Sex determination in *Bombyx mori*

Fumi Ohbayashi, Masataka G. Suzuki† and Toru Shimada*

Department of Agricultural and Environmental Biology, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan
†Present address: Laboratory of Molecular Entomology and Baculovirology, The Institute of Physical and Chemical Research (RIKEN), 2-1 Hirosawa Wako, Saitama 351-0198, Japan

We describe here the current status and future prospects of sex-determination studies in the silkworm, *Bombyx mori*. The sex of *Bombyx* is strongly controlled by the presence of the W chromosome. Although several classical studies suggested that a presumptive feminizing gene (*Fem*) is located at a limited portion of the W chromosome, the *Fem* gene has not yet been cloned. Recently, the homologues of *Drosophila* sex determining gene *doublesex* was found in *Bombyx* and analysed. The *doublesex* in *Bombyx* (*Bmdsx*) is clearly regulated by sex-specific splicing, as is *Drosophila doublesex*. In spite of the fact that the upstream system in sex determination is very different between *Drosophila* and *Bombyx*, they may share a common downstream gene, *doublesex*. Future studies should try to locate the molecular cascade from the W chromosome to *Bmdsx* and identify the terminal sex-specific genes regulated by BmDSX.

A number of different mechanisms for sex determination exist among living organisms. It is possible to classify sex-determining systems into two categories. One is the environmental sex-determination (ESD) system, in which the primary signal is supplied from the environment, and the other is the genetic sex-determination (GSD) system. The GSD systems include several types of mechanisms. Even within the class of insects alone, sex can be determined by various mechanisms: by Y(W) chromosomal factors, by autosomal factors, by the number of X chromosomes, by haploidy versus diploidy, or by infection of microorganisms.

In *Drosophila melanogaster*, the first signal of sex-differentiation is the ratio of X chromosomes to sets of autosomes (A). The X:A ratio, a balance mechanism in which X chromosomal gene products are titrated against autosomal gene products, governs sex determination[1]. Although the sex-determining mechanism of *D. melanogaster* is one of the best-understood pathways, the balance mechanism observed in *Drosophila* is not common in any other previously examined insect. Many other species adopt the epistatic sex-determination mechanism[2]. For example, even in Diptera, an epistatic maleness factor found in the Y chromosomes of several species, such as the housefly *Chironomus thummi*[3], *Musca domestica* (standard strains)[4], *Ceratitis capitata*[5], *Lucilia cuprina*[6] and *Bactorocera tryoni*[7] determines sex by an epidtatic male-determining factor on the Y chromosome. The mosquito *Culex tritaeniorhynchus* has no sex chromosome, and its male sex is determined by a dominant gene on an autosome[8].

Bombyx mori is a female-heterogametic organism (ZZ in male, ZW in female)[9] that appears to have a feminizing gene (*Fem*) on the W chromosome. It was reported by Hasimoto[10] that the sex of *Bombyx* is controlled by the presence/absence of the W chromosome. The W chromosome possesses a strong ability to determine the female sex in *Bombyx* as described below.

The genetics of *Bombyx* has a history of long standing. In *B. mori*, 400 or more visible mutations have been identified, including sex-dependent mutations and reproductive abnormalities, and over 200 of them have been placed on linkage maps[11,12]. Furthermore, it is easy to generate polyploids and gynandromorphs artificially in this insect. Therefore, *Bombyx* should be a suitable material for studies on sex determination. Because the sex determination of *Bombyx* is a model of epistatic systems in invertebrates, clarification of the sex-determining mechanism of this insect would be helpful for a comprehensive understanding of sex determination in invertebrates. Furthermore, determination and expression of sex in *Bombyx* is important not only in basic biology but also in practical applications. To produce silk, males are more efficient than females, in whom the resources are utilized for oogenesis rather than silk protein synthesis. Thus, if we could regulate sex in an artificial way, it would be possible to produce a much higher yield of silk or other useful biomaterials more efficiently. Recently, molecular genetic studies have been conducted for the sex determination of *Bombyx*. In this article, we review the classical and molecular genetics of sex determination in *Bombyx*.

Sex chromosomes and the sex determinants on the W chromosome

Sex-determining function of the W chromosome

Lepidoptera do not have a male-specific sex chromosome, but another system, ZW female/ZZ male or ZO

*For correspondence. (e-mail: shimada@ss.ab.a.u-tokyo.ac.jp)

female/ZZ male, is adopted depending on the species or strains. Although there are 5 suborders in Lepidoptera, namely, Zeugloptera, Dacnonypha, Exoporia, Monotrysia, and Ditrysia, the W chromosome has been found only in Ditrysia[13]. Ditrysia contains both ZW species and ZZ species. In Trichoptera, the order most closely related to Lepidoptera, only the ZZ type has been reported. Therefore, it is reasonable that the W chromosome emerged after the divergence between Ditrysia and other suborders in Lepidoptera. In spite of the fact that the W chromosome is a newly acquired chromosome, it plays a very important role in sex determination in *Bombyx*[10].

The triploid ZZW+3A and tetraploid ZZWW+4A become female[10], and tetraploid ZZZW becomes female as well. This situation is different from that in *Drosophila*. In *Drosophila*, XXY+3A produces an intersex, and XXXY+4A produces a female individual in the same way as a normal female (XX+2A) does. This is why the sex of *Bombyx* has been considered to be controlled by an epistatic system that is different from the *Drosophila*'s balance system. Tazima and his colleagues succeeded in establishing various kinds of chromosome aberrations, mainly translocations, for the W chromosome. Their efforts resulted in several kinds of sex-limited genetic markers, for example, sex-limited p^B, sex-limited p^{Sa}, sex-limited *Y*, sex-limited *Ze*, and sex-limited $+^{w-2}$ (refs 14, 15). Furthermore, deletion of the feminizing gene region on the W chromosome was exploited using X-ray irradiation[16]. Tazima[16] concluded that the feminizing factor is located in a restricted area on the W chromosome. So far, however, nobody has succeeded in cloning the feminizing gene (*Fem*) predicted by Tazima. Hirokawa[17] found an intersex mutation (*Isx*) controlled by the W chromosome and speculated that *Isx* is an allele at *Fem*.

It is not very easy to identify the W chromosome under a microscope even though the epistatic function of the W chromosome is clear. In some other lepidopteran species, the W chromosome has been cytologically identified[18]. In *Bombyx*, however, the W chromosome does not have any morphological characteristics compared with other chromosomes. In addition, it is known that the W chromosome contained many retrotransposable elements that are dispersed not only on the W chromosome but also on autosomes, as described below.

Molecular structure of the W chromosome

Abe and his colleagues[19,20] (including the authors) tried to obtain some DNA fragments from the W chromosome. They compared the genomic DNA between females and males by using the random-amplified polymorphic DNA (RAPD) method and found several female-specific RAPD fragments. By using these fragments as probes, they obtained lambda and BAC clones corresponding to three regions on the W chromosome. They determined the nucleotide sequences and noticed that the cloned sequences were fully occupied by various kinds of transposable elements, especially LTR-type and non-LTR-type retrotransposons. What is the significance of the fact that the W chromosome contains so many transposable elements? In humans and plants, retrotransposable elements are also accumulated on the Y chromosome[21,22]. It can be explained that the Y/W chromosome cannot easily exclude the inserted sequences because it is not recombined with the X/Z chromosome. Another explanation is that the W chromosome has so few functional genes that retrotransposition does not cause any serious damage to the survivability and adaptability of the individuals. In any case, the W chromosome is full of retrotransposable elements, and we therefore cannot easily analyse the molecular structure of the W chromosome to reach the feminizing gene.

The Z chromosome – partner of the W chromosome

As described above, it is speculated that the W chromosome evolved after the split of the suborder Ditrysia and other suborders in Lepidptera. If this is true, the prototype of the sex chromosomes might be ZZ/ZO in Lepidoptera. For example, the number of the Z chromosomes would be counted in the ZZ/ZO species. Although it is unknown whether the W chromosome was differentiated from the Z chromosome or it was reconstituted from fragments of autosomes, the W chromosome probably shares some homologous regions with the Z chromosome because the W and Z chromosomes can be paired during the meiotic division of oocytes. The authors have cloned two Z-linked genes, *T15.180a* and *Bmkettin*. The amounts of their mRNAs were approximately two times as much in males as in females in somatic tissues[23,24]. There have also been several reports indicating absence of the dosage compensation in lepidopteran insects other than *Bombyx*. It is known that dosage compensation is essential in *Drosophila* and mammalian animals. For example, the lack of several genes regulating dosage compensation leads to lethality in *Drosophila*. Why can lepidopterans survive without a dosage-compensation mechanism? A possible answer to this question is that the Z chromosome contains only genes that do not require dosage compensation. If this is true, the functions of the Z-linked genes should be somewhat biased. In some species that do not show dosage compensation, it appears that there is a close relationship between an absence of dosage compensation of Z-linked genes and sexual dimorphism in phenotype such as mate-selection and courtship behaviour[25]. The final answer will be obtained from large-scale analyses of the structure and expression of the Z chromosome.

Bombyx homologues of *doublesex* and other sex-determining genes in *Drosophila*

Outline of the genetic cascade for sex determination in Drosophila

The sexual differentiation of vertebrates is strongly controlled by sex hormones circulating in blood. The sex hormones are steroids secreted from gonads. In contrast, the sexes of insects are autonomously determined in each cell. This is why we can observe sex mosaics or gynandromorphs in various insects. In *Bombyx*, sex mosaics are induced genetically by mosaicism genes, *mo* and *mo-t*, or supercooling treatment of the eggs[26] (Figure 1 *a*). In the sex mosaics of *Bombyx*, the sex-specific proteins SP1 and vitellogenin are expressed only in the female cells of the fat body, and neighbouring male cells are not affected by female cells (Figure 1 *b*). Whereas the sex of each cell is determined by the presence or absence of the *Fem* on the W chromosome in *Bombyx*, *Sex-lethal* (*Sxl*) is a key gene for sex determination in somatic cells in *Drosophila*. *Sxl* can be activated only when the X/A ratio exceeds 1 (ref. 1). The default sex in *Drosophila* is male, in which *Sxl* is inactive. In the somatic sex-determination pathway, the target gene of the SXL protein is the *transformer* (*tra*) gene. The activity of the *Sxl* locus leads to the synthesis of the active product of the *transformer* (*tra*) gene by directing the female-specific pattern of splicing of the *tra* primary transcript[27]. Because of the absence of the functional SXL protein, the male fly is not able to synthesize the functional TRA protein. The *tra* gene product is a splicing activator, leading to female-specific splicing of its target gene. Together with the cofactor TRA2, the *transformer2* (*tra2*) gene product, the TRA protein, promotes female-specific splicing of the bifunctional gene *doublesex*. The resultant of the sex-specific transcripts encodes the female- or male-specific gene product DSXF or DSXM. Both DSXF and DSXM are transcription factors and locate at the bottom of the sex-determination cascade. The products of DSXF and DSXM control the activity of the final target genes necessary for sexual differentiation.

In this section, we report several *Bombyx* homologues of the *Drosophila* sex-determining genes, *dsx* and others.

Bombyx homologues of the Drosophila sex-determining genes

Many homologues of the *Drosophila* sex-determining genes have been already found in *Bombyx*. First, a homologue of the master sex switch gene, *Sxl*, was found by Niimi and his colleagues. It is, however, not sex-dependently expressed in somatic tissues, suggesting that it plays a sex-independent role in soma of *Bombyx* (Niimi, personal communication). The homologues of *Sxl* were also found in non-drosophilid dipteran insects; *Chrysomya*[28], *Megaselia*[29], *Musca*[30] and *Ceratitis*[31]. Although the structure of these *Sxl* homologues is very well conserved, they are not sex-dependently expressed. The

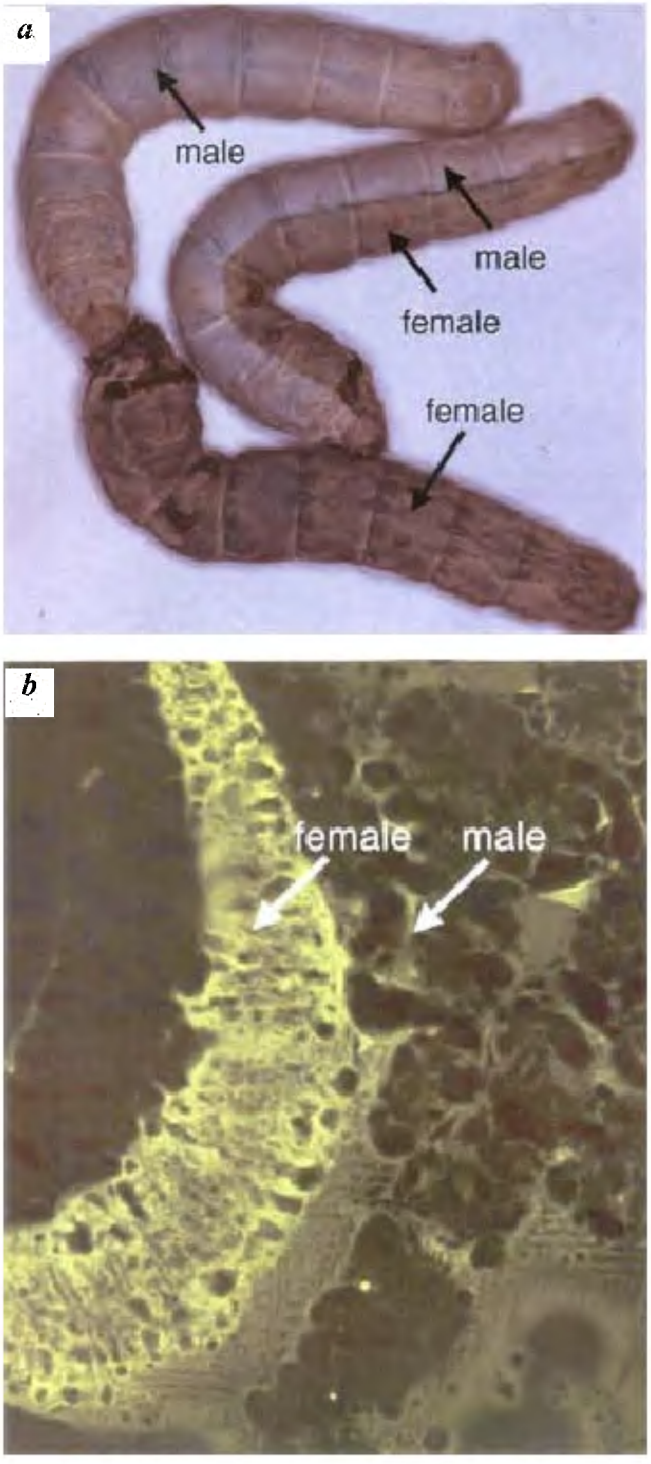

Figure 1. ***a***, Sex mosaics induced by the *mo* mutant. Fifth instar larvae; black skin is marked by a female-specific gene, WpSa. ***b***, Frozen section of the larval fatbody in a sex mosaic was stained by the anti-SP1 antibody. Fluorescence indicates FITC linked to a second antibody, namely, the existence of the SP1 protein.

Sxl homologues seem to have no relation to the sex, except for *Drosophila Sxl*.

The second sex-determining gene is *tra* in *Drosophila*. Among *tra* homologues in several *Drosophila* species, the coding region has evolutionary diversity to an unusually high degree[32]. In contrast, *tra2* appears to be widely conserved in species much more distantly related[33,34]. It is, however, unclear whether or not the *tra2* homologues regulate sex determination in each organism. Although we have not yet found the *tra* homologue in *Bombyx*, we found a homologue of *tra2* in the EST database (URL: http://www.ab.a.u-tokyo.ac.jp/silkbase/) of *Bombyx* (Nakagawa *et al.*, unpublished). So far, there is no evidence that supports sex-related function of *Bombyx tra2* homologue.

The sex of the nervous system is determined by the *fruitless* (*fru*) gene in *Drosophila*. We found a *Bombyx* homologue of *fru* in the EST database. The *Bombyx fru* homologue yields many mRNA isoforms as *Drosophila fru* does. However, neither the sex-specific mRNA nor sex-specific protein has been found in the *Bombyx fru* homologue (Ohbayashi *et al.*, unpublished data). Among all the homologues of the *Drosophila* somatic sex-determining genes, only the *dsx* homologue is a candidate gene that may regulate the sex in *Bombyx* as explained in the next section.

Bmdsx, a doublesex homologue and its sex-specific splicing

Although *Sxl*, *tra*/*tra2*, and *fru* seem to be *Drosophila*-specific sex-determinants, *dsx* has functional homologues in other metazoan organisms, for example *mab-3* in the nematode, *Caenorhabditis elegans*. *Drosophila dsx* and *C. elegans mab-3* share a number of properties, and both of them are conserved structurally and functionally. Both the genes contain a DM domain, a zinc finger-like DNA-binding motif[35,36]. Furthermore, the two genes directly regulate yolk protein gene transcription[37,38]. The homologues of *dsx* have also been found in human, mouse, chicken, and turtle, among vertebrates. Most *dsx* homologues are considered to regulate sexual differentiation in each organism. Thus, if the *Bombyx* has a *dsx* homologue, it might also regulate sex differentiation. We have isolated a *dsx* homologue in *Bombyx*, which was named *Bmdsx*, by using the EST database. We obtained evidence that *Bmdsx* is transcribed into sex-specific mRNA isoforms due to differential processing of pre-mRNA[39], the same as *Drosophila dsx*. The amino acid sequences of female-and male-type *Bmdsx* cDNAs encode common amino-terminal regions and sex-specific carboxyl termini (Figure 2). The common regions contain the DM domain. The DM domain of BmDSX shows 80% of identity with that of *Drosophila* DSX. Other functional regions of DSX, such as the OD2 domain, are also well conserved between *Bombyx* and *Drosophila*. In *Drosophila*, the OD2 domain is known to be necessary for the oligomerization of DSX and is supposed to be related to DNA-binding cooperativity when the proteins bind regulatory sites in target DNA[40]. Thus, BmDSX would also bind to target DNA by forming oligomers. Although the actual expression level of *Bmdsx* mRNA was higher in gonads and pheromone glands than in other tissues, *Bmdsx* mRNA was sex-specifically expressed in various tissues at larval, pupal, and adult stages in the silkworm. As *dsx* in *Drosophila*, *Bmdsx* expresses male- and female-specific transcripts, and the exon–intron structure is largely conserved[39,41]. Although the function of the *Bmdsx* has not yet been clarified and the primary signal in sex determination is substantially different between *Bombyx* and *Drosophila*, our findings indicate that the *Bmdsx* would also regulate sexual differentiation in *Bombyx,* as does the *Drosophila dsx* gene.

As shown in Figure 2, the sex-specific difference in the splicing pattern of pre-mRNA from *Bmdsx* resembles that of *dsx* in the female-specific exon(s), which is skipped in the male-specific transcript. Then, has the regulatory mechanism of sex-specific RNA splicing at the *dsx* gene been conserved during evolution?

Our study showed that the answer to this question is negative. In *Drosophila dsx*, male-specific splicing occurs under the default splicing condition. Two splicing activators, TRA and TRA-2, are required for female-specific splicing. However, in *Bombyx Bmdsx*, unlike *Drosophila dsx*, female-specific splicing occurs under the default condition. And it was revealed that both TRA and TRA-2 are not concerned with sex-specific splicing of *Bmdsx* pre-mRNA[41]. Therefore, repression of the default processing pattern would be necessary for generating a male-specific transcript. Accordingly, it is supposed that the *dsx* homologue is an ancient and conserved gene and that different upstream regulators have been utilized in different taxonomic groups during evolution. In order to clarify the function of *Bmdsx* in *Bombyx*, we have to either knock out the endogenous gene or express it ectopically.

Terminal genes in sexual differentiation

The final phenotypes of the sex-determining genes are sexual dimorphisms in morphological, physiological, and behavioural characteristics. It is, however, not well understood which of the target genes of the sex-determining gene products are in *Drosophila*, *C. elegans* and mammals. In *Drosophila*, it is only known that the DSX protein regulates the transcription of yolk protein genes, *yp1*, *yp2* and *yp3*, directly. However, the *yp* genes are not only the targets of DSX because *dsx* mutations affect not only oogenesis but also sex-dependent cuticular hydrocarbons and sexual behaviour[42]. So far, only a few candi-

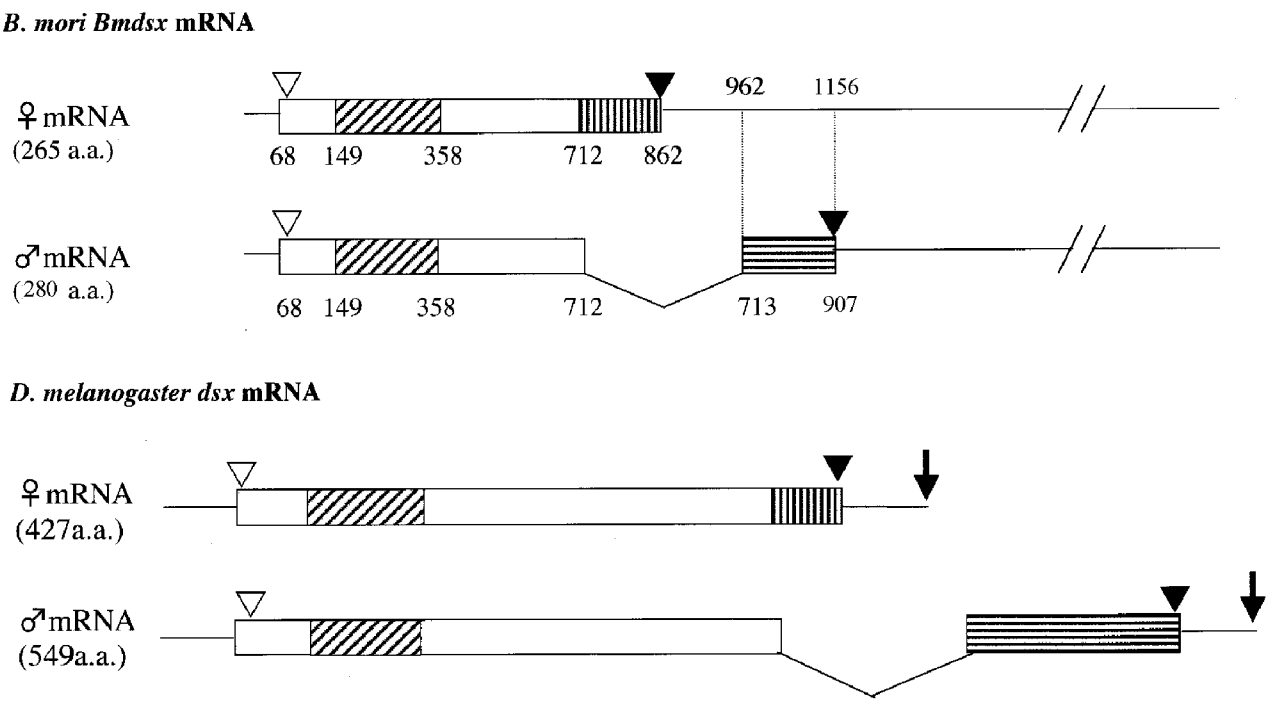

Figure 2. Comparison between male and female mRNA. ***a***, *B. mori Bmdsx*. Open reading frames (ORFs) are boxed. The DNA binding domain is represented by an oblique line. The male- and female-specific regions are indicated by a vertical and a horizontal line, respectively; ***b***, *Drosophila melanogaster dsx*. The arrows indicate the polyadenylation sites of the female and male transcripts. A white reversed triangle denotes an initiation codon, and a black one indicates a termination codon.

date genes can be suggested as the targets of the sex-determining mechanism in *Bombyx*. The first candidate is the gene encoding vitellogenin, a yolk protein precursor. The vitellogenin gene is located on an autosome and specifically transcribed only in females. Yano *et al.*[43,44] examined the 5′ regulatory region of the vitellogenin gene, but its sex-specific regulatory mechanism is still unknown. The second candidate is the gene for SP1, the female-specific storage protein. The *SP1* gene is located on the 23rd chromosome. It is transcribed in both sexes until the fourth instar but only in females in the fifth instar larvae. The gene homologous to *Drosophila yp* genes is the *ESP* gene encoding the egg-specific protein. The *ESP* gene is located on chromosome 19 and transcribed only in follicle cells in the ovary. The *ESP* gene is the third candidate regulated by the BmDSX protein. On the other hand, there are several proteins expressed only in males. One is the pheromone-binding protein in male antennae. Sandler *et al.*[45] found several bombykol-binding proteins in male antennae. Another male-specific protein is the testis-specific tubulin found by Mita *et al.*[46].

Since *Bombyx* has more sex-specific proteins than *Drosophila*, it is probable that new target genes in *Bombyx* will be found and the common regulatory mechanism of the sex-specific transcription will be elucidated in the near future.

Conclusions

Recent genomic analysis of *Bombyx* revealed several new genes that may play important roles in sex determination. Especially the *doublesex* homolog, *Bmdsx*, is strongly suggested to be a dual switch gene at the terminal of the sex-determining cascade in *Bombyx*. Although the sex of *Bombyx* is epistatically determined by the W chromosome, the *Fem* gene, whose existence has been predicted, has not yet been identified. The mechanisms whereby the W chromosome regulates the sex-specific splicing of *Bmdsx* and the BmDSX protein realizes the final sex-specific gene expression should be clarified on the basis of molecular biological and genetical approaches.

1. Cline, T. W., *Trends Genet.*, 1993, **9**, 385–390.
2. Marin, I. and Baker, B. S., *Science*, 1998, **281**, 1990–1994.
3. Hagele, K., *Chromosoma*, 1985, **9**, 167–171.
4. Hilfiker-Kleiner, D., Dubendorfer, A., Hilfiker. A. and Nothiger, R., *Development*, 1994, **120**, 2531–2538.
5. Willhoeft, U. and Franz, G., *Genetics*, 1996, **144**, 737–745.
6. Bedo, D. G., *Chromosoma*, 1982, **87**, 21–32.
7. Shearman, D. C. A. and Formmer, M., *Insect Mol. Biol.*, 1998, **7**, 355–366.
8. Baker, R. H. and Sakai, R. K., *J. Hered.*, 1976, **67**, 289–294.
9. Tanaka, Y., *J. Coll. Agric. Sapporo*, 1916, **6**, 1–33.
10. Hasimoto, H., *Jpn. J. Genet.*, 1933, **8**, 245–247 (in Japanese).
11. Doira, H., *Fac. Agric. Kyushu Univ.*, Fukuoka, Japan, 1992.

12. Goldsmith, M. R., in *Molecular Model Systems in the Lepidoptera* (eds Goldsmith, M. R. and Willkins, A. S.), Cambridge Univ. Press, New York, 1995, pp. 21–76.
13. Traut, W. and Marec, F., *Q. Rev. Biol.*, 1996, **71**, 239–256.
14. Tazima, Y., *Jpn. J. Seric. Sci.*, 1943, **14**, 69–97 (in Japanese).
15. Tazima, Y., *Jpn. J. Breed.*, 1951, **1**, 47–50 (in Japanese).
16. Tazima, Y., *Bull. Seric. Exp. Sta. Jpn.*, 1944, **12**, 109–179 (in Japanese).
17. Hirokawa, M., *Bull. Fukushima Seric. Exp. Sta.*, 1995, **28**, 1–104 (in Japanese with English summary).
18. Traut, W., Sahara, K., Otto, T. D. and Marec, F., *Chromosoma*, 1999, **108**, 173–180.
19. Abe, H., Kanehara, M., Terada, T., Ohbayashi, F., Shimada, T., Kawai, K., Suzuki, M., Sugasaki, T. and Oshiki, T., *Genes Genetic Syst.*, 1998, **73**, 243–254.
20. Abe, H., Ohbayashi, F., Shimada, T., Sugasaki, T., Kawai, S., Mita, K. and Oshiki, T., *Mol. Gen. Genet.*, 2000, **263**, 916–924.
21. Tilford, C. A. *et al.*, *Nature*, 2001, **409**, 943–945.
22. Shibata, F., Hizume, M. and Kuroki, Y., *Chromosome Res.*, 2000, **8**, 229–236.
23. Suzuki, M. G., Shimada, T. and Kobayashi, M., *Heredity*, 1998, **81**, 275–283.
24. Suzuki, M. G., Shimada, T. and Kobayashi, M., *Heredity*, 1999, **82**, 170–179.
25. Gruta, J. W. and Taylor, O. R., *Evolution*, 1980, **34**, 688–695.
26. Ebinuma, H., Kobayashi, M., Kobayashi, J., Shimada, T. and Yoshitake, N., *Genet. Res. Camb.*, 1988, **51**, 223–229.
27. Nagoshi, R. N., McKeown, M., Burtis, K. C., Belote, J. M. and Baker, B. S., *Cell*, 1988, **53**, 229–236.
28. Muller-Holtkamp, F., *J. Mol. Evol.*, 1995, **41**, 467–477.
29. Sievert, V., Kuhn, S. and Traut, W., *Genome*, 1997, **40**, 211–214.
30. Meise, M., Hilfiker-Kleiner, D., Dubendorfer, A., Brunner, C., Nothiger, R. and Bopp, D., *Development*, 1998, **125**, 1487–1494.
31. Saccone, G., Peluso, I., Artiaco, D., Giordano, E., Bopp, D. and Polito, L. C., *Development*, 1998, **125**, 1495–1500.
32. O'Neil, M. T. and Belote, J. M., *Genetics*, 1992, **131**, 113–128.
33. Dauwalder, B., Amaya-Manzanares, F. and Mattox, W., *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 9004–9009.
34. Chandler, D., McGuffin, M. E., Piskur, J., Yao, J., Baker, B. S. and Mattox, W., *Mol. Cell. Biol.*, 1997, **17**, 2908–2919.
35. Erdman, S. E. and Burtis, K. C., *EMBO J.*, 1993, **12**, 527–535.
36. Raymond, C. S., Shamu, C. E., Shen, M. M., Seifert, K. J., Hirsch, B., Hodgkin, J. and Zarkower, D., *Nature*, 1998, **391**, 691–695.
37. Coschigano, K. T. and Wensink, P. C., *Genes Dev.*, 1993, **7**, 42–54.
38. Yi, W. and Zarkower, D., *Development*, 1999, **5**, 873–881.
39. Ohbayashi, F., Suzuki, M. G., Mita, K., Okano, K. and Shimada, T., *Comp. Biochem. Physiol. B.*, 2001, **128**, 145–158.
40. An, W., Cho, S., Ishii, H. and Wensink, P. C., *Mol. Cell Biol.*, 1996, **16**, 3106–3111.
41. Suzuki, G. M., Ohbayashi, F., Mita, K. and Shimada, T., *Insect Biochem. Mol. Biol.*, 2001, **31**, 1201–1211.
42. Waterbury, J. A., Jackson, L. L. and Schedl, P., *Genetics*, 1999, **152**, 1653 1667.
43. Yano, K., Sakurai, M. T., Izumi, S. and Tomino, S., *FEBS Lett.*, 1994, **356**, 207–211.
44. Yano, K., Sakurai, M. T., Watabe, S., Izumi, S. and Tomino, S., *Biochim. Biophys. Acta*, 1994, **1218**, 1–10.
45. Sandler, B. H., Nikonova, L., Leal, W. S. and Clardy, J., *Chem. Biol.*, 2000, **7**, 143–151.
46. Mita, K., Nenoi, M., Morimyo, M., Tsuji, H., Ichimura, S., Sawai, M. and Hamana, K., *Gene*, 1995, **162**, 329–330.

ACKNOWLEDGEMENTS. Our studies on the sex determination in *B. mori* have been financially supported by JSPS, MAFF, and BRAIN, Japan. We thank Drs K. Mita, H. Abe, and M. Kobayashi for helpful discussions.