Molecular analysis of the deletion mutants in the E homeotic complex of the silkworm *Bombyx mori*

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Summary

The E loci in *Bombyx mori* are expected to contain a homeotic gene complex specifying the identities of the larval abdominal segments. However, the molecular structure of this complex remains to be determined. We have started to analyze the structural changes in the E complex mutations. We used three newly isolated *Bombyx* homeobox genes as probes. These genes are probably homologues of the *Ultrabithorax* (*Ubx*), *abdominal-A* (*abd-A*) and *Abdominal-B* (*Abd-B*) in the *Drosophila* bithorax complex, because the amino-acid sequences of the homeobox regions in these *Bombyx* genes are almost identical to those of *Drosophila* genes. We found that the *Bombyx Ubx* and *abd-A* genes are deleted in the *EN* chromosome, and the *Bombyx abd-A* gene is deleted in the *E Ca* chromosome. From these results, we conclude that the *Bombyx* E complex consists of the *Ubx*, *abd-A* and possibly *Abd-B* genes, which may play similar roles to their homologues in the *Drosophila* bithorax complex.

Key words: *Bombyx mori*, homeotic gene, the E complex, Ultrabithorax, abdominal-A, Abdominal-B.

Introduction

Genetical studies revealed that the identities of the body segments are determined by homeotic genes in many kinds of insects (Ouweneel, 1976). In the fruit fly *Drosophila melanogaster*, the red flour beetle *Tribolium castaneum* (Beeman, 1987) and the silkworm *Bombyx mori* (Hashimoto, 1941), homeotic genes are clustered in homeotic gene complexes. In *Drosophila*, the Antennapedia complex (ANT-C) (Wakimoto and Kaufman, 1981) and the bithorax complex (BX-C) (Lewis, 1978), which are located on the right arm of the third chromosome, specify the identities of the body segments or parasegments. ANT-C determines identities of the head to the middle thoracic segment and BX-C determines identities of the posterior thoracic segment and all the abdominal segments. Molecular studies of the structure of ANT-C and BX-C revealed that these gene complexes consist of homeobox genes (Gehring and Hiromi, 1986). In BX-C, three homeobox genes, *Ubx*, *abd-A* and *Abd-B* are involved in the determination of the abdominal segments in *Drosophila* (Duncan, 1987). In *Tribolium*, similar homeotic genes are known to determine the identities of the body segments. Beeman demonstrated that six loci of homeotic genes are clustered in the second linkage group of *Tribolium* (Beeman, 1987) and these loci include elements with homology to the homeotic genes in the ANT-C and BX-C in *Drosophila*.

The E loci in *B. mori* contain homeotic genes specifying the identities of the larval abdominal segments (Hashimoto, 1941; Tazima, 1964). This homeotic gene complex is thought to be located on the 0.0 locus of the sixth chromosome linkage group in *Bombyx*, and over thirty types of mutations were found and analyzed for pseudoallelism. All of these mutations are dominant and induce expression of the extra markings or the supernumerary legs in the abdominal segments. Most of the mutations in the E complex are lethal in the homozygous condition.

The E complex reveals one interesting aspect different from the homeotic gene complexes of *Drosophila*. Most of the E mutations cause a particular directional shift, anterior to posterior or vice versa, on both the dorsal and ventral sides of larvae. However, some E mutations cause shifts in one direction on the dorsal side and in the opposite direction on the ventral side (Itikawa, 1943; Tazima, 1964). Since such an independent determination on the dorsal side and ventral side in larvae has not been observed in *Drosophila*, there must be differences in the regulatory mechanisms that specify abdominal segments between *Bombyx* and *Drosophila*.

To understand how the E complex specifies the identities of body segments on the dorsal side and ventral side of larvae independently, it is necessary to know the molecular structure of the E complex. Since some mutations in the E complex are associated with
phenotypes similar to those of the mutations in the bithorax complex, we assumed that the E complex might be analogous to the *Drosophila* bithorax complex. In this report, we describe the isolation of three *Bombyx* genomic fragments that are probably homologues of the Ubx, *abd-A* and *Abd-B* in *Drosophila*. Analyses with the two types of mutant chromosomes, *E* and *E*<sub>C</sub>, showed that the *Bombyx* Ubx and *abd-A* genes are deleted in the *E* chromosome, and the *Bombyx* *abd-A* gene is deleted in the *E*<sub>C</sub> chromosome. These results suggest the E complex consists of the homeobox genes and is similar to the bithorax complex in *Drosophila*.

**Materials and methods**

**Animals**

*Bombyx* eggs and larvae of mutant strains f12 carrying *E*<sub>N</sub> chromosome and f21 carrying *E*<sub>C</sub> chromosome were gifts from Dr H. Doira, Institute of Genetic Resources, Kyushu University. Embryos were developed at 25°C for about 5 days to stages 22 or 25 (Takami and Kitazawa, 1990) and dissected out from eggs under a binocular microscope. Larvae of a commercial strain of *B. mori* (Kin-Shu × Sho-Wa from the Kanebo Silk Co., Kasugai City, Japan) were reared at 27°C on an artificial diet from Kyodo Shiryo Co. (Yokohama, Japan).

**Cloning of the Bombyx Ubx, *abd-A* and *Abd-B* genes**

Isolation of *Bombyx Ubx* gene was performed following the PCR amplification method as described (Kamb et al., 1989). DNA fragments containing homeobox regions were amplified from genomic DNA with fully degenerated primers that correspond to two consensus amino-acid sequences, 5′ELEK-EFH3′ and 5′IKWFQN3′, in the homeodomain. The reaction mixture of the PCR amplification was incubated in a Perkin Elmer Cetus thermocycler for 25 cycles; 1 min at 94°C, 2 min at 37°C, and 3 min at 55°C (Sakiki et al., 1985). After amplification, the DNA fragments were subcloned into a plasmid vector pGEM3Zf (+). Clones containing *Bombyx Ubx* were identified by sequencing. Clones carrying longer genomic fragments were isolated from a genomic library with a probe that was amplified from the subclone. The *Bombyx* genomic library (2 × 10<sup>5</sup> clones) was constructed by size fractionating a *MboI* partial digest of posterior silk gland DNA from *Bombyx* (the Kanebo strain, Kin-Shu × Sho-Wa) and ligating the 30-45 kb fraction into the *BamH* site of pWE15 cosmid vector (Stratagene Co.) (Evans and Wahl, 1987). Hybridization was performed at 65°C for 2 hours using Amersham Rapid Hybridization kit. After blots were washed in 2 × SSC containing 0.1% SDS (sodium dodecyl sulfate) at 65°C for 3 hours, positive clones were detected by autoradiography. Since the screening probe hybridized to a 7.0 kb *EcoRI* fragment, this fragment was subcloned into plasmid vector and sequenced with fully degenerated primers that correspond to two consensus amino-acid sequences, 5′ELEK-EFH3′ and 5′IKWFQN3′, in the homeodomain. Since the screening probe hybridized to a 7.0 kb *EcoRI* fragment, this fragment was subcloned into plasmid vector and sequenced with fully degenerated primers that correspond to two consensus amino-acid sequences, 5′ELEK-EFH3′ and 5′IKWFQN3′, in the homeodomain.

**For the isolation of *Bombyx* *abd-A* and *Abd-B* genes, the *Bombyx* genomic cosmid library was screened by cross-hybridization with the homeobox regions of the *abd-A* and *Abd-B* genes of *Drosophila*. The homeobox region of *Drosophila* *abd-A* was amplified from *abd-A* genomic DNA p53 with two primers, 5′GCCCGCCAAAAAGGAGGAG3′ and 5′CTCACCTGTTCATTATTTCCGTG3′ (Regulski et al., 1985). *Drosophila Abd-B* probe was amplified from *Abd-B* cDNA E61 by PCR with two primers 5′CAGGTTCGCCGGAAGAGAGGG3′ and 5′GGTGAGGTGTCGCCGTCG3′ (Zavortink and Sakonju, 1989). The hybridization conditions were the same as described above. The *Drosophila* *abd-A* probe hybridized to a 2.5 kb HindIII fragment, and the *Drosophila* *Abd-B* probe hybridized to a 5.0 kb HindIII fragment. Therefore these fragments were subcloned and sequenced as described above.

**Extraction of DNA from the posterior silk glands of the fifth instar larvae and whole embryos**

The heterozygous *E*<sup>N</sup>/*E* and *E*<sup>C</sup>/*E*<sup>C</sup> DNAs and the wild-type +/+ DNA for Southern blot analysis were extracted from the posterior silk glands of the fifth instar larvae by the method described previously (Gross-Bellard et al., 1973). We started DNA extraction from two pairs of the posterior silk glands which were stored at −80°C. Posterior silk glands were ground under liquid nitrogen and suspended in 500 µl of digestion buffer (100 mM NaCl, 10 mM Tris-HCl (pH 8.0), 25 mM EDTA (pH 8.0), 10% SDS and 0.1 mg/ml Proteinase K) and incubated at 50°C for 12 hours. DNAs were extracted from a pipette tip and dissolved in TE buffer (10 mM Tris-HCl (pH 8.0) and 1 mM EDTA). Quantities of DNA were estimated by diphenylamine reaction (Burton, 1956).

For PCR analysis, DNAs were prepared from the heterozygous *E*<sup>N</sup>/*E* and *E*<sup>C</sup>/*E*<sup>C</sup> embryos and the wild-type embryos by the method described (Jowett, 1986). After the embryos were dissected under a binocular microscope in 1 × SSC at room temperature, mutant and wild-type embryos were washed in 1 × SSC twice and stored at −80°C. Since embryos with the +/+ genotype or *E*<sup>N</sup>/*E* and *E*<sup>C</sup>/*E*<sup>C</sup> genotype cannot be distinguished, we extracted DNA from individual embryos of apparent wild-type phenotype (either *E*<sup>N</sup>/*E* or *E*<sup>C</sup>/*E* or *) or +/+,* *E*<sup>N</sup>/*E*<sup>C</sup>* or *) and apparent *E*<sup>N</sup>/*E* or *E*<sup>C</sup>/*E*<sup>C</sup> phenotype. Each embryo was suspended in 25 µl of a buffer containing 10 mM Tris-HCl (pH 7.5), 60 mM NaCl, 50 mM EDTA, 0.15 mM spermine and 0.15 mM spermidine, and ground with a pipette tip. After 25 µl of a solution containing 1.25% SDS, 0.3 M Tris-HCl (pH 9.0), 0.1 mM EDTA, 5% sucrose, and 0.75% freshly added diethylpyrocarbonate were added, solutions were incubated for 40 minutes at 60°C. SDS and protein were precipitated by centrifugation after addition of 30 µl of 8 M potassium acetate and cooling for 45 minutes on ice. DNAs were precipitated by adding 2 volumes of ethanol and dissolved in 25 µl of TE buffer.

**Southern blot analysis of heterozygous mutant DNA**

After each 3 µg of DNA extracted from the *E*<sup>N</sup>/*E* or *E*<sup>C</sup>/*E* and +/+ embryos were digested with EcoRI, Southern blot analyses were performed sequentially with randomly primed specific DNA probes which are located 3′ to each homeodomain. A 296-bp DNA probe was prepared for the analysis of *Bombyx* Ubx by PCR amplification from cloned *Bombyx* Ubx DNA with two primers, 5′TGAAAGACGCAAGA-GAAACGCGC3′ and 5′TCCAACAGTTATTTGCTGATCC3′. A 341-bp DNA was amplified for the analysis of the *Bombyx* *abd-A* gene with two primers 5′AACAG- GCTCGCCCGCGAAAGAGAGG3′ and 5′CCCTCACAACAAGCACAGCACCTCC3′. A 341-bp DNA was amplified for the analysis of the *Bombyx* *abd-B* gene with two primers 5′AACACAATTCGAAGAACGACAG3′ and 5′GA- TACAAGCTTCTCCCGTGAATT3′. Hybridization was performed at 65°C for 2 hours with Amersham Rapid
Hybridization kit and blots were washed in 0.3 x SSC buffer containing 0.1% SDS at 65°C. Hybridization patterns were analyzed and extents of hybridization were estimated with a Bio-Image Analyzer (BAS 2000, Fuji Photo Film Co., LTD).

**PCR analysis of homozygous mutant DNA**

DNAs extracted from individual embryos were subjected to 25 cycles of amplification by PCR, at an annealing temperature of 45°C, using the mixed primers used in the preparation of the probes just 3' to the homeodomains of the Bombyx Ubx, Bombyx abd-A and Bombyx Abd-B genes as described above. After amplified products were separated by electrophoresis on a 2% agarose gel and transferred to membrane filter by Vacuum Blot (LKB Co.), blots were used for rehybridization with the probes for the Bombyx Ubx, Bombyx abd-A and Bombyx Abd-B genes. Hybridization was performed at 65°C for 2 hours and blots were washed in 2 x SSC containing 0.1% SDS for 30 minutes.

**Results**

**The phenotypes of EN and ECa mutations**

Itikawa (1943) reported that embryos homozygous for the EN (new additional crescent) mutation express many thoracic-type appendages in would-be abdominal segments and die at the late embryonic stages. As shown in Fig. 1B, homozygous EN/EN embryos express thoracic-type legs from the first to the seventh abdominal segments (A1 to A7) and intermediate thoracic/abdominal-type legs in the A8 segment. Itikawa (1943) also reported that the nerve commissures and the tracheae in the first to the eighth abdominal segments in the embryos homozygous for EN show the patterns similar to those in the thoracic segments of normal embryos. From these observations, Itikawa proposed that the first to the seventh or eighth abdominal segments in homozygous EN/EN embryos are transformed to the thoracic-type segments.

In Drosophila, the bithorax complex consists of three homeobox genes, the Ubx, abd-A and Abd-B. Lewis (1978) reported that the functional deficiency of the Ubx and abd-A (DfUbx109) causes the transformation of most abdominal segments to the thoracic-type segments in Drosophila. From the similarity of the phenotype between the homozygous EN embryo and DfUbx109 embryo, we assumed that the E complex has a similar structure and function to the bithorax complex in D. melanogaster.

Itikawa (1943) also reported that the embryos homologous for ECa (additional crescent) reveal three pairs of normal thoracic legs in the thoracic segments, but no abdominal legs in the abdominal segments. The abnormality of the homozygous ECa embryos is shown in Fig. 1C. We speculated that the A3 to A6 segments, which normally reveal abdominal legs, might have transformed to other abdominal segments that have no leg. Which abdominal segment repeats at the position of the A3 to A6 segments in the embryos homozygous for ECa was not determined.

![Fig. 1. Phenotypes of Bombyx mori (A) wild-type, (B) homozygous EN/EN and (C) homozygous ECa/ECa embryos. The wild-type embryo has three pairs of thoracic legs from the first to the third thoracic segments (T1 to T3) and four pairs of abdominal legs from A3 to A6 segments. The embryo homozygous for EN expresses thoracic-type legs from A1 to A7 segments (arrowheads) and intermediate thoracic/abdominal-type legs on A8 segment (arrow). The homozygous ECa embryo does not express any abdominal legs (arrowheads). Embryos were approximately 2 mm long.](image-url)
Isolation of Bombyx homologues of Drosophila Ubx, Abd-A and Abd-B

To determine whether the E complex consists of homeobox genes similar to Drosophila Ubx, Abd-A and Abd-B, we screened the Bombyx genome to isolate the homologues. First we performed PCR amplification to isolate of Bombyx homeobox genes using the degenerated oligonucleotides primers corresponding to the consensus amino-acid sequences in homeodomain (see Materials and Methods). With this method, we expected to obtain homologues of Drosophila Ubx, Abd-A and Abd-B genes, because Ubx and Abd-A genes contain the consensus amino-acid sequences. Although we sequenced many clones prepared by subcloning of the amplified DNA, we could obtain only the gene that seems to be a homologue of the Drosophila Ubx gene. The 86 amino acids encoded by the Ubx gene might also contain an intron at the same position.

To isolate of Bombyx homologues of Abd-A and Abd-B genes, the genomic library was screened by cross-hybridization with the homeobox regions of the Abd-A and Abd-B genes of Drosophila. Figs 3 and 4 show the comparison of the nucleotide and amino-acid sequences between Bombyx genes and Drosophila cDNAs. The nucleotide sequences of the homeobox and flanking regions of the Bombyx Abd-A (nucleotide position 13 to 276) were 77% homologous to those of Drosophila Abd-A gene. The 86 amino acids encoded by the Bombyx Abd-A gene were identical to those of Drosophila Abd-A cDNA (Karch et al., 1990). A homologue of Abd-A was isolated from the locust Schistocerca gregaria and the amino-acid sequences including the homeodomain and its flanking regions were found identical to the corresponding regions of Drosophila Abd-A (Tear et al., 1990). Although the Drosophila Abd-A gene has a short intron at the position just 5' to the homeobox as indicated in Fig. 2 (Kornfeld et al., 1989). Since the putative amino acid sequences encoded by the Bombyx Ubx following the corresponding position were homologous to those of Drosophila Ubx, the Bombyx gene might also contain an intron at the same position.

To isolate of Bombyx homologues of Abd-A and Abd-B genes, the genomic library was screened by cross-hybridization with the homeobox regions of the Abd-A and Abd-B genes of Drosophila. Figs 3 and 4 show the comparison of the nucleotide and amino-acid sequences between Bombyx genes and Drosophila cDNAs. The nucleotide sequences of the homeobox and flanking regions of the Bombyx Abd-A (nucleotide position 13 to 276) were 77% homologous to those of Drosophila Abd-A gene. The 86 amino acids encoded by the Bombyx Abd-A gene were identical to those of Drosophila Abd-A cDNA (Karch et al., 1990). A homologue of Abd-A was isolated from the locust Schistocerca gregaria and the amino-acid sequences including the homeodomain and its flanking regions were found identical to the corresponding regions of Drosophila Abd-A (Tear et al., 1990). Although the Drosophila Abd-A gene has a short intron at the position just 5' to the homeobox as indicated in Fig. 2, this intron was absent in the Abd-A homologue from Schistocerca. Since the putative amino-acid sequences in the 5' flanking region of the Bombyx Abd-A gene were also identical to the sequences of Drosophila Abd-A.
Fig. 3. Comparison of Bombyx 
abd-A genomic sequences (upper, Bm) with cDNA 
sequences of Drosophila abd-A (lower, Dm). Sequences 
encoded by the abd-A gene from Drosophila 
are from the reference by Karch et al. 
Markings are the same as in 
Fig. 2. The sequence has been 
entered in the EMBL 
database, accession no. X62620.

Fig. 4. Comparison of Bombyx 
Abd-B genomic sequences (upper, Bm) with cDNA 
sequences of Drosophila Abd-B 
(lower, Dm). Sequences 
encoded by the Abd-B gene from Drosophila 
are from the reference by Zavortink and 
Sakonju. Markings are the 
same as in Fig. 2. The sequence has been 
entered in the EMBL 
database, accession no. X62619.

A cDNA, the Bombyx abd-A gene probably does not 
have an intron at the 3' flanking region of the 
homeobox.

The homology of the nucleotide sequences between 
Bombyx Abd-B gene (nucleotide position 19 to 234) 
and Drosophila Abd-B cDNA was as high as those of 
the abd-A and Ubx genes (79% homologous). In the 
homeodomains of both Abd-B genes, only one amino 
acid was different, but the asparagine residue in 
Bombyx has a similar property to the glutamine residue.
in *Drosophila*. The *Drosophila* Abd-B gene also contains an intron in the homeobox region (DeLorenzi et al., 1988). Since the putative amino-acid sequences in the homeobox region of *Bombyx* Abd-B gene were identical to the sequences of cDNA of the *Drosophila* Abd-B gene, the *Bombyx* Abd-B gene probably does not have this intron. Compared with the similarities of the Ubx and abd-A genes between *Bombyx* and *Drosophila*, the homology of the amino-acid sequences of the 3' flanking region of the Abd-B genes was not so high.

These conservations of the homeodomain amino-acid sequences between *Bombyx* and *Drosophila* suggest that these *Bombyx* homologues may have functions analogous to their *Drosophila* counterparts. Therefore, we name these genes *Bm Ubx*, *Bm abd-A* and *Bm Abd-B*, respectively.

**Analysis of the EN chromosome**

To examine the abnormalities in the EN chromosome, we performed a Southern blot analysis of the heterozygous EN/++ chromosome and an amplification analysis by PCR of the homozygous EN/EN chromosome. The homozygous EN/EN chromosome is the best for analyses of chromosomal abnormalities, but sufficient quantities of DNA for a Southern analysis cannot be easily obtained because of the embryonic lethality of homozygosity. Therefore, first we performed a Southern blot analysis on heterozygous EN/++ DNA. We used a probe fragment just 3' to the homeodomain of the *Bombyx* genes (Fig. 5A-5C) to prevent a cross-hybridization. The Bm Ubx probe hybridized to 6.7 kb fragments from both heterozygous EN/++ and wild-type +/+ DNAs digested with EcoKl, but a quantitative analysis revealed that the extent of hybridization (as determined by the intensity of signals from hybridized bands) with heterozygous EN/++ DNA was about a half of that with wild-type +/+ DNA (Fig. 5A). Also, the heterozygous EN/++ DNA contained only a hah0 amount of a 7.4 kb fragment hybridized with Bm abd-A probe compared to wild-type +/+ DNA (Fig. 5B). However, the extent of hybridization with the Bm Abd-B probe was almost the same for EN/++ and wild-type DNAs (Fig. 5C).

These results strongly suggested that the Bm Ubx and abd-A regions are deleted in the EN chromosome of *Bombyx*. To confirm this further, we examined whether the DNA fragments just 3' to the homeodomains of the *Bombyx* genes could be amplified from the homozygous EN/EN chromosome by PCR (Fig. 5D-5F). Amplified DNA fragments were detected by Southern blot analysis with specific probes to the *Bombyx* homeobox genes. In the case of Bm Ubx gene, the DNA fragment was amplified from DNAs extracted from three individual wild-type embryos, while no appropriate product was amplified from DNAs from three individual embryos homozygous for EN/EN. Similar results were
obtained in the case of the Bm abd-A gene; no amplified product was detected from DNAs extracted from embryos homozygous for E°/E°. However, amplifications from the Bm Abd-B gene were detectable from DNAs extracted from embryos homozygous for E°.

**Analysis of the ECa chromosome**

Structural and functional analysis of the E complex was carried out also on ECa chromosome. Southern blot hybridization revealed that the extent of hybridization with the Bm Ubx probe in the heterozygous ECa/+ DNA was the same as in wild-type +/- DNA (Fig. 6A), while that of the Bm abd-A in the heterozygous ECa/+ DNA was a half of that of wild-type +/- DNA (Fig. 6B). For the Bm Abd-B gene from the heterozygous ECa/+ chromosome, two fragments (5.0 kb and 1.5 kb) of a comparable intensity were detected (Fig. 6C). Since the total intensity of Bm Abd-B hybridization on the 5.0 kb and 1.5 kb fragments from the heterozygous chromosome was the same as the intensity of 1.5 kb fragment from the wild-type chromosome, restriction polymorphisms with the heterozygous chromosome might have caused the two fragments. By PCR amplification on the homozygous ECa/ECa chromosome, no product was detected for the Bm abd-A gene (Fig. 6E), while appropriate products were detected for the Bm Ubx (Fig. 6D) and Abd-B genes (Fig. 6F). These results suggest that the Bm abd-A gene is deleted in the ECa chromosome.

**Discussion**

**Conservation of amino-acid sequences of the homeodomains between Bombyx and Drosophila**

We have isolated Bombyx Ubx, abd-A and Abd-B genes, and shown that the amino-acid sequences including the homeodomain and its flanking regions of these genes are almost identical to those of Drosophila Ubx, abd-A and Abd-B. The conservation of the homeodomain amino-acid sequences between Bombyx and Drosophila suggests that Bombyx homologues may have similar functions to those of Drosophila.

We have also isolated the Antennapedia (Antp), Sex combs reduced (Scr), engraved (en), invected (in) homologues from Bombyx (W. Hara and Y. Suzuki, unpublished data; K. Ueno, unpublished data; Hui et al., 1991). The homeodomain sequences of the Bombyx Antp and Scr were also homologous to those of Drosophila Antp and Scr, respectively. Four highly conserved domains, including the homeodomain, were identified in en and in proteins from Bombyx and Drosophila (Hui et al., 1991). These conservations suggest that the common amino-acid sequences of the homeodomain and flanking regions are necessary for specifying the body segments.

**The E° and ECa mutations and their phenotypes**

Since the homeodomain is important to express the function of the homeoprotein and our results indicate that the homeodomain regions of the Bm Ubx and abd-
A genes are deleted in the $E^N/E^N$ chromosome, we speculate that complete functional deficiency of the Bm Ubx and abd-A genes may have caused the transformation of the A1 to A7 segments to the thoracic type segments in the homozygous $E^N$ embryos (Fig. 1B). Observation of intermediate thoracic/abdominal-type legs in the A8 segment suggests that the Bm Abd-B gene may be functional in the homozygous $E^N$ embryos.

In Drosophila, the deletion mutations of the Ubx and abd-A genes such as DfUbx$^{109}$ cause the transformation of the A1 to A7 segments to the thoracic-type segments (Lewis, 1978), and the A8 segment expressed abdominal-type phenotype in DfUbx$^{109}$ mutant. The similarity of the phenotypes of the homozygous $E^N/E^N$ embryos and the DfUbx$^{106}$ embryos suggests that the Bm Ubx, abd-A and Abd-B genes have similar functions to the Drosophila genes.

In Drosophila, functional deficiency of the abd-A gene is thought to cause the transformation of the A2-A7 segments to A1 type segments (Lewis, 1978). By analogy, we infer that a complete deficiency of abd-A function in homozygous $E^N/E^N$ embryos may have caused the transformation of the A2-A7 segments to A1 type segments, which have no abdominal legs.

The structure of the E complex
These analyses on $E^N$ and $E^C_a$ chromosomes suggested that the deletions of Bm Ubx and/or abd-A genes are responsible for the phenotypes of these E mutants and functions of these genes are analogous to those of homologues in the BX-C. No chromosomal abnormality was detected at the homeobox region of the Bm Abd-B gene in either the $E^N$ or $E^C_a$ chromosome. Nevertheless, we speculate that the E complex also contains the Bm Abd-B gene for the following reasons. It is known that the $E^{DD}$ (Double stars) causes abnormal specification of segments flanking the A5 segment (Takasaki, 1947). This phenotype seems to resemble the phenotype associated with Abd-B mutation in Drosophila (Duncan, 1987). Therefore, the E complex in Bombyx probably consists of Ubx, abd-A and Abd-B genes and the functions of these homeobox genes resemble those of the Drosophila BX-C.

Unity and diversity of the homeotic gene complex between Bombyx and Drosophila
From our results, we think that the E loci accommodate a gene complex analogous to BX-C. Lewis has suggested the hypothesis that 'leg-suppressing' genes ancestral to the bithorax complex are responsible for removing the legs from abdominal segments of millipede-like ancestors during the evolution of the arthropod (Lewis, 1978). The $E^N/E^N$ embryo with its many thoracic-type legs may appear to express atavistic characteristics by the deletion of some 'leg-suppressing' genes.

Recently, we demonstrated that the Ne locus at 1.4 genetic units from the E loci probably contains Bombyx Antp gene (T. Nagata, unpublished data). This observation suggests that the Ne locus may constitute a complex analogous to Drosophila ANT-C. Therefore, Bombyx also has two homeotic gene complexes corresponding to the ANT-C and BX-C of Drosophila.

The E complex is different in one important respect from the Drosophila BX-C. Some E mutations like $E^{P}P/E^{P}P$ (Kp supernumerary legs) (Hashimoto, 1941) and $E^{D}/+$ (Double crescents) (Hashimoto, 1930) cause shifts in one direction on the dorsal side and to the opposite direction on the ventral side (Tazima, 1964). Since such an independent determination of the two sides is not seen in Drosophila, there must be differences between the Bombyx E complex and the bithorax complex of Drosophila in the regulatory mechanisms that specify abdominal segments. Further detailed analyses are required to determine these differences.

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