Evaluation of oxidative stress mechanisms and the effects of phytotherapic extracts on Parkinson's disease *Drosophila PINK1^{B9}* model

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Short title: Oxidative stress markers and TL in *Dm* PD model

List of abbreviations

PD, Parkinson's disease; *Dm*, *Drosophila melanogaster*; WT, wild type; PINK1, PTEN-induced putative kinase 1; PTEN, Phosphatase and tensin homolog; *Mpe*, *Mucuna pruriens* extract; *Wse*, *Withania somnifera* extract; TL, telomere length; LTR, long terminal repeat; GSH-T, total glutathione; GSSG, oxidized glutathione; SOD, superoxide dismutase; SOD-c, cytosolic SOD1, SOD-m, mitochondrial SOD2; HTT, *HeT-A*, *TART*, *TAHRE*

Abstract

Oxidative stress is commonly observed in both idiopathic and genetic cases of Parkinson's disease (PD). It plays an important role in the degeneration of dopaminergic neurons, while and it has been associated to altered telomere length. There is currently no cure for PD and antioxidative plant extracts, such as *Mucuna pruriens* and *Withania somnifera*, are commonly used in Ayurveda to treat PD patients. In this study, we evaluated two enzymatic markers of oxidative stress, glutathione (GSH) system and superoxide dismutase (SOD), and telomere length in a *Drosophila melanogaster* model for PD ($PINK1^{B9}$). This evaluation was also performed after treatment with the phyto-extracts. $PINK1^{B9}$ mutants showed a decrease in GSH amount and SOD activity and unexpected longer telomeres compared to WT flies. *Mucuna pruriens* treatment seemed to have a beneficial effect on the oxidative stress conditions. On the other hand, *Withania somnifera* treatment did not show any improvements in the studied oxidative stress mechanisms and even seemed to favor the selection of flies with longer telomeres. In conclusion, our study suggests the importance to test antioxidant phyto-extracts in $PINK1^{B9}$ model to identify beneficial effects for PD.

Keywords

Drosophila melanogaster, Parkinson's disease, $PINK1^{B9}$ mutants, phytotherapic preparations, telomere length, oxidative stress

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disease characterized by the selective loss of dopaminergic neurons in the *substantia nigra pars compacta*. Although the majority of PD cases are sporadic, both genetic and environmental factors contribute to PD etiology. The identification of autosomal recessive forms of PD caused by mutations in genes encoding for proteins involved in mitochondrial quality control have highlighted the importance of oxidative stress in PD (1, 2). Loss of function mutations in *PINK1* cause early-onset autosomal recessive PD. *PINK1* encodes for a mitochondrial protein kinase that regulate mitochondrial integrity; therefore, mutations in this gene are linked to mitochondrial dysfunction and consequent oxidative stress (3, 4).

Increased oxidative stress often leads to a decrease in antioxidant pathways, such as glutathione (GSH) and superoxide dismutase (SOD), resulting in altered oxygen consumption and perturbed redox homeostasis (5, 6). Mitochondrial dysfunction results in the production of large amounts of reactive oxygen species causing oxidative damage to cellular components including telomeres (7). In fact, oxidative stress accelerates telomere shortening, and antioxidants can reverse this phenotype (8). Although telomere length (TL) has often been speculated as a prognostic factor for various diseases, including Parkinson's disease, it remains unclear if the TL shows any correlation to pathological state (9-13).

In the present work, we employed a fruit-fly *Drosophila melanogaster* (*Dm*) mutant for *PTEN*-induced putative kinase 1 ($PINK1^{B9}$) gene as a model of PD to study two markers of oxidative stress, GSH amount and SOD enzymatic activity, and telomere length.

$PINK1^{B9}$ *Dm* model recapitulates the essential features of PD providing information regarding its pathogenic molecular basis and mitochondrial dysfunction (14, 3, 4, 15) and has been used to study neuronal dysfunction and molecular aspects of neurodegeneration (16). To note, impairment of mitochondrial morphology and function has been observed not only in the brain but also in the supercontractile muscles such as the heart in mammals (1) and the crop in *Dm* $PINK1^{B9}$ (15).

Although the mechanisms of formation and maintenance of telomeres are different in *Dm* and humans, several studies have proposed *Dm* as a useful model to study telomere function in humans and as an excellent translational model to study the association between telomeres length and human pathologies (17-20).

Dm lacks telomerase and chromosome length is maintained by the targeted transposition of three non-long terminal repeat (LTR) retrotransposons, *HeT-A*, *TART*, and *TAHRE* (collectively abbreviated as HTT), to chromosome ends. The telomere length and the composition of such retrotransposons' repeats can vary significantly between either chromosomes or fly phenotypes (21).

PD cannot be cured, and the medical treatments available so far are primarily useful to regulate the disease symptoms. Novel effective drugs or strategies for PD treatment are urgently warranted. The use of plant extracts is largely employed worldwide in traditional medicine, constituting the basis of health-care in many societies, to treat disparate pathologies (22). The therapeutic properties of two plant extracts, *Mucuna pruriens* (*Mpe*) and *Withania somnifera* (*Wse*), have been investigated in several models (23) and have been already proved useful for PD treatment due to their antioxidative properties (24-26). Moreover *Mpe*, whose seeds contain glutathione (25, 27), has been shown to rescue motor and mitochondrial impairment in $PINK1^{B9}$ PD model (4), while *Wse* has been shown to recover both motor and no motor symptoms in *Dm* LRRK2 mutants (28, 29).

In the present study we characterized endogenous antioxidant GSH and enzyme SOD and assessed telomere length in *Mpe* and *Wse* treated $PINK1^{B9}$ *Dm* model.

MATERIALS AND METHODS

Flies

Adult wild-type (WT) Oregon-R and $PINK1^{B9}$ mutant *Dm* males were obtained from Bloomington Stock Center (Indiana University, Bloomington, IN, USA). After eclosion, WT and $PINK1^{B9}$ males were separated from females. WT and mutant flies were reared on a standard corn-meal-yeast-agar medium in controlled environmental conditions (25°C; 60% relative humidity; light/dark=12/12 hours). In addition, four groups of mutant $PINK1^{B9}$ flies were reared on a standard medium supplemented with a methanolic extract of *Mucuna pruriens* or *Withania somnifera* (kindly supplied by Natural Remedies Ltd, Bangalore, India) at 0.1% w/w, as larvae and adult (L^+/A^+) or as adult only (L^-/A^+), as described elsewhere (4, 29).

It has to be noted that, according to data in literature reporting impairment of mitochondrial morphology and function not only in the brain but also in the supercontractile muscles such as the heart in mammals (1) and the crop in *Dm* $PINK1^{B9}$ (15), both oxidative stress markers and telomere length analyses have been performed on the whole insects (10-15 days old).

Quantification of total GSH and GSSG in *Dm* homogenates

The amount of total glutathione (GSH-T) and oxidized glutathione (GSSG) in whole *Dm* homogenates was quantified using a glutathione colorimetric detection kit (Arbor Assays, Eisenhower Place Ann Arbor, MI, USA) as per manufacturer's instruction. Free or reduced glutathione (GSH) was calculated by subtracting GSSG from GSH-T fraction. Data from each of the different insect groups (WT, untreated $PINK1^{B9}$, L^-/A^+ *Mpe*- or *Wse*-treated, L^+/A^+ *Mpe*- or

Wse-treated) were obtained from a pool (15-20) of whole flies. Experiments were conducted in triplicate.

Quantification of cytosolic and mitochondrial SOD activity in *Dm* homogenates

The activity of cytosolic SOD1 (SOD-c) and mitochondrial SOD2 (SOD-m) in whole *Dm* homogenates was quantified according to the manufacturer's protocol (Arbor Assays, Eisenhower Place Ann Arbor, MI, USA). Data from each of the different insect groups (WT, untreated *PINK1*^{B9}, L^{-}/A^{+} *Mpe*- or *Wse*-treated, L^{+}/A^{+} *Mpe*- or *Wse*-treated) were obtained from a pool (8-10) of whole flies. Experiments were conducted in triplicate.

DNA extraction

Genomic DNA was extracted from a pool (8-10) of whole flies using GenElute Mammalian Genomic DNA Miniprep Kit (Sigma, Saint Louis, Missouri, USA).

qPCR

Genomic copy number of telomeric (*HET-A*, *TART* and *TAHRE*) retrotransposon was estimated by qPCR conducted using DNA Engine Opticon 2 Real-Time Cycler (Bio-Rad, Hercules, CA, USA). qPCR reactions were conducted in a total volume of 20 µl containing: 20 ng genomic DNA, 10 µl iQ™ SYBR® Green Supermix (Bio-Rad, Hercules, CA, USA) and 6 pmol of each primer. Primer sequences were obtained from Walter et al. (21). Reactions were performed using the following conditions: 3 min at 95°C followed by 40 cycles with 10 sec at 95°C and 1 min at 58°C. Experiments were conducted in triplicate. Threshold cycle values (Ct) were normalized against RpS17 copy number. The generated data were analyzed applying ΔΔCT method.

Statistical analysis

Data subjected to statistical analysis were evaluated by the one-way ANOVA test (Statistica for Windows, version 7.0; StatSoft, Tulsa, OK, USA). Post-hoc comparisons were conducted with the Tukey test and p values < 0.05 were considered significant. Unpaired T-test analysis was performed in TL comparisons. Data are expressed as average ± standard error of the mean (SEM). TL length data are reported as fold change.

RESULTS

GSH /GSSG pathway in *PINK1*^{B9} mutant

Drosophila PINK1 loss-of-function mutation leads to abnormalities in mitochondrial morphology and electron transport chain (30-32). As mitochondria are a primary source of reactive oxygen species, PINK1 deficiency results in increased oxidative stress (33, 34). We monitored the amount of glutathione, a crucial antioxidant, to assess the oxidative stress in *PINK1*^{B9} *Dm* model of PD (henceforth, *PINK1*^{B9} mutants). We measured both total glutathione (GSH-T) and its oxidized form (GSSG). As shown in Fig. 1 *PINK1*^{B9} mutant flies significantly differed from WT in both the amounts of GSH-T (50.45 ± 7.20 and 107.51 ± 10.74 millimole/insect, respectively; p=0.0003) and of GSSG (17.66 ± 3.82 and 31.06 ± 3.33 millimole/insect, respectively; p=0.0134). Furthermore, we also measured the percentages of free and oxidized form of GSH (Table 1). In line with our prediction, we observed that mutant flies firstly, express a lower amount of total GSH and secondly, a greater percentage of glutathione is in oxidized form. Next, we reasoned if pharmacological treatment of *PINK1*^{B9} mutant flies with *Mpe* and *Wse* could alter the antioxidant GSH pathway. Accordingly, we treated the flies with *Mpe* and *Wse* either only at the adult stage (L^-/A^+) or from larval stage (L^+/A^+). We have previously shown that the *Mpe* treatment restores the locomotor function and overall morphology of the *PINK1*^{B9} mutant flies (4). As shown in Fig. 1, *Mpe* treatment significantly increased the amount of total GSH (80.31 ± 4.56 for L^-/A^+ and 85.14 ± 9.40 millimole/insect for L^+/A^+, respectively; p=0.0012 and p=0.0075) compared to the untreated *PINK1*^{B9} mutant flies. Moreover, as shown in Table 1, *Mpe* treated flies showed a decrease in the percentage of the oxidized GSH (34.16% and 36.62%, in L^-/A^+ and L^+/A^+, respectively). We observed that the *Mpe* treatment ameliorated the glutathione levels even when the flies received the treatment only in adult stage. Furthermore, the GSH-T in *PINK1*^{B9}mutant flies which received *Mpe* from larval stage was comparable to control flies. *Wse* has strong antioxidant activity and we and others have shown the beneficial effects *Wse* treatment in various neurodegenerative models (29, 35, 36).

In contrast to *Mpe* treatment, the amount of total GSH (30.91 ± 5.53 mmole/insect; p = 0.0370) and GSSG (5.03 ± 1.20 mmole/insect; p = 0.0012) reduced significantly in *PINK1*^{B9} mutant flies which received *Wse* only in adult stage (L^-/A^+). Interestingly, despite the decrease in the total GSH observed following the *Wse* administration, the free GSH was 80.55% and the oxidized GSH only 19.45% (Table 1). The *PINK1*^{B9}-*Wse* L^+/A^+ flies did not differ from the untreated mutant flies for the total GSH (p = 0.6026) as well as for the oxidized GSH (p = 0.5814).

Table 1. Percentage of GSH/GSSG in different *Dm* homogenates

***Dm* phenotype**	**Free GSH**	**GSSG**
WT	59.38	40.62
Pink1^{B9}	46.16	53.84
Pink1^{B9}-Mpe (L^-/A^+)	65.84	34.16
Pink1^{B9}-Mpe (L^+A^+)	63.38	36.62
Pink1^{B9}-Wse (L^-/A^+)	80.55	19.45
Pink1^{B9}-Wse (L^+/A^+)	40.41	59.59

SOD pathway in *PINK1^{B9}* mutants

Next, we measured the effects of administration of *Mpe* and *Wse* on the SOD antioxidant system. We measured the cytosolic (SOD-c) and the mitochondrial (SOD-m) values in the whole fly. As shown in Fig. 2, *PINK1^{B9}* mutants showed a significantly lower enzymatic activity of both the SOD-c (0.082 ± 0.002 U/insect and 0.107 ± 0.002 U/insect, respectively; p=6.2E 10^{-7}) and the SOD-m (*i.e.*, 0.002 ± 0.0002 U/insect and 0.004 ± 0.0002 U/insect, respectively; p=4.9E 10^{-6}) compared to WT flies.

PINK1^{B9} flies which received *Mpe* showed a significant increase in the SOD-c activity when treated, both, at adult stage (*i.e.*, *PINK1^{B9}-Mpe* (L^-/A^+), SOD-c: 0.116 ± 0.002 U/insect; p=1.2E 10^{-8}) and from larval stage (*i.e.*, *PINK1^{B9}-Mpe* (L^+/A^+), SOD-c: 0.150 ± 0.001 U/insect; ; p=7.2E 10^{-10}).

The SOD-m activity in *PINK1^{B9}* mutant flies remained unchanged after *Mpe* treatment (p=0.1218 and p=0.1007 for *PINK1^{B9}-Mpe* L^-/A^+ and L^+/A^+, respectively; Fig. 2).

The *Wse* administration to adult *PINK1^{B9}* mutants (L^-/A^+), caused an increase in SOD-c activity (p=0.0003), while SOD-m activity remained unchanged (L^-/A^+ flies, p=0.7818). Finally, *Wse* (L^+/A^+)-treated flies showed no difference in the SOD-c activity (p=0.4623), but a decrease in the SOD-m activity (p=0.0017) compared to the untreated *PINK1^{B9}* mutants (Fig. 2).

Telomere length in *PINK1^{B9}* mutants

We computed genomic copy number of HTT-array as a measure of telomere length. We predicted to observe telomere shortening as a result of mitochondrial abnormalities in *PINK1^{B9}* mutant flies. Contrary to our expectations, we observed significantly higher number of copies of both *TART* (p = 0.0069) and *TAHRE* (p = 0.0164) but not *HET* (Fig. 3).

To investigate the effects of *Mpe* and *Wse* treatments on telomere length, we compared *PINK1^{B9}* mutants treated as adult only (L^{-}/A^+) or as larvae and adult ($L^{+/}A^+$) with *PINK1^{B9}* untreated flies.

The copy numbers of the telomeric retrotransposons remained unchanged after *Mpe* treatment (Fig. 4).

On the other hand, mutants treated with *Wse* (L^-/A^+) showed a significant increase in copy number of *TART* (from 22.6 to 42.0; $p = 0.046$) and a significant decrease in *TAHRE* copy number (from 16.3 to 11.5; $p = 0.019$) as compared to untreated $PINK1^{B9}$ mutants. The other retrotransposon did not show a significant difference in copy number between untreated and treated mutants (Fig. 4).

DISCUSSION

PD is mostly a sporadic disease, but an increasing number of evidences support an extensive and complex contribute of the interaction between genetics, environmental factors and the normal process of aging to PD pathogenesis (37). However, to date only a small part of the genetic component of PD has been recognized and studied (38). Genetic mutations in *PINK1* (39) and *PARK2* genes, the most known and studied genetic mutations in PD, result in an increased oxidative stress.

In our study, as predicted, the analysis of the two pathways GSH/GSSG and SOD-c/SOD-m demonstrate that $PINK1^{B9}$ mutants present a greater oxidative stress as compared to WT. The amount of total GSH was decreased more than 50%, while the SOD activity was decreased by around 20%. Thus, in whole fly, the GSH pathway is likely to be affected more than the SOD pathway. Accordingly, given the components and especially the contents of glutathione in the extract (25, 27), *Mpe* is more effective in rescuing the oxidative stress, even if both *Mpe* and *Wse* are reported to have a clear antioxidant effect (24-26). Moreover, Whitworth and collaborators (40) reported that oxidative stress is a major contributing factor in sporadic PD and that glutathione counteracts the oxidative stress effects.

Several studies have investigated telomere length as a possible biomarker for PD. In this respect, it has been recently shown that there is no consistent evidence of shorter telomeres in PD (13). Unexpectedly, we found that $PINK1^{B9}$ mutants had elongated HTT-array (increased copy number of *TART* and *TAHRE*) compared to WT Oregon-R. In particular, it is known that *HeT-A* is the most abundant element at *Drosophila* telomeres (19), as observed in our WT flies, while *TART* and *TAHRE* appeared the most abundant elements in $PINK1^{B9}$ mutants. The observation that *PINK1* PD model has elongated HTT-array is consistent with the results shown by Degerman and colleagues (12). These authors did not detect a significant difference in TL between 168 patients with idiopathic parkinsonism (including 136 PD, 17 with Progressive Supranuclear Palsy (PSP), 15 with Multi System Atrophy (MSA)) at baseline and 30 controls. Nevertheless, they found a significant

correlation between TL at diagnosis and cognitive phenotype in the follow-up of PD and PSP patients, with longer TL mean at diagnosis in patients with dementia onset within three years. Another study showed that both short and long telomeres increase risk of amnestic mild cognitive impairment (41). Furthermore, longer telomeres are often associated with increased risk of different cancer types (42).

Therefore, contrary to what is commonly believed, not only the shortening of telomeres is associated with pathological states but also telomere elongation. The possible telomere elongation effects are certainly less studied, but it has been hypothesized, for example, that telomere elongation may have a negative effect on cell division rates (43).

It could be speculated that in certain diseases the cells put in place a sort of "prevention" or "protection system" against adverse situations (such as oxidative stress), lengthening or maintaining the TL, to the detriment then of their proper functioning.

A situation of oxidative stress had already been associated with a lengthening, and not a reduction, of TL in *Dm* by Korandová and colleagues (20). In fact, the authors observed an extended TL in flies chronically exposed to non-/sub-lethal doses of paraquat, a redox cycling compound used to induce oxidative stress. The authors hypothesized that this phenomenon may happen thanks to an adaption mechanism or because of a positive selection favoring individuals with longer telomeres. The results of our study are in line with what was observed by Korandová and colleagues (20), associating a state of oxidative stress to elongated HTT-array in $PINK1^{B9}$ mutants compared to WT. Interestingly, $PINK1^{B9}$ mutants showed a significantly lower amount of total GSH, a greater percentage of GSSG and significantly lower cytosolic and mitochondrial SOD enzymatic activities when compared to WT.

Our results show that $PINK1^{B9}$ mutants present an oxidative stress state, and that this is associated with an increased TL compared to WT. We investigated whether $PINK1^{B9}$ mutants' treatment with two plant extracts, *Mpe* and *Wse*, with known antioxidant properties, could be able to modify these parameters. Accordingly, von Zglinicki (8) proposed that oxidative stress play a role in telomere loss, which is considered even larger than DNA replication. In this respect, large amounts of reactive oxygen species causing oxidative damage to cellular components, including telomeres, are likely to depend on mitochondrial dysfunction (7); the latter was previously shown to be characterizing the $PINK1^{B9}$ *Dm* mutants (3, 4).

The results obtained show that the mutant flies treated with *Mpe* tend to re-establish conditions of GSH/GSSG pathway similar to WT. By recalling the contents of glutathione in the extract (25), data are in agreement with those reported in literature (40).

Since *PINK1* gene mutations are linked to mitochondrial dysfunction, in particular complex I deficiency (32, 44) and *Mpe* is able to rescue mitochondrial morphology in *Dm PINK1*^{B9} (3, 4), we suggest that *Mpe* rescue properties may be due to increased complex-I activity and to the presence of nicotinamide adenine dinucleotide and coenzyme Q-10, as reported by Manyam et al. (45), thus interfering with the mechanisms responsible of energy production.

Mpe treatment was also able to rescue the cytosolic SOD activity, while it had no effect on the mitochondrial SOD activity. Unexpectedly, in L^+/A^+ cytosolic activity was greater than what found in WT. This would seem to be assisted by the availability of the glutathione from the *Mpe* extract. Conversely, the effects of the *Wse* treatment are less clear on both pathways GSH/GSSG and SOD-c/SOD-m, no matter of the treatment length (L^-/A^+ vs. L^+/A^+). In fact, by considering the GSH/GSSG pathway, the dramatic decrease in the GSH-T amount compared not only to WT, but also to untreated *PINK1*^{B9}, is paralleled by a similarly dramatic increase of TART copy number compared to both WT and untreated mutants. Although more experiments are needed to better elucidate *Wse* treatment effects, it seems that *Wse* is not effective in counteracting oxidative stress (especially in relation to the metabolism of glutathione) in spite of its well-known antioxidant effects (24-26) in this *Dm* model of PD. One possible explanation for *Wse* results can be attributed to the apoptosis inductive effect (46, 47). Apoptotic cell death is considered as a protective mechanism to clear cytotoxic debris and to protect surrounding healthy cells. Interestingly, the effects observed on TL in the flies treated with *Wse* perfectly match with the observation that *W. somnifera* root extract enhances telomerase activity in the human HeLa cell line (48). It should be noted that *TART* is one of the *Dm* telomeric retrotransposons encoding also a Pol protein with reverse transcriptase (RT) activity, shared with human telomerase (18).

Overall, we show that the *Dm PINK1*^{B9} PD model benefits from *Mpe*, but not *Wse* administration. We put another piece into 'the telomere length biomarker hypothesis' puzzle from an evolutionary perspective and highlight that the conserved mechanism of telomere elongation in neurodegenerative state can be studied using fruit fly. Moreover, according to our previous studies (4, 15), we can speculate that *Mpe* interferes with the antioxidant pathways which rescues the mitochondrial functioning from oxidative stress. Protective effect of these phyto-extracts used in traditional medicine could be framed into effective pharmacotherapeutic strategy.

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Author Contributions

B. Baroli, E. Loi, P. Solari, P. Zavattari and A. Liscia designed research; B. Baroli, P. Solari. E. Loi, L. Moi and P. Muroni performed research; S. Kasture, M. D. Setzu, P. Zavattari and A. Liscia contributed to reagents and analytical tools; B. Baroli, E. Loi, P. Solari, M. D. Setzu and L. Moi analyzed data; A. Kasture, S. Kasture, P. Zavattari and A. Liscia wrote the paper.
All the Authors reviewed and approved the manuscript.

Competing Interests

The authors declare no competing interests.

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Legend to figures

Figure 1. GSH/GSSG metabolic potential of *Drosophila melanogaster* homogenates. Comparison of total (in white) and oxidized (in gray) glutathione amounts found in total bodies homogenates of *Dm* flies (WT, untreated *PINK1*^{B9} and *Mpe*- (M) or *Wse*- (W) treated *PINK1*^{B9} as L^{-}/A^{+} (-/+) or L^{+}/A^{+} (+/+)).

Mean values ± SEM (vertical bars). a and b indicate a significant difference ($p < 0.05$; Tukey test subsequent to one-way ANOVA) compared to WT and untreated *PINK1*^{B9} flies, respectively.

Figure 2. SOD metabolic potential of *Drosophila melanogaster* homogenates. Comparison of cytosolic (in white; SOD-c) and mitochondrial (in gray; SOD-m) superoxide dismutase activity found in different homogenates (total bodies) of *Dm* flies flies (WT, untreated *PINK1*^{B9} and *Mpe*- (M) or *Wse*- (W) treated *PINK1*^{B9} as L^{-}/A^{+} (-/+) or L^{+}/A^{+} (+/+)).

Mean values ± SEM (vertical bars). a and b indicate a significant difference ($p < 0.05$; Tukey test subsequent to one-way ANOVA) compared to WT and untreated *PINK1*^{B9} flies, respectively.

Figure 3. Telomere length comparison between WT and *PINK1*^{B9} flies. Bar plots represent copy number relative to Rps17 with upper/lower limits. Asterisks indicate statistically significant differences relatively to WT (*$p < 0.05$, **$p < 0.01$).

Figure 4. Telomere length comparison between *Mpe*- (M) or *Wse*- (W) treated *PINK1*^{B9} as L^{-}/A^{+} (-/+) or L^{+}/A^{+} (+/+)) and untreated *PINK1*^{B9} flies. Bar plots represent copy number relative to Rps17 with upper/lower limits. Asterisks indicate statistically significant differences relatively to untreated flies (*$p < 0.05$).

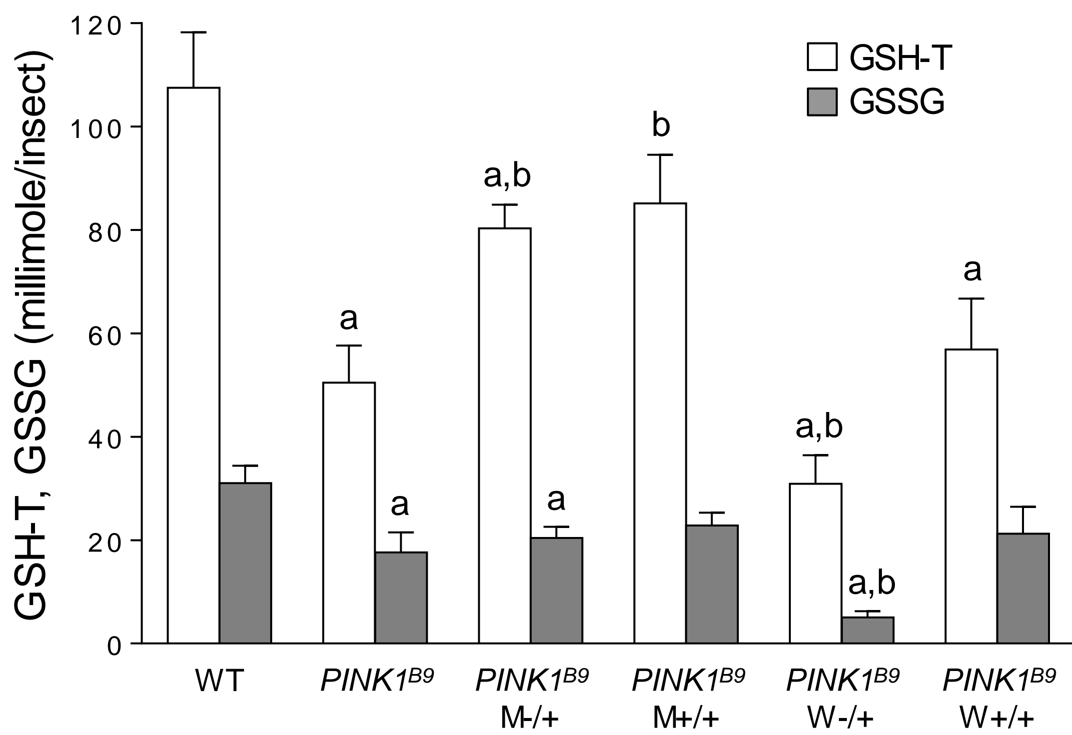

Fig. 1

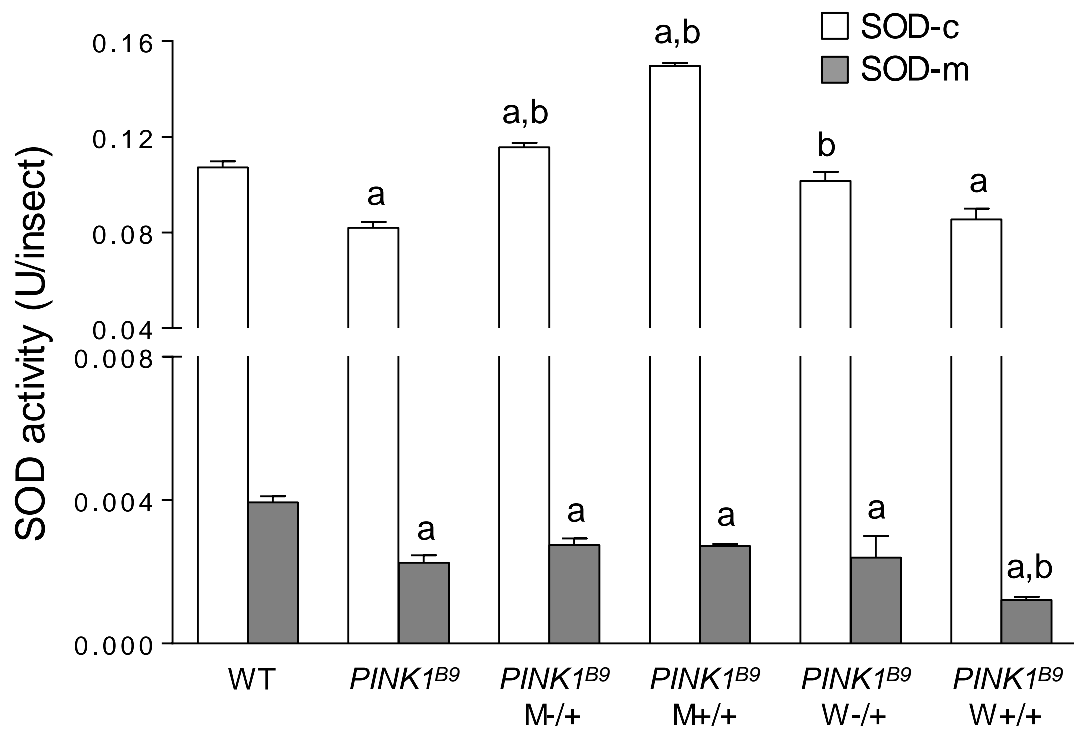

Fig. 2

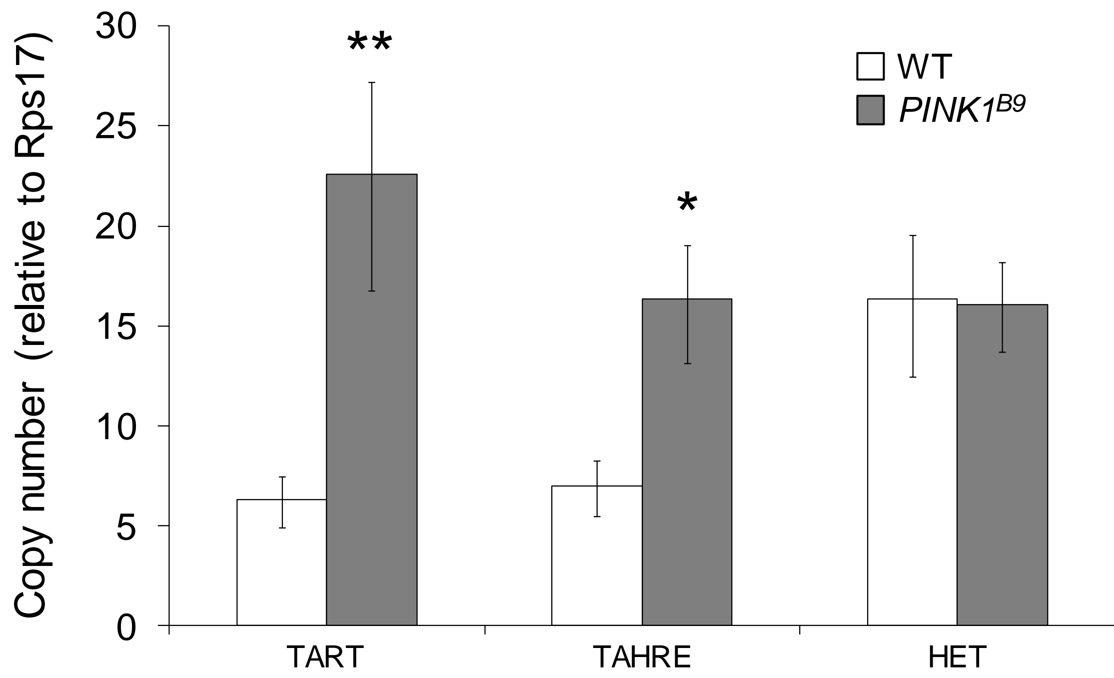

Fig. 3

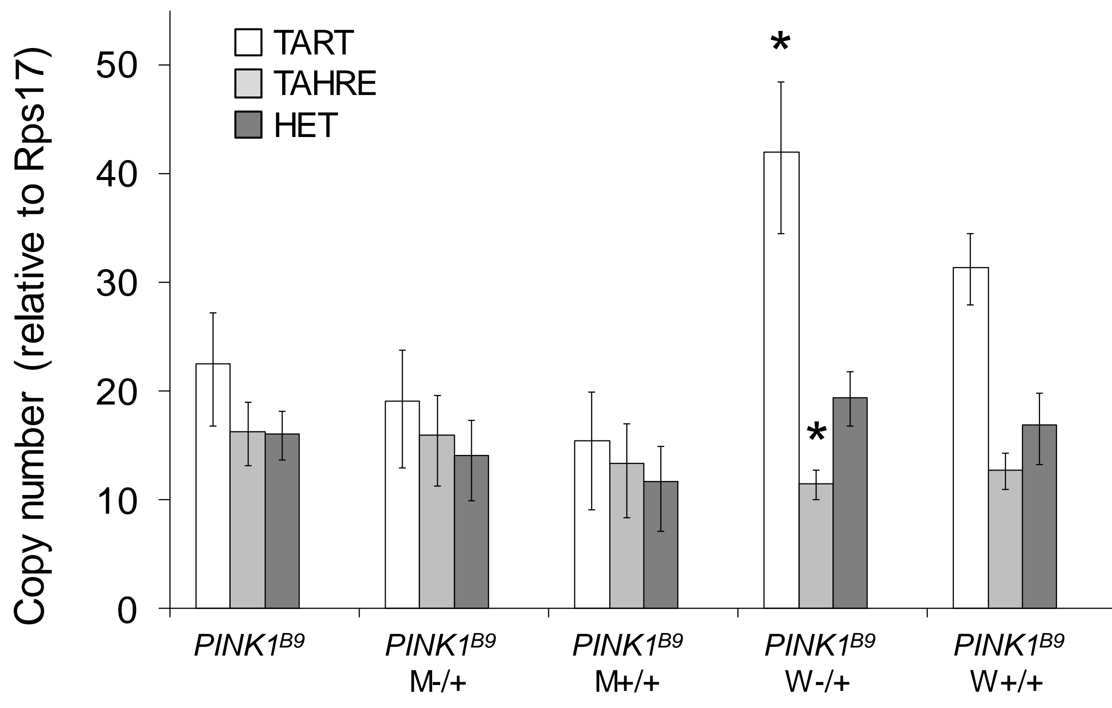

Fig. 4