Trends in Entomology
Vol. 15, 2019

Original Article

Reconsideration of the abnormal larval behaviors used to characterize a strain, *lemon lethal*, of the silkworm *Bombyx mori* as a model of Parkinson's disease

Hiroaki Abe*

Department of Biological Production, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183-8509, Japan.

ABSTRACT

A mutant strain of the silkworm *Bombyx mori* (L.) (Lepidoptera: Bombycidae) has been proposed as a model for Parkinson's disease. The larvae of homozygous lemon lethal (*lem^{l}*) mutants, deficient in sepiapterin reductase, are reported to show abnormal behaviors such as head shaking and feeding inability after the first ecdysis. It was observed that although the *lem^{l}*/*lem^{l}* larvae had an appetite, they could not bite off pieces of mulberry leaves because of their abnormally soft mandibles. Further observations of *lem^{l}*/*lem^{l}* larvae revealed normal movement. Taken together, these data indicate that the *lem^{l}* strain is not suitable for use as a Parkinson's disease model.

KEYWORDS: silkworm, Parkinson's disease, *Bombyx mori*, xanthine oxidase, abnormal behavior.

INTRODUCTION

Parkinson's disease is a progressive disorder of the central nervous system (CNS), characterized by a reduced concentration of the neurotransmitter dopamine in the brain [1]. This dopamine deficit is caused by the premature death of dopamine-containing neurons in a region of the midbrain, leading to debilitating problems with tremor, muscular rigidity, and slowness of movement [2]. In insects, dopamine is involved in cuticlar sclerotization and melanization in addition to its role as a neurotransmitter [3]. Precursors for sclerotization are derived from the amino acid tyrosine in three enzymatic steps. First, the tyrosine is hydroxylated to 3,4-dihydroxyphenylalanine (L-Dopa) by tyrosine hydroxylase (TH). The L-Dopa is then decarboxylated to dopamine by dopa decarboxylase, followed by the acylation of the dopamine amino groups with either acetate or β-alanine to form the cuticle tanning precursors catecholamines N-acetyldopamine (NADA) or N-β-alanyldopamine (NBAD), respectively (Figure 1). Dopamine, the central molecule for both sclerotization and melanization, can also be channeled into the pathway for melanin production [3].

The silkworm, *Bombyx mori* (L.) (Lepidoptera: Bombycidae), is a lepidopteran model insect [4] that has been completely domesticated and is no longer found in the wild. However, a presumed ancestral species, *B. mandarina*, can still be found in mulberry fields. The silkworm has been used in genetic studies since the birth of genetics in the early 1900s, primarily in Japan. *B. mori* is a convenient experimental model organism because a large variety of mutants are available in the egg, larval, pupal, and adult stages [5].

A draft sequence of the genome of the p50 (standard) strain of *B. mori* was constructed by 3-fold whole-genome shotgun (WGS) sequencing in Japan [6]. Moreover, a draft sequence of the p50 strain was constructed by 5.9-fold WGS sequencing in China [7]; ultimately, full-scale genome sequencing was performed [8].

*Email id: wfem@cc.tuat.ac.jp

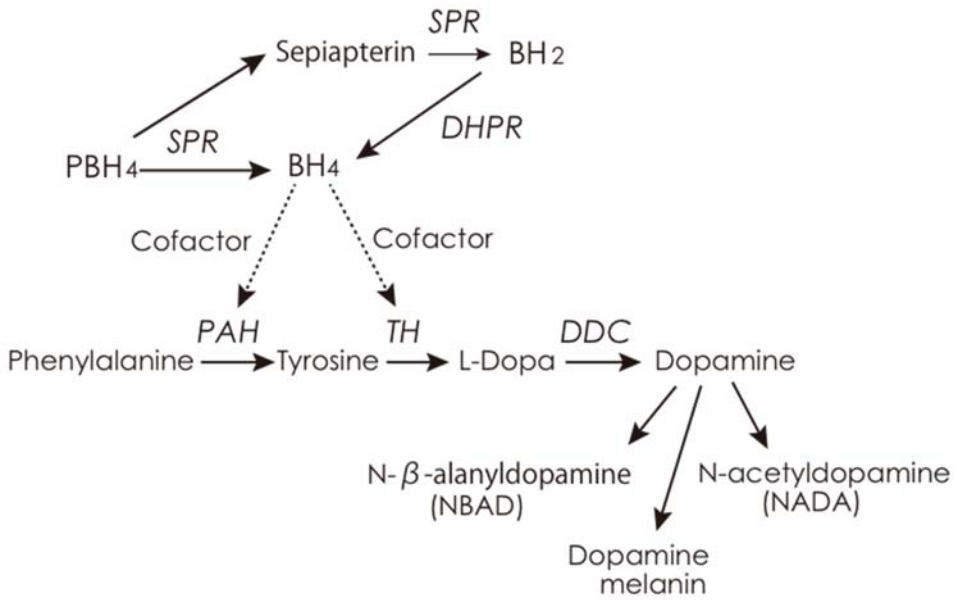

Figure 1. Pathways for dopamine biosynthesis along with its related products from phenylalanine. PBH_4, 6-pyruvoyl-tetrahydropterin; BH_4, tetrahydrobiopterin; L-Dopa, 3,4-dihydroxyphenylalanine; SPR, sepiapterin reductase; PAH, phenylalanine hydroxylase; TH, tyrosine hydroxylase; DDC, Dopa decarboxylase; BH_2, dihydrobiopterin; DHPR, dihydrobiopterin reductase.

Two mutant strains of the silkworm *B. mori* have been proposed as models for Parkinson's disease. One strain is the *op* mutant, which has translucent larval integument [9]. In wild-type *B. mori*, uric acid, one of the end products of nitrogen metabolism, is synthesized in the larval fat body. Then, the uric acid is transported to the epidermis and accumulates as urate granules in epidermal cells and renders the larval skin opaque [10]. To date, more than 30 translucent mutants have been reported [11] with the responsible genes identified for the *oq* [12], *og* [13], *od* [14], and og^z [15] mutants. Through maicroarray analysis, Tabunoki *et al.* [9] identified a novel uric acid synthesis-modulating pathway, from DJ-1 to xanthine dehydrogenase (XDH) that is repressed in the *op* mutant. Human DJ-1 is considered to be the causative gene in PARK7-linked familial Parkinson's disease [16]. Xanthine oxidase (XO) catalyzes xanthine to uric acid [17]. Moreover, Tabunoki *et al.* [9] show that levels of DJ-1 and TH mRNA are lower in the brain of *op* mutant and propose that the *op* mutant is a potential *B. mori* model for Parkinson's disease. To support this proposal, the authors report that the *B. mori op* mutant exhibits spontaneous and pronounced translucency during the larval stage and demonstrates occasional unique actions such as vibration [9]. A movie on comparing these actions between *op* and wild-type larvae is included in their report. However, how these actions compare with those of other strains is not shown. Moreover, they present no evidence to suggest this action is unique to *op*/*op* individuals. Upon viewing their movie, no actions that appeared to be unique to the *op* mutant are seen [18]. Abe [18] concluded that occasional unique actions of *op*/*op* larvae were simply reactions to the touch of other larvae and an "occasional unique action" is likely to be incorrect. Moreover, xanthine oxidase injection did not rescue the phenotype of *op* mutant larvae [18]. In addition, Tabunoki *et al.* [9] reported *op*/*op* male individual's infertility. However, the fertilized eggs when using *op*/*op* male moths can be obtained [18]. Therefore, it may be necessary to reconsider the uric acid synthesis-modulating pathway from DJ-1 to XDH and the use of the *op* strain as a Parkinson's disease model [18].

Additionally, several genes considered to be involved in human diseases, including Parkinson's disease, were identified in the genome of *B. mori*. Lemon (*lem*) and its lethal allele lem^l are *B. mori*

mutants with irregular body coloration. The *lem* silkworms display yellow body coloration in larval stages and grow to adult stage [5]. In contrast, the *lem*^{l} larvae can hatch from eggs and grow normally in the first instar but die in the second instar [19-21]. Meng *et al.* [22] demonstrated that mutations in the *B. mori* sepiapterin reductase gene (*BmSpr*) are responsible for the *lem* and *lem*^{l} phenotypes. Sepiapterin reductase (SPR) catalyzes the biosynthesis of tetrahydrobiopterin (BH_4), an essential cofactor for phenylalanine hydroxylase and TH (Figure 1). The human SPR gene could be a causative gene for PARK3, familial Parkinson's disease [23]. *lem* and *lem*^{l} might provide the first insect model for human SPR deficiency diseases [22]. To suggest the relationship between *lem*^{l} and SPR deficiency disease, Meng *et al.* [22] provided the following observations: (i) 'After the first ecdysis, the *lem*^{l} larvae stop feeding, shake their heads frequently, and die within 3 days,'; (ii) 'In general, patients with BH_4 deficiency present progressive neural deterioration, convulsions, abnormal movements, and difficulty in swallowing'; (iii) 'Abnormal symptoms observed in *lem*^{l} larvae, such as head shaking and feeding inability after the first ecdysis, are strikingly similar'. However, these descriptions do not match other observations of *lem*^{l} indicating these larvae do not appear to have neural and muscular defects in feeding. Umeya and Tsujita [20] reported that *lem*^{l} larvae try to feed on the mulberry leaves actively. Based on detailed observations using dissection, they concluded that *lem*^{l} larvae were unable to feed on leaves because of incomplete hardening of the mandible [20]. It is believed that this observation brings into question the "abnormal behavior" of *lem*^{l} larvae reported by Meng *et al.* [22]. Meng *et al.* [22] cited no references describing this "abnormal behavior" reported by Umeya and Tsujita [20] and did not show examples in movies or figures.

In this report, the abnormal behaviors and actions of the mutant that was reported as a *B. mori* model for Parkinson's disease are reconsidered. The larval behavior of *lem*^{l} was observed and filmed.

MATERIALS AND METHODS

Silkworms

This study used the *lem*^{l} (a65) strain maintained at the Institute of Genetic Resources, Faculty of Agriculture, Kyusyu University (NBRP silkworm database, http://www.shigen.nig.ac.jp/silkworm base/). The a65 strain includes *lem*^{l}/*lem*^{l}, *lem*^{l}/+, and +/+ individuals because this strain results from crossing of a *lem*^{l}/+ female moth with a *lem*^{l}/+ male moth. Homozygotes of *lem*^{l} individuals were distinguishable from *lem*^{l}/+ and +/+ individuals after the first ecdysis. The second instar *lem*^{l}/*lem*^{l} larvae had a yellowish body color.

Video recordings

Videos of the actions and behaviors of the silkworms were made using a Sony Handycam HDR-PJ590V digital HD video camera recorder (Sony Corp., Tokyo, Japan). Moreover, to show the actions of larvae, figures from the videos were obtained. Furthermore, the two short movies showing the biting behavior of second instar *lem*^{l}/*lem*^{l} larvae are available from YouTube (https://www.youtube.com/watch?v=qZ4NRTiudi4 and https://www.youtube.com/watch?v=ZK8wkmYgD2w).

RESULTS

Biting behavior of *lem*^{l} larvae

Normal silkworm larvae locomote and search to find the margin of mulberry leaf using slow wagging of the head to the right and left. When they find the margin of a mulberry leaf, they stop and immediately begin to bite. The normal larvae can bite off pieces of mulberry leaves and swallow them at great speed, rhythmically. In contrast, it was observed that the 2nd instar *lem*^{l} larvae could bite into the mulberry leaves but could not tear off a piece because of the soft mandibles. The mandibles of normal larvae are blackish brown and hard. In contrast, the mandibles of 2nd instar *lem*^{l} larvae were yellowish and not completely hardened. The 2nd instar *lem*^{l} larvae continued biting at the same point of the leaf margin (Figures 2 and 3). After some time, the larvae gave up and moved a short distance to another point. The 2nd instar *lem*^{l} larvae were able to locomote, search for and find mulberry leaves, and grasp the margin of the leaf using thoracic and abdominal legs. Although the 2nd instar *lem*^{l} larvae could not bite off pieces of mulberry leaves, the series of movements observed were normal rather than abnormal (Videos in YouTube). The 2nd instar *lem*^{l} larvae died approximately 3 days after the first ecdysis (Figure 4).

Figure 2. Biting behavior of 2nd instar *lem*^{l} larvae. In the center of the screen, the yellowish larvae are *lem*^{l}/*lem*^{l} individuals. Other blackish larvae are *lem*^{l}/+ or +/+ individuals. The *lem*^{l} larvae continued biting at the margin of the mulberry leaf but could not bite off a piece.

Figure 3. Biting behavior of 2nd instar *lem*^{l} larvae. In the center of the screen, the yellowish larva with the abdomen covered by a leaf is *lem*^{l}/*lem*^{l}. The blackish larvae (*lem*^{l}/+ or +/+) survived and grew.

DISCUSSION

Behaviors of *lem*^{l} larvae

The behavior of 2nd instar *lem*^{l} larvae was observed and filmed. The *lem*^{l} larvae had a hearty appetite. Although written in Japanese, these behaviors were previously reported by Umeya and Tsujita [20]. However, it was considered that the behavioral characteristics of this *lem*^{l} strain might have changed over many decades of its breeding and maintenance. As results, the series of movements (searching, locomotion, and biting) of 2nd instar *lem*^{l} individuals was normal except for their inability to bite off the mulberry leaves. These results suggest that biosynthesis of neural dopamine in the CNS is not essential for these behaviors in 2nd instar *lem*^{l} larvae. If dopamine in the CNS is essential for these behaviors, it cannot exclude the possibility that a slight amount of residual dopamine reserved in the CNS during the 1st instar is sufficient for these behaviors in 2nd instar. Iino *et al.* [24, 25] discovered two possible routes for forming BH_4 from 6-pyruvoyl-tetrahydropterin (PBH_4) in *B. mori*. Therefore, another possibility is that BH_4 in the CNS is synthesized by an enzyme other than SPR.

Figure 4. The 2nd instar larvae of the *lem*^{l} (a65) strain approximately 3 days after the first ecdysis. The yellowish *lem*^{l}/*lem*^{l} larvae (arrows) are dead.

In addition, one of the main symptoms of Parkinson's disease is tremor, which is the uncontrollable shaking of the hand or arm. If a silkworm were to show symptoms similar to those of Parkinson's disease, the thoracic, abdominal, and caudal legs would shake, preventing maintenance of its posture. However, the 2nd instar *lem*^{l} larvae maintain their posture by grasping plants with their abdominal and caudal legs, can move, and can shake their heads normally.

Meng *et al.* [22] reported that, "oral inoculation of BH_4 effectively improved the feeding ability of the *lem*^{l} larvae and enabled them to grow normally through the larval developmental stage, suggesting that the loss of BmSPR activity reduced BH_4 to a lethal level in the *lem*^{l} larvae." However, they did not describe how the BH_4

rescued the *lem*^{l} larvae. Does BH_4 affect the CNS, the cuticle, or both? They compared the abnormal symptoms such as head shaking and feeding inability with those of neural deterioration in humans but did not refer to the structure and softness of mandible. Therefore, readers of Meng *et al.* [22] will be impressed by the effects of BH_4 on the CNS and the restoration of appetite in *lem*^{l} larvae. However, the principal factor responsible for the rescue of *lem*^{l} larvae is likely the hardening of the mandibles, as the *lem*^{l} larvae had an appetite before BH_4 treatment. Establishment of a silkworm strain lacking TH expression selectively in the CNS would be useful for studying the function of neural dopamine.

Parkinson's disease is a human disorder of the CNS, characterized by a decreased concentration of the neurotransmitter dopamine in the brain [1]. However, the administration of dopamine itself is not a useful therapeutic treatment, as the blood-brain barrier prevents dopamine from entering the brain. Therefore, the usual treatment for Parkinson's disease is L-Dopa, a dopamine precursor (Figure 1), which can enter the CNS [26]. Insects are similar to higher vertebrates in possessing a well-developed blood-brain barrier (or hemolymph-CNS barrier) [3, 27-30]. It is unclear why Meng *et al.* [22] used dopamine instead of L-Dopa in their experiments, as L-Dopa is also used in insects (such as *Drosophila*) as dopamine supplementation to the CNS [31, 32]. However, Meng *et al.* [22] observed that the oral inoculation of dopamine also effectively improved the survival rate and feeding ability of *lem*^{l} larvae and concluded that an SPR-deficiency-induced lack of dopamine resulted in the abnormal behavior observed in the *lem*^{l} larvae. In fact Meng *et al.* [22] should have used L-Dopa for their experiment if they were going to use the *lem*^{l} strain as a model of Parkinson's disease. However, surprisingly, they state that "The results clearly showed that, similar to BH_4, dopamine administration effectively increased the survival rate of *lem*^{l} larvae, because this treatment drastically improved their feeding abilities." In *B. mori*, is the oral administration of dopamine (not L-Dopa) effective in the CNS? If so, the blood-brain barrier of *B. mori* is different from one of human, and *B. mori* could not use as a model of Parkinson's disease. Their results cannot be explained at present, but dopamine administered by feeding might play multiple roles in the larvae of *B. mori*.

A recent review by Tabunoki *et al.* [33] ended with the question. "Do you now believe that silkworm can be used as human disease models?" The answer to this question is as follows. Several strains may be possibly used as human disease models. However, at the present, the *op* [9] and *lem*^{l} [19-21] strains are not suitable for use as Parkinson's disease models. Additionally, the basis that the causative gene for familial Parkinson's disease (PARK3) is human SPR gene became doubtful. A large association study of the SPR gene could not find any association with Parkinson's disease in a worldwide sample [34, 35]. Despite the reports that the abnormal behavior of *lem*^{l} [22] and occasional unique action of *op* [9] mutants are similar to the feature of Parkinson's disease, any action or behavior considered specific to these mutants was not observed. The observations in this study indicate that caution is warranted when relating the actions or behaviors of silkworms to those characteristics of human diseases. However, these reports [9, 22] provided the opportunity to observe and reconsider the normal actions and behaviors exhibited by silkworm larvae.

CONCLUSION

Abnormal behaviors such as head shaking and feeding inability of lemon lethal (*lem*^{l}) mutant silkworm reported by Meng *et al.* [22] were not observed in this study. Meng *et al.* [22] observed that oral inoculation of 'dopamine' effectively improved feeding ability of *lem*^{l}/*lem*^{l} larvae and concluded that a lack of 'dopamine' resulted in the abnormal behavior. In *B. mori*, is the oral administration of dopamine (not L-Dopa) effective in the central nervous system? If so, the blood-brain barrier of *B. mori* is different from one of human. It is concluded that Meng *et al.* [22] should have used 'L-Dopa' for their experiment if they were going to use the *lem*^{l} strain as a model of Parkinson's disease.

ACKNOWLEDGMENTS

Some silkworm strains were provided by the National BioResources Project, Japan. The author would like to thank Enago (www.enago.jp) for English language review.

CONFLICT OF INTEREST STATEMENT

The author declares that there is no conflict of interest.

REFERENCES

1. Bernheimer, H., Birkmayer, W., Hornykiewicz, O., Jellinger, K. and Seitelberger, F. 1973, J. Neurological Sciences, 20, 415.
2. Devine, M. J., Plun-Favreau, H. and Wood, N. W. 2011, Nat. Rev. Cancer, 11, 812.
3. Klowden, M. J. 2013, Physiological Systems in Insects (Third Edition), pp. 682. Academic Press, San Diego, CA, USA.
4. Goldsmith, M. R., Shimada, T. and Abe, H. 2005, Annu. Rev. of Entomol., 50, 71.
5. Banno, Y., Fujii, H., Kawaguchi, Y., Yamamoto, K. 2005, A Guide to the Silkworm Mutants 2005: Gene Name and Gene, Institute of Genetic Resources, Kyushu University, Fukuoka, Japan.
6. Mita, K., Kasahara, M., Sasaki, S., Nagayasu, Y., Yamada, T., Kanamori, H., Namiki, N., Kitagawa, M., Yamashita, H., Yasukochi, Y., Kadono-Okuda, K., Yamamoto, K., Ajimura, M., Ravikumar, G., Shimomura, M., Nagamura, Y., Shin-I, T., Abe, H., Shimada, T., Morishita, S. and Sasaki, T. 2004, DNA Res., 11, 27.
7. Xia, Q., Zhou, Z., Lu, C., Cheng, D., Dai, F., Li, B., Zhao, P., Zha, X., Cheng, T., Chai, C., Pan, G., Xu, J., Liu, C., Lin, Y., Qian, J., Hou, Y., Wu, Z., Li, G., Pan, M., Li, C., Shen, Y., Lan, X., Yuan, L., Li, T., Xu, H., Yang, G., Wan, Y., Zhu, Y., Yu, M., Shen, W., Wu, D., Xiang, Z., Yu, J., Wang, J., Li, R., Shi, J., Li, H., Li, G., Su, J., Wang, X., Li, G., Zhang, Z., Wu, Q., Li, J., Zhang, Q., Wei, N., Xu, J., Sun, H., Dong, L., Liu, D., Zhao, S., Zhao, X., Meng, Q., Lan, F., Huang, X., Li, Y., Fang, L., Li, C., Li, D., Sun, Y., Zhang, Z., Yang, Z., Huang, Y., Xi, Y., Qi, Q., He, D., Huang, H., Zhang, X., Wang, Z., Li, W., Cao, Y., Yu, Y., Yu, H., Li, J., Ye, J., Chen, H., Zhou, Y., Liu, B., Wang, J., Ye, J., Ji, H., Li, S., Ni, P., Zhang, J., Zhang, Y., Zheng, H., Mao, B., Wang, W., Ye, C., Li, S., Wang, J., Wong, G. K. and Yang, H. 2004, Science, 306, 1937.
8. The International Silkworm Genome Consortium. 2008, Insect Biochem. Mol. Biol., 38, 1036.
9. Tabunoki, H., Ono, H., Ode, H., Ishikawa, K., Kawana, N., Banno, Y., Shimada, T., Nakamura, Y., Yamamoto, K., Satoh, J-I. and Bono, H. 2013, PLoS One, 8(7), e69130. doi:10.1371/journal.phone.0069130.
10. Tamura, T. and Akai, H. 1990, Cytologia, 55, 519.
11. Komoto, N. and Tamura, T. 2011, Sanshi-Konchu Biotec, 80, 75 (In Japanese).
12. Komoto, N. 2002, Insect Biochem. Mol. Biol., 32, 591.
13. Komoto, N., Sezutsu, H., Yukuhiro, K., Banno, Y. and Fujii, H. 2003, Insect Biochem. Mol. Biol., 33, 417.
14. Fujii, T., Abe, H., Katsuma, S., Mita, K. and Shimada, T. 2008, Insect Biochem. Mol. Biol., 38, 1072.
15. Fujii, T., Ozaki, M., Masamoto, T., Katsuma, S., Abe, H. and Shimada, T. 2009, Genes Genet. Syst., 84, 147.
16. Bonifati, V., Rizzu, P., van Baren, M. J., Schaap, O., Breedveld, G. J., Krieger, E., Dekker, M. C., Squitieri, F., Ibanez, P., Joosse, M., van Dongen, J. W., Vanacore, N., van Swieten, J. C., Brice, A., Meco, G., van Duijin, C. M., Oostra, B. A. and Heutink, P. 2003, Science, 299, 256.
17. Bursell, E. 1967, Adv. Insect Physiol., 4, 33.
18. Abe, H. 2016, Trends Entomol., 12, 81.
19. Suzuki, K. 1950, Jpn. J. Genetics, 25, 95 (In Japanese with English summary).
20. Umeya Y, Tsujita M. 1951. Genetical and histo-cytological studies on the letal yellow larva in the silkworm. Bulletin of the Imperial Sericultural Experiment Station 13: 329-345 (In Japanese with English summary).
21. Tsujita, M. 1955, Jpn. J. Genetics, 30, 107 (In Japanese with English summary).
22. Meng, Y., Katsuma, S., Daimon, T., Banno, Y., Uchino, K., Sezutsu, H., Tamura, T., Mita, K. and Shimada, T. 2009, J. Biol. Chem., 284, 11698.
23. Takazawa, C., Fujimoto, K., Homma, D., Sumi-Ichinose, C., Nomura, T., Ichinose, H. and Katoh, S. 2008, Biochemical and Biophysical Research Communications, 367, 787.

24. Iino, T., Sawada, H., Tsusue, M. and Takikawa, S. 1996, Biochim. Biophys. Acta, 1297, 191.
25. Iino, T., Takikawa, S. I., Yamamoto, T. and Sawada, H. 2000, Arch. Biochem. Biophys., 373, 442.
26. Djaldetti, R. and Melamed, E. 2001, J. Neurol., 248, 357.
27. Treherne, J. E. 1985, Blood-Brain Barrier. In Comprehensive Insect Physiology Biochemistry and Pharmacology, Vol. 5, pp. 115-137.
28. Nation, J. L. 2015, Neuroanatomy in "Insect Physiology and Biochemistry" (Third Edition), pp 644. CRC Press, Taylor & Francis Group.
29. Schwabe, T., Bainton, R. J., Fetter, R. D., Heberlein, U. and Gaul, U. 2005, Cell, 123, 133.
30. Stork, T., Engelen, D., Krudewig, A., Silies, M., Bainton, R. J. and Klambt, C. 2008, J. Neuroscience, 28, 587.
31. Riemensperger, T., Isabel, G., Coulom, H., Neuser, K., Seugnet, L., Kume, K., Iche-Torres, M., Sassar, M., Strauss, R., Preat, T., Hirsh, J. and Birman, S. 2011, Proc. Natl. Acad. Sci. USA, 108, 834.
32. Neckameyer, W. S. 1996, Developmental Biol., 176, 209.
33. Tabunoki, H., Bono, H., Ito, K. and Yokoyama, Y. 2016, Advance Publication doi:10.5582/ddt.2016.01011
34. Sharma, M., Maraganor, D. M., Ioannidis, J., Riess, O., Aasly, J. O., Annesi, G., Abahuni, N., Bentivoglio, A. R., Brice, A., van Broeckhoven, C., Chartier-Harlin, M-C., Destée, A., Westenberger, A., Elbaz, A., Farrer, M. J., Ferrarese, C., Gibson, M. J., Gispert, S., Hattori, N., Jasinska-Myga, B., Klein, C., Lesage, S., Lynch, T., Lichtner, P., Lambert, J-C., Lang, A. E., Mellick, G. D., De Nigris, F., Opala, G., Quattrone, A., Riva, C., Rogaeva, E., Ross, O. A., Satake, W., Silburn, P. A., Theuns, J., Toda, T., Tomiyama, H., Uitti, R. J., Wirdefeldt, K., Wszolek, Z., Gasser, T. and Krüger, R. 2011, Neurobiology of Ageing, 32(11), 2108.e1-2108.e5.
35. Lohmann, E., Koroglu, C., Hanagasi, H., Dursun, B., Tasan, E. and Tolun, A. 2012, Parkinsonism and Related Disorders, 18, 191.