recurrent parent to increase the possibility of exchange. Only female of Bc1, Bc3 BC_x (x is odd number) of *sch* were used because 1/2 or 1/4 female individuals expressed *sch* in those generations (Fig. 4).

The individuals whose phenotype was same as recurrent parent except the aim gene were selected to mate to the recurrent parent. The gene proportion from *Hb* would be increased rapidly along with backcrossing. If there were *n* genes difference between recurrent parent and donor parent, after *m* times backcrossing, the proportion of homozygotes will account for $[1-(1/2)^m]^n$ (Xu *et al.*, 1994).

The sericulturists in the world now cooperate in the silkworm genomic plan. A dense molecular linkage map will be complete soon. The NILs will be very useful in the gene location and positional cloning in the future.

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