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Mutations in PARKIN (pdr-1) Results in Increased Reproductive Fitness in C. elegans

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Mutations in PARKIN (*pdr-1*) results in increased reproductive fitness in *C. elegans*.

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By

Tracy Barhydt

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CERTIFICATION OF APPROVAL

I certify that I have read *Mutations in PARKIN (pdr-1) results in increased reproductive fitness in C. elegans* by Tracy Barhydt and I approved this thesis to be submitted in partial fulfillment of the requirements for the degree: Master of Sciences in Biology at Dominican University of California and the Buck Institute of Aging.

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Abstract

Parkinson's disease (PD) is largely an idiopathic disease that includes contributions from both genetic and environmental factors, with aging itself being the largest risk factor. These combined susceptibility factors are believed to accumulatively contribute to initiation and progression of Parkinson's disease.

Although most cases of PD are idiopathic, rare genetic forms of the disease do exist. In these cases, an important unanswered question is why mutations that cause the disease have come to be maintained within the human population? One possibility is based on what is known as the Antagonist Pleiotropic Theory (APT) of Aging, a theory that states that genes that confer reproductive benefit early in life may result in detrimental effects with post-reproductive aging.

Mutations in the PARKIN gene are associated with PD in humans. We have made a series of observations in the model organism *Caenorhabditis elegans* in which, the worms carrying a mutation of the homozygous gene, *pdr-1*, displayed increased reproductive fitness. This suggests that familial parkin mutation may have a beneficial effect in terms of natural selection even though its known clinical effect in PD patients bearing this mutation is detrimental. This data provides the first clues as to why this particular PD-related mutation may be maintained within the population. I have investigated the mechanism of this newly identified phenotype and if environmental factors alter this affect. Specifically, we have asked whether increased fitness may be due to enhanced mitochondrial function during reproduction. Additionally, we contemplate if the environmental factor manganese (Mn), which has been identified as a risk factor for the disease, alters reproductive fitness in relation to this gene mutation.

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Introduction

Parkinson's disease (PD): sporadic versus familial forms

Parkinson's disease (PD) affects about 2% of the population and is the second most common neurological disease in people over the age of 65 (Singer et al, 2005, Tanaka, 2010, and Vries and Przedborski, 2013). PD is largely considered an idiopathic disorder that includes contributions from genetic and environmental factors, as well as aging itself. Indeed aging is the number one risk factor for the disorder. Although the majority of PD cases are idiopathic, rare inherited forms do exist as a consequence of both autosomal dominant and recessive mutations. Dominant forms of monogenic parkinsonism are caused by mutations in the alpha-synuclein (SNCA) and leucin-rich repeat kinase 2 (LRRK2) genes. Autosomal recessive forms of early-onset (<45 years of age) PD also exist and include those caused by mutations in PARKIN (Park 2), PTEN induced putative kinase 1 (PINK, Park 6) and DJ-1 (Park7). In both idiopathic and familial forms of the disorder, PD is characterized by a decline in motor dysfunction, akinesia, rigidity with gait and balance impairment. This is due to selective loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) (Harrington et al, 2010 and Vives-Bauza and Przedborski, 2010, Eng-King and Skipper, 2007, Frank et al, 2008). Younger individuals including those manifesting recessive mutations are generally observed to demonstrate a slower decline of motor function whereas in older individuals, motor dysfunction and gait imbalances worsen more rapidly (Obeso et al 2010).

Although PD is classically diagnosed by the onset of motor dysfunction, the concept of pre-motor PD has recently gained wide support (Kitada et al, 1998, Forno, 1996). There is increasing evidence that olfactory dysfunction, sleep abnormalities, cardiac sympathetic denervation, constipation, depression and pain may antedate the onset of motor signs in PD (Obeso et al, 2010). However, the loss in motor function in PD is still the major target for clinical

treatment. Increased evidence suggests an involvement of loss of mitochondrial function in association with characteristic SN DA cell loss (Exner et al, 2012, Vives-Bauza and Przedborski).

Parkin and its role in maintaining mitochondrial function

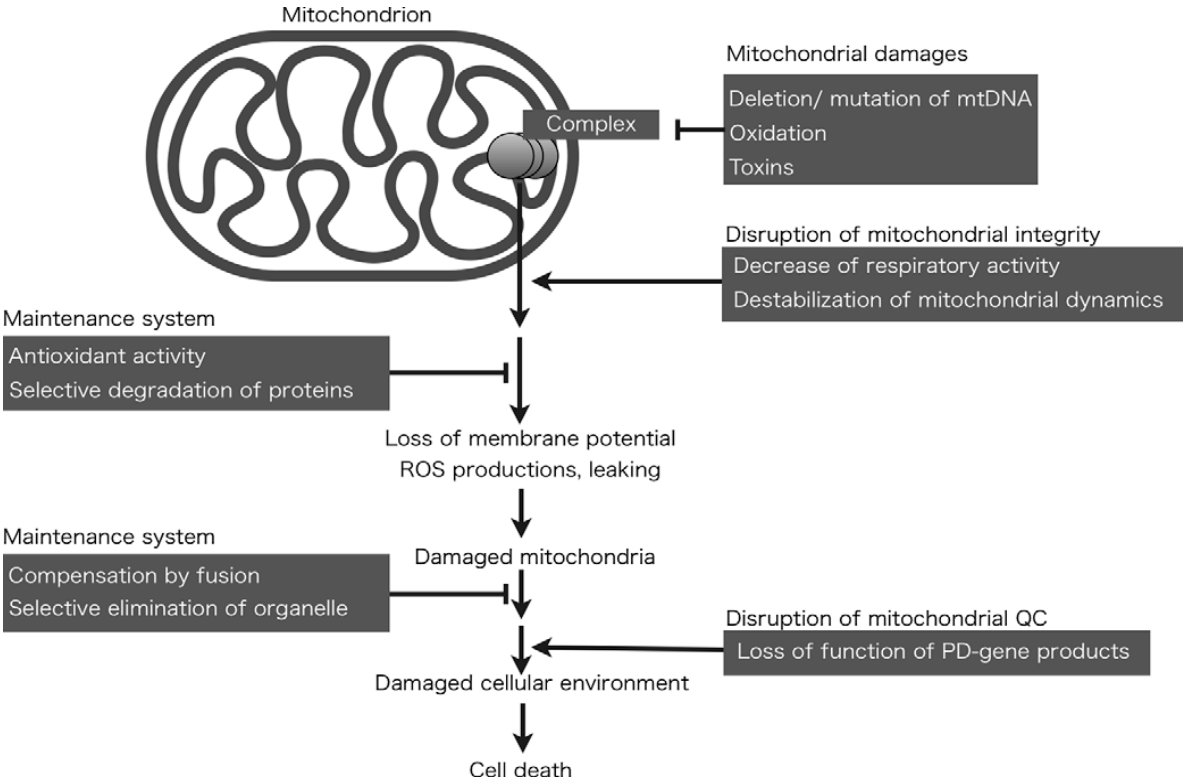
Mutations in the PARK2 gene (parkin) results in a familial autosomal recessive juvenile parkinsonism, that is the most common genetic form of early-onset PD (Frank J.S Lee and Fang Liu, 2008 and Harrington et al, 2010). These include both homozygous or compound heterozygous recessive mutations. PARKIN mutations result clinically in early onset parksonism, with patients displaying a good response to dopamine replacement therapy via Levodopa treatment similar to that observed in patients with later onset, sporadic forms of PD. In most patients with familial PD, the average age of onset is in the early 30's, although there have been reported cases of age of onset being as late as 70 years of age (Bonifati, 2012).

The PARK2 gene encodes the parkin protein. Structurally, the protein consists of an N-terminal ubiquitin like domain, (UBL) a central linker region or unique parkin domain (UPD), and a C-terminal RING domain that consists of two RING finger motifs separated by an in-between RING (IBR) domain. In its capacity as an E3 ligase, PARKIN plays an important role in the ubiquitination targeting of select protein substrates for proteasomal degradation. Each domain of the PARKIN gene construct has been shown to be involved in the ubiquitylation activity. For example, substrate recognition, PARKIN stability for ubiquination and proteasome binding have all been implicated to require the UBL domain (Springer et al, 2005, Frank et al, 2008).

Recent studies have suggested that parkin also plays an important role not only in the targeting of select protein moieties for degradation, but also whole organelles including the mitochondria. In this case, loss of PARKIN dysfunction would result in increased numbers of damaged mitochondria per cell which could have effects on overall neuronal health and function (Cook et

al, 2012, Head et al., 2011). Familial PARKIN mutations are indeed associated with increased mitochondrial defects within midbrain dopamine neurons (Kim and Pallanck, 2012).

Mitochondrial ATP production normally supports many important neuronal functions including the generation of axonal and synaptic membrane potential. Dendritic mitochondria support synapse density and plasticity. Mitochondrial loss from these regions can inhibit synaptic transmission owing to insufficient ATP supply or losses in calcium homeostasis (Zu-Hang Sheng et al, 2012). Maintaining mitochondrial function is important for overall neuronal health, and its loss can contribute to neurodegeneration. Damaged mitochondria may be eliminated via lysosomal autophagy or “mitophagy” in response to mitochondrial “stress”. The parkin protein has been shown to localize to the outer mitochondrial membrane and to drive mitophagy of damaged mitochondria by marking them for turnover by the lysosome (Exner et al,2012 and Kitada et al,1998)



Schematic of maintenance system of PARKIN: dysfunctional mitochondria eliminated by mitophagy. With the loss of E3 ligase function, the accumulation of damaged mitochondria occurs, which can result in cell death. (Tanaka, 2010)

Manganese toxicity: a risk factor for PD linked to parkin dysfunction

Manganese (Mn) is an essential metal for many physiological processes including optimal brain development. While essential for physiological functions, excessive Mn uptake can lead to a condition that clinically resembles PD called 'manganism' (Au et al, 2009). Mn has also been shown to be a risk factor for development of PD itself following long-term, lower level exposure (Settivari et al, 2009). Mn has been demonstrated to elicit its selective dopaminergic neurotoxic effects via increases in oxidative stress and loss of mitochondrial function.

Mn uptake occurs via a specific transporter called the divalent metal transporter 1 (DMT-1). This transporter is also involved in the uptake of other divalent metals including iron, cadmium, and nickel (Roth et al, 2010). DMT-1 is a substrate for PARKIN; when levels of the transporter

are too high, it is ubiquitinated by parkin for proteasomal degradation. When PARKIN activity is reduced as occurs in association with mutation, this can result in elevated levels of DMT-1 and increased Mn uptake (Settivari et al, 2009 and Au et al, 2009, Guilarte, 2010) In this manner, PARKIN mutation could theoretically elicit elevated cellular Mn levels that can contribute to mitochondrial damage and subsequent dysfunction.

The antagonistic pleiotropic theory (AP), suggests that variants of longevity genes have been favored over evolutionary time owing to benefits on early life fitness traits (Williams, 1957). This theory further suggests that genes may have beneficial reproductive traits early in life and deleterious effects later in life, such as involvement in the development of neurological diseases. In age-structured populations, an optimal brood size version of sex allocation theory is most appropriate, because trade-offs between brood size and the time until the next episode of reproduction may favor immediate brood size (Stine et al, 1990). This suggests that positive effect of early fitness from an allele would outweigh negative deleterious effects, such as PD, later in life. However, alleles without genetic disease-causing outcomes in the population haven't been identified in the population (Albin, 1993, Estes et al, 2004). Existing PARKIN mutations that confer shorter lifespan associated with disease may have a positive effect on some aspect early in life.

C. elegans as a model organism

Caenorhabiditis elegans has been extensively used in the past two decades as a model organism for studying various aspects of aging and neurodegenerative disease. Advantages of its use for this purpose include its short-lifespan, the ease of producing and raising large numbers of progeny, and its transparency which facilitates morphological analysis. Straight-forward means of gene manipulation in order to establish stable mutant lines has provided an experimental platform for analyzing loss-of function mutations and potential genetic and

environmental interactions (Hope, 1999). *C. elegans* are also amenable to RNA interference (RNAi) which allows selective knock down of the expression of most genes in the *C. elegans* genome via the culturing of worms on *Escherichia coli*-containing bacterial RNAi clones or by soaking in a solution of double stranded RNA (Hope,1999).

In terms of its use as a model to study PD, unlike other model systems which contain tens of thousands (*Drosophila*) to millions of neurons (mammals), the adult *C. elegans* hermaphrodite has only 8 dopaminergic neurons out of a total of 302 neurons throughout the body (Harrington et al, 2010). This leads to easier manipulation of these neurons within the worm to study the effect of genetic and/or environmental manipulations. Importantly for our studies, *C. elegans* contains a homolog of the human PARKIN gene termed *pdr-1* (Parkinson's disease-related gene- 1) which encodes a 386 amino acid protein similar in structure to the 465 amino acid human parkin protein (Springer et al, 2010). The *C.elegans* homolog contains similar domain characteristics as the human PARKIN, with the overall homology being 41% similarity. Baumeister's group have shown conservation in the ubiquitylation complex in *pdr-1* (Springer et al, 2005)

In the Lithgow laboratory, preliminary data generated by Suzanne Angeli Ph.D., demonstrated that worm strains containing PARKIN mutations displayed increased reproduction on Day 1 and Day 2 compared to wild-type (N2) worms. This data provided me with a platform for my two year Master's thesis work to further the investigation of this mutant on reproductive fitness. The suggestion that the PARKIN mutant in *C. elegans* has increased reproductive fitness fits in with the optimal brood size version of the sex allocation theory; in this theory, trade-offs between brood size and the time until the next episode of reproduction favor immediate brood size (Stine et al, 1990). We postulated that due to effects on brood size, positive effects on early fitness as a consequence of the PARKIN mutation outweigh negative deleterious effects later in life because of total life positioning in the population. In other words, although PARKIN mutations

may have an overall shorter lifespan, this is outweighed by a positive effect of the mutation on reproductive fitness earlier in life. This provides a possible explanation as to why such a mutation would be maintained in a population (Springer et al, 2005). I have further begun to explore whether enhanced mitochondrial function may be a contributing factor to this affect and what impact altered handling of the environmental factor manganese (Mn) has as a consequence of PARKIN mutation.

Materials and Methods

C. elegans was used as a model organism for all experiments. The strains *pdr-1*(*lg103*) and wild-type (N2) were a gift from Benjamin Wolozin (Boston University School of Medicine, Boston, Massachusetts.) The strain *pdr-1(gk448)*[GL325] and N2[GL] were purchased from the *Caenorhabditis* Genetics Center (CGC) at the University of Minnesota, St. Paul. [GL325] was back crossed five times by Suzanne Angeli PhD (Lithgow and Andersen laboratories) to ensure the elimination of any mutations that might modify the phenotype. It should only reveal a homozygous background. RNA interference (RNAi) clones were selected from the Open Biosystems library.

Standard media preparation

Unless otherwise stated, worms were grown at 20°C on nematode growth media (NGM) plates; 17g bacto agar, 3g NaCl, 2.5 g bacto peptone, 25 ml KH_2PO_4, 1 ml $MgSO_4$, 1 ml $CaCl_2$, 1 ml cholesterol, and 925 ml H_2O for 1 L NGM (Hope, 1999). *C. elegans* were fed the common laboratory attenuated *E. coli* strain OP50. OP50 is an *E. coli* strain that is auxotrophic for uracil. This bacterial culture was grown, seeded, and stored according to protocols listed in *C. elegans: A Practical Approach* (Hope 1999).

RNA interference (RNAi) media

Standard media was prepared as stated above. Once the media had cooled and the remaining reagents added, an inducer, IPTG, and the antibiotics, ampicillin and tetracycline, were added according to the procedures outlined by Alavez (Alavez et al, 2011). RNAi is performed by "feeding" worms RNAi-containing bacterial clones.

Clones were selected from the Open Biosystems library (stored at -80˚C). To retrieve the clones, a sterile needle was used to pick bacteria and then streaked onto an LB plates containing ampicillin and tetracycline. This plate was then incubated at 37°C overnight. A single colony was then transferred using sterile technique to a tube containing autoclaved LB. 1000 µg/mL Ampicillin and 12 µg/mL tetracycline were added at a ratio of 1 µl of antibiotic per 1 mL of LB. 0.5M isopropyl β-D-thio-galactopyranoside (IPTG) was added. The tubes were placed in a rack on an orbital shaker in a 37˚C incubator overnight.

RNA interference allows knock out of mRNAs encoding select gene products; libraries of *E. coli* have been produced containing bacterial clones capable of expressing RNAi complementary to most genes in the C. *elegans* genome. (Avery 1993).

Worm culture

Care was taken to keep all individual worm strains separate from other strains to assure no mixing of strains and to keep cultures free of bacterial and fungal contamination. Worms were passaged on medium (6 cm) plates, and stored at 20˚C. The developmental stage from egg to gravid adult is three days. To assure the worm cultures had adequate bacteria for feeding and to reduce the potential for starvation which leads to the worms burrowing in the agar, eggs were picked from the plates every three days. This technique of transferring the eggs was used to ensure multi-stage populations. For synchronous populations, gravid adults were moved to a fresh medium plate for two hours. The adults were then removed, yielding synchronous egg populations. Synchronized populations were alternatively obtained using a hypochlorite solution (see below).

Hypochlorite synchronization

Worms were floated off culture media plates using M-9 solution, 3 g KH_2 PO_4, 6 g Na_2HPO_4, 5 g NaCl, per 1 liter (Hope, 1999). M-9 liquid mixed with worms was removed using a sterile pipette and transferred to a 15 mL conical tube. The tube was centrifuged at 1,700 RPM for 50 seconds, and the supernatant aspirated. A hypochlorite solution (5 mL KOH, 4 mL NaClO, and 41 mL water) was added to the tube which was hand-shaken for approximately 5 minutes, or until the bodies of the adult worms were no longer visible. The tube was centrifuged again, and the hypochlorite solution removed. This was performed twice. The resulting eggs were washed twice with M-9 solution, followed by centrifugation and aspiration. The last step involved a MilliQ water rinse step of 10 mL added to the 15 mL tube. The eggs were placed in glass dishes containing fresh S-basal or M-9 and incubated at the appropriate temperature for 16 hours yielding synchronous arrested L1 larvae.

Exposure of C. elegans to manganese chloride ($MnCl_2$)

$MnCl_2$ solution was added to standard NGM media from a stock solution of freshly prepared 3M $MnCl_2$ (3g/5mL) dissolved in MilliQ H_2O (pH 3.0). Once the OP50 was seeded onto 35 mm plates, the plates were swirled to achieve an even lawn of bacteria. The worms were placed on the plates with and without $MnCl_2$ as L4's.

Fitness assays*:*

Hermaphrodite self-fertility brood size (fecundity: total number of eggs laid)

To determine the number of viable progeny produced self-fertilization, individual fourth-stage larvae (L4) were transferred to Standard NGM plates spotted with 80 µl of *E. coli* (strain OP50). Ten replicates of each worm strain (*pdr-1* mutant or RNAi knockout versus N2) were grown on 35 mm Standard NGM plates. The initial transfer on Day 1 was at the L4 stage. Each subsequent transfer was conducted daily until egg laying subsided, usually between days 4 and 5. On the final day of transfer, progeny on each plate was quantified at the L4/young adult stage (Walker et al, 2000)

Time of first egg lay (α-time)

Five replicates of both wild-type N2 and *pdr-1* mutants, *pdr-1*(*lg103*) and *pdr-1*(*gk448*), or *pdr-1*(RNAi) strains were used for these studies. For each plate, two gravid adults were transferred to the plates for a 1 hour egg-laying session. The adults were then removed. The progeny were observed approximately 65 hours later, by which time the original eggs had grown to gravid adults. Each plate was observed every 30 minutes after the 65 hour time point until all the plates contained eggs and timing of the first egg laid were recorded.

Laboratory natural selection (competition) assay

We performed laboratory natural selection (competition) experiments by transferring wild-type (N2) worms mixed with *pdr-1(lg103) or pdr-1(gk448)* on the same agar plates over multiple

culture generations. Duplicate populations were established containing 50 N2 and 50 *pdr-1* mutant eggs laid within 60 min of each other from age-synchronous parental populations on 10 mm plates. All populations were maintained at 20°C. A total of 50 eggs were transferred to new nematode growth medium (NGM) plates for each subsequent generation up to twelve generations. Under these conditions, the worm populations never starved. As there were no males in these populations, all progeny were the result of self-fertilization. Worms from multiple generations were genotyped by polymerase chain reaction (PCR) to determine the frequency of *pdr-1(lg103)* and *pdr-1*(*gk448)*, compared to the wild-type in the population (see below, Jenkins et al, 2004)

Laboratory natural selection (competition) assay with starvation cycle

Population studies were conducted as stated above. The plate of the dual population was stored at 20°C within the starvation cycles. The plate was monitored for food which was exhausted by Day 5 then maintained for another two days at 20°C. Two plugs of the same diameter were removed from opposite sides of this plate and transferred to another spotted plate of a larger diameter (100 mm). Approximately 48 hours later, 50 eggs were transferred to initiate the next generation and 50 adult worms were used to determine allele frequency by genotyping by PCR (Walker et al, 2000; Jenkins et al, 2004).

Polymerase chain reaction (PCR)

The goal of the PCR screening procedure was to identify levels of DNA from the deletion mutant versus the N2 strain to estimate percentage of each in the population. By PCR amplifying a region of the gene of interest (*pdr-1*) and analyzing on a 2% agarose gel electrophoresis, the smaller deletion was observed on the gel as a band with faster mobility than the larger wild-type amplicon and the ratio of the two can be assessed to determine contribution to the population. Each band represented a single worm DNA. Standard PCR protocols were used. PCR

amplification used the Promogen^{TM}GoTAq polymerase and was performed on a Bio-Rad PCR thermocycler. The *pdr-1*(*lg103*) primers were 5'-TGTCTGATGAAATCTCTATA -3' and 5'-TACGACGTCTGACCCATAATGT-3', with an annealing temperature of 51.0°C. The expected size for N2 (WT) was about 1200 bp and the *pdr-1*(*lg103*) expected size was 100 bp.
The following was primers sets used for *pdr-1*(*gk448*) 5'UTR-F: ACAAAAACATGGGGCTTCAA and 5'-ATCCGAGGTCGAAGATCACTT-3' with an annealing temperature of 54°C. The expected size for WT is about 800 bp used to backcross with *pdr-1*(*gk448*). The expected size for *pdr-1*(*gk448*) is 450 bp.

Lifespan assays

Lifespan assays were performed in duplicate as previously described (Alavez et al, 2011). $MnCl_2$ plates contained a final volume of 10 mM from a stock of 3M $MnCl_2$ (See above method for *C*. *elegans* exposure to $MnCl_2$). At the developmental L4 stage, worms were placed on either non-treated or $MnCl_2$-treated plates. All worms were scored as live vs. dead (no movement when touched with a platinum wire pick) every two-three days until all worms were dead. Lifespan data was plotted as a survival curve using Graphpad Prism™, and compared using a log rank test implemented in Graphpad Prism™ (Alavez et al, 2011).

Thermotolerance stress test

As Day 1 adults, duplicates of both N2 wild-type and *pdr-1*(*lg103*) and *pdr-1*(*gk448*) strains were exposed at 35°C for 6 hours followed by worm scoring. Groups of 20–30 animals were transferred to NGM plates with ample food and then placed into an incubator with the temperature maintained at 34.5 ± 1°C. Worms were scored every hour until considered dead and removed from the plate when they no longer responded to prodding with the worm pick.

Mitochondria membrane potential (MMP) via TMRM

Tetramethylrhodomine methyl ester (TMRM) is a lipophilic cation whose mitochondrial uptake is dependent on plasma or mitochondrial membrane potential (Yoneda et al, 2004,Settivar et al, 2009). Measurement of mitochondrial TMRM levels was used as a gauge of mitochondrial membrane potential and functional state. A 50 um solution of TMRM was prepared in DMSO and added to the nematode growth media at a final concentration of 0.1 mM (Yoneda et al, 2004) in the absence and presence of 20 mM $MnCl_2$ to assess effects on MMP in *pdr-1* versus N2 worms. Animals were transferred at the L4 stage to TMRM plates for approximately 24 hours and worms imaged by fluorescent microscopy Olympus BX15. Worms were paralyzed with 2 mM levamisol and mounted on slides with agar pads. The entire worm was captured and scored for fluorescence and length using 20x magnification. Image J was used to determine the mid-line length of all worms (Image J score), and the length in mm was calculated in Microsoft Excel™ with the fluorescent density.

Endogenous elemental analysis using Inductively coupled plasma (ICP)

Two to three replicates were prepared for each condition. A mass culture of worms was treated with hypochlorite solution (1 ml 5M KOH, 0.8 ml hypochlorite, 8.2 ml H_2O). Initially, the culture was washed three times with M9 buffer, then washed with MilliQ laboratory water, then transferred to a glass petri-dish containing M9 solution overnight at room temperature to arrest at the L1 larval stage (Page et al, 2012). To assure that the M9 media was not contributing to any increased elemental values, after the worms were washed off the mass culture with M9, a solution containing 150 mM choline chloride,1 mm HEPES, was used to remove M9 and any residual bacteria in the gut of the worms. ICP was conducted as previously described by our laboratory (Page et al, 2012),

Worms used for elemental analysis were washed off plates with M9 buffer without disturbing the bacterial lawn. The worms were pelleted by centrifugation (1700 x g, 45 sec) and washed three

times with M9 buffer over a total of 20 min to promote clearance of gut content. Statistical analysis was completed using Microsoft^{tm} Excel software. A two-tailed T-test for two samples with unequal variance was used to analyze the control and treated populations for each element; p values of less than 0.05 were considered significant. Elemental values for treated populations were compared to the mean value for the control population to produce the percentage change from control values [(element level for exposure) – (mean element level for control)) / (mean element level for control)] used to calculate the mean percentage change from control for the graphical representation of the data.

Results

pdr-1 mutants confer reproductive fitness

Prior to my arrival in the Andersen and Lithgow laboratories as a Master's student, Suzanne Angeli Ph.D (Lithgow and Andersen laboratories) obtained preliminary data suggesting that the mutant, *pdr-1*(*lg103*), an in-frame deletion, (Springer et al, 2010) had an increased early reproduction, day 1 and day 2, in the reproductive stage compared to wild-type (Angeli, unpublished data) By the third day to day five, the wild-type were laying more eggs. These results are suggested to follow early reproductive fitness. The total broodsize *C*. *elegans* normally has a reproductive period of up to five days with a total number of progeny of approximately 300.

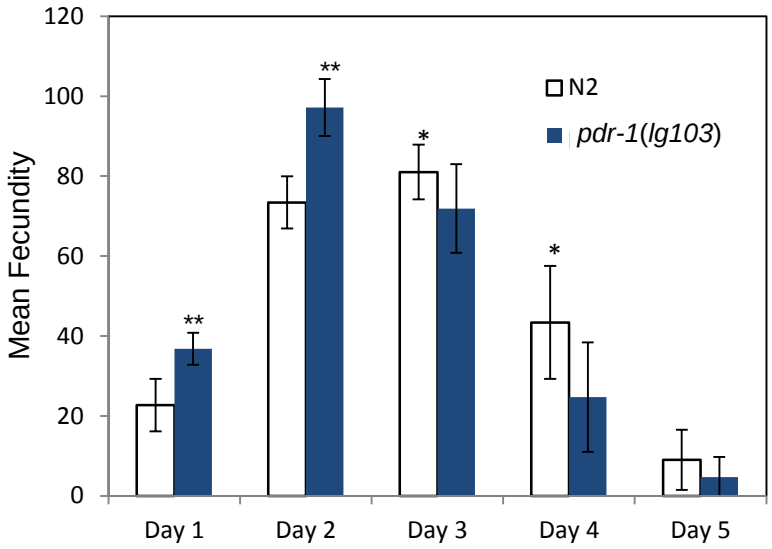


Figure 1. *pdr-1(lg103)* confers increased early fecundity. N=10 **p<0.005 *p<0.05 (T-test). Error bars indicate standard deviation of ten replicates. Three biological replicates completed. Total progeny approximately 250.

In order to further investigate the *pdr-1* gene, I evaluated another allele, *pdr-1*(*gk448*) to determine if it too displayed similar characteristics as the *pdr-1*(*lg103*) in terms of fecundity. The mutant strain *pdr-1*(*gk448*) carries deletion at the ATG start codon site, producing a null allele, or a complete loss of function. The previous protocol used for *pdr-1*(*lg103*) was followed for *pdr-1*(*gk448*).

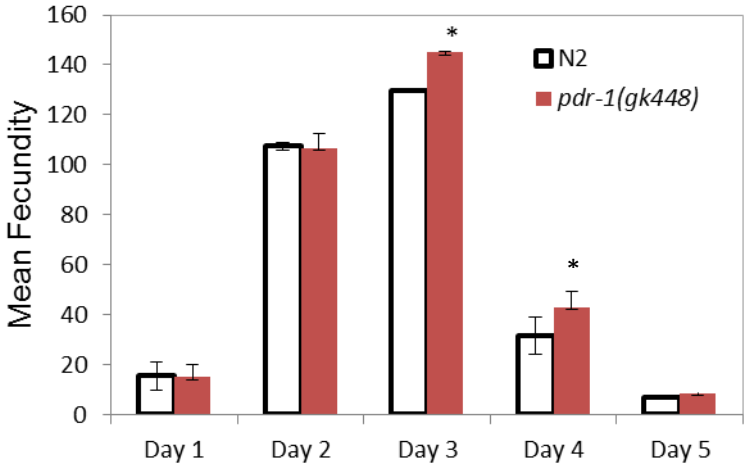


Figure 2. *pdr-1*(*gk448*) confers increased late fecundity. Increased fecundity is noted at day 3 for this *pdr-1* allele as well as an increase in the <u>total</u> number of progeny. N=10. This represents pooled data from three biological replicates. *p<.05

Shown in Figure 2, mean fecundity during Day 3 for *pdr-1*(*gk448*) was the most significant compared to N2; whereas, the phenotype for *pdr-1*(*lg103*) had the most viable eggs laid on Day 1 and Day 2. There are also differences in the total progeny for *pdr-1*(*gk448*). The total broodsize for *pdr-1*(*gk448*) was 319 compared to wild-type broodsize of 291. *Pdr-1*(*lg103*) broodsize was comparable to the wild-type. Indeed, during every reproductive day from day 3, *pdr-1*(*gk448)* demonstrated increased progeny versus the wild-type N2. Taken in total, these data suggest that while the mean fecundity for the *pdr-1*(*gk448*) displayed a different phenotype, which could be a result of complete loss of function.

We also conducted brood size (fecundity) assays in *pdr-1*(RNAi)-treated worms. The worms were fed dsRNAi bacteria with counting the daily and total broodsize for the animals. The RNAi treated worms showed increased fecundity at Day 2 compared to N2 ($p<0.05$). These results showed similarities as the mutant *pdr-1*(*lg103*). However, the total number of reproductive days were shorter, 4 versus 5 days. (Figure 3).

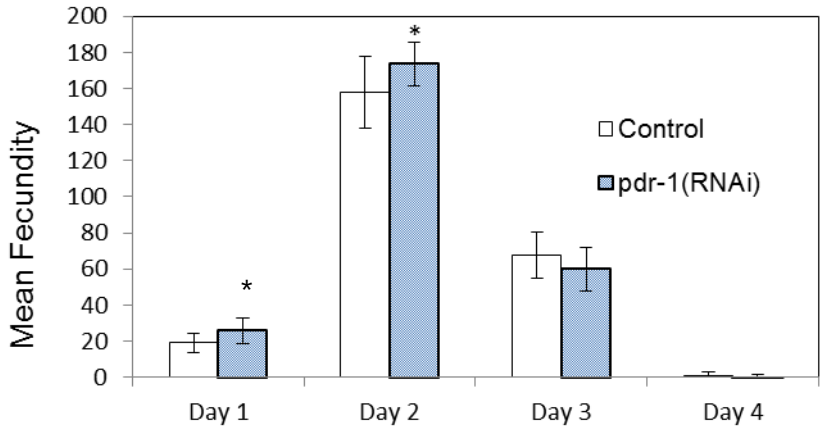


Figure 3. Fecundity following *pdr-1*(RNAi) Total broodsize for control vector versus *pdr-1*(RNAi) treated worms, 245 and 259, respectively. *p<.0.05 N=10.

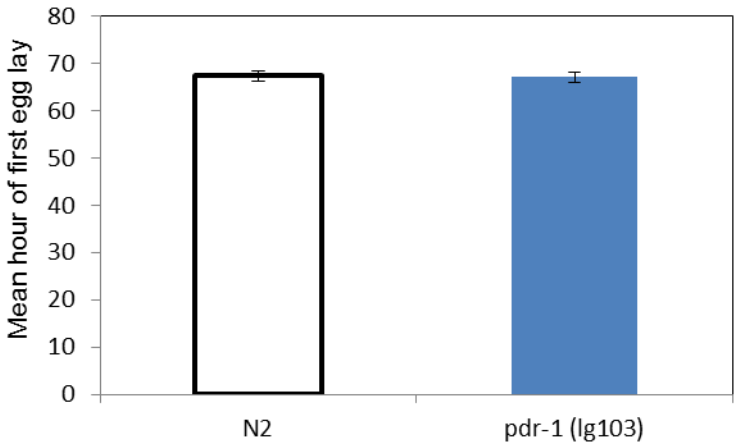


Figure 4. Time of first egglay (α-time) *pdr-1*(*lg103*) vs N2. Data shows mean time in hours to first egg lay at 20°C; no significant difference between N2s and the *pdr-1* mutant. Values are pooled from biological triplicates. N=5

Increased progeny number (fecundity) could stem from timing of the first egg laid or a decrease in development from egg to adult. In Figure 4, the differences of mean hour for time of first egg lay were not significant between *pdr-1*(*lg103*) and wild-type, which suggested the strains are laying eggs at a similar time.

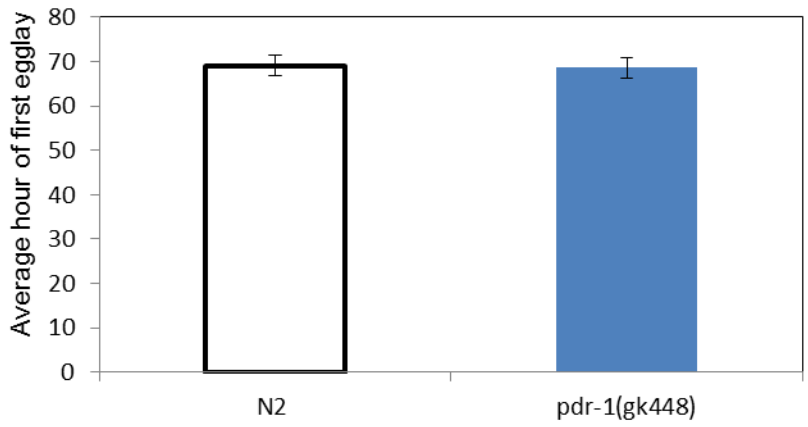
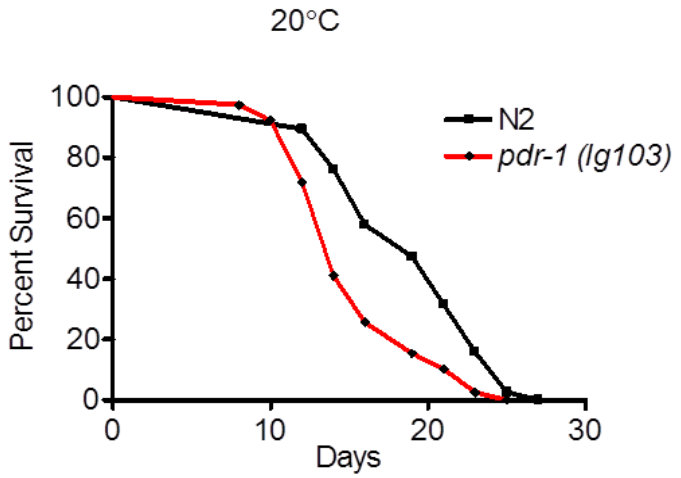


Figure 5. Time of first egg lay (α time) of *pdr-1*(*gk448*) versus N2. No significant difference. Values are pooled from biological triplicates. N=5.

Pdr-1(*gk448*) compared to wild-type showed no significant difference in the time of first egg laid. (Figure 5)

A



B

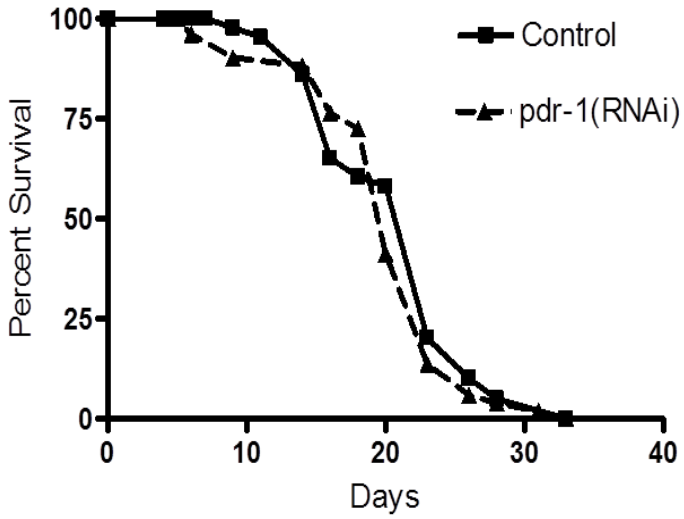


Figure 6. Lifespan survival curves. (A) The *pdr-1*(*lg103*) lifespan (conducted by Susan Angeli) demonstrates a shorter lifespan versus N2 ($p<0.05$, Logrank test). (B) *pdr-1*(RNAi)-treated worms display no significant difference between N2 and pdr-1(*lg103*). Data represents two biological replicates.

pdr-1(lg103) has reduced lifespan

Pdr-1(*lg103*) demonstrated a shorter lifespan compared to N2 (Figure 6). These data (increased fecundity but shorter lifespan) in the *pdr*-1 mutant worms fits with the antagonist pleiotropy theory as it relates to aging, suggesting that altered expression of some genes or genetic pathways may be beneficial early in life but deleterious later in life. *Pdr-1*(RNAi) lifespan in contrast show no significance difference in lifespan. This may reflect a difference between partial *pdr-1* activity versus efficiency of the RNAi.

				Logrank test	
	Experiment	Median Lifespan	Total # of worms	p-value	Chi square
A	N2	19	38		
	pdr-1(lg103)	14	39	***p=0.0006	11.65
B	N2	18	69		
	N2 +10mM Mn	18	77	p=0.9496	0.0040
C	*pdr-1*(*lg103*)	16	51		
	pdr-1(*lg103*)+10mM	20	71	*p=0.0079	7.06
	N2	15	22		
D	*pdr-1*(*gk448*)	13	36	p=01809	1.790
	N2	15	22		
E	N2 +10mM Mn		34	p= 0.0510	3.807
	pdr-1(*gk448*) 0 mM Mn	13	36		
F	*pdr-1*(*gk448*) +10mM		59	p=0.3247	0.9698
	Control vector	23	41		
G	*pdr-1*(RNAi)	20	51	p= 0.3124	1.021

Table 1. Lifespan analysis for *pdr-1* mutants and *pdr-1*(RNAi). A and G Represents data from Figure 6. B-G corresponds to Figures 16 and 17, respectively.

pdr-1(lg103) confers a fitness advantage

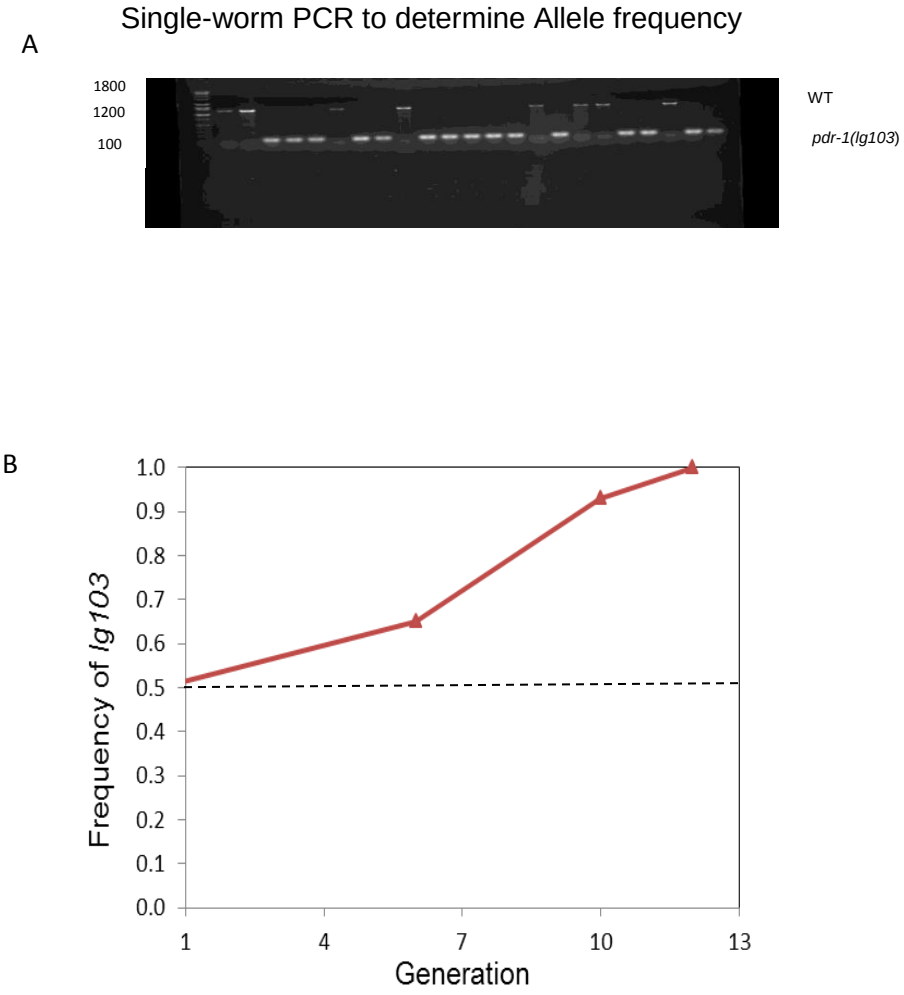


Figure 7. Laboratory natural selection (competition without nutritional stress) assay *pdr-1*(*lg103*) versus wild-type (N2). A) A representation of F1 PCR genotyping from combined population. Each band represents single worms DNA. B) Data is representative of three biological replicates. N=50

A laboratory natural selection assay was conducted to assess whether enhanced brood size in the *pdr-1*(*lg103*) worms conferred a reproductive advantage resulting in a selective advantage in the combined population. The *pdr-1*(*lg103*) was found to constitute 100% of the population by

the twelfth generation (Figure 7). The *pdr-1*(*lg103*) band size is 100 base pairs; whereas the wild-type band size showed ~1200 base pairs.

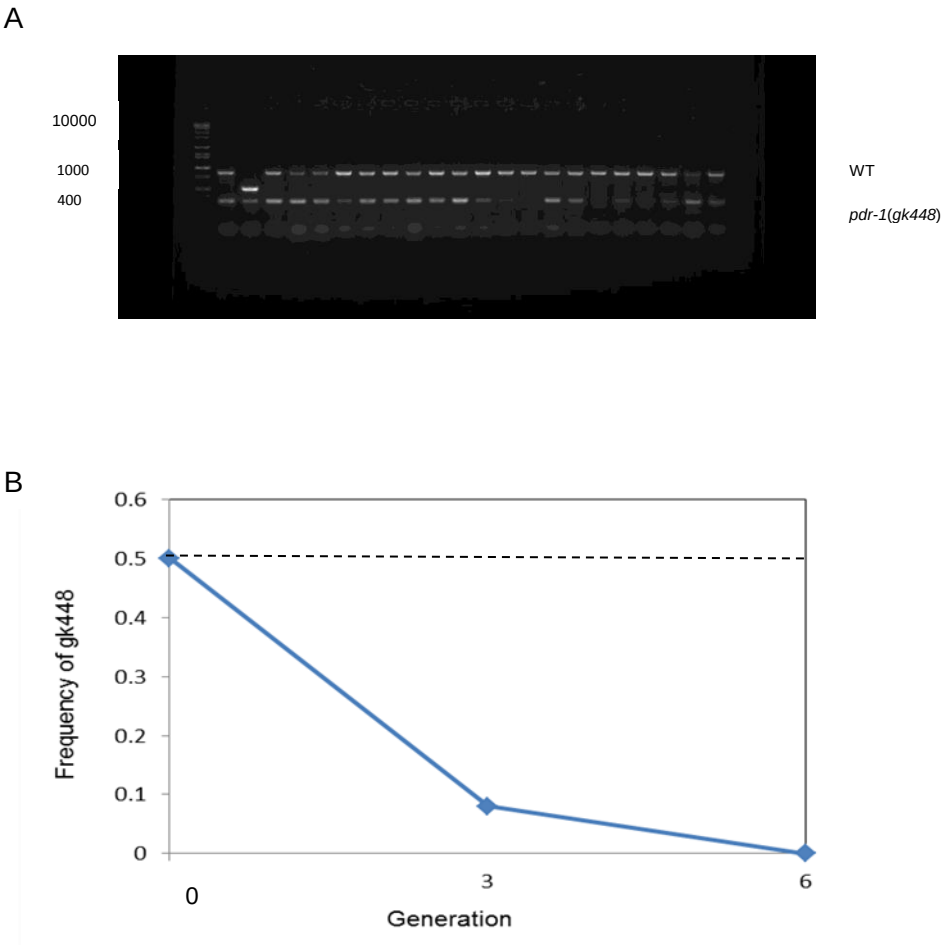


Figure 8. Laboratory natural selection (competition without nutritional stress) assay in *pdr-1(gk448)*. A) A representation of an agarose gel showing F3 genotyping between wild type and *pdr-1*(*gk448*). Each band represents a single worms DNA. B) Graphical data of gel results. Data represents two biological samples. N=50. The band size for *pdr-1*(*gk448*) is 400 bp.

In contrast, data showed that by generation 6, the wild type strain out-competed the *pdr-1*(*gk448*) containing strain (Figure 8). This is in keeping with the brood size data

demonstrating that in this mutant, fecundity was elevated on Day 3 rather than Day 2 as for the other *pdr-1* mutant. Although, these results suggest validation of the brood size effect, they only represent one biological sample and need to be repeated to confirm results.

To mimic conditions in the wild, worms were allowed to deplete all of the food on the plate then starved for an additional two days. This replicates the food restricted environment of the worm's natural habitat. Each starvation cycle consisted of approximately two reproductive cycles or two generations. As shown in Figure 9, the *pdr-1*(*lg103*) out competed the wild-type population by the third starvation cycle. In contrast, *pdr-1*(*gk448*) appeared to be outcompeted by wild-type at later cycles (Fig. 10), although the data is not conclusive. This experiment should be repeated for confirmation.

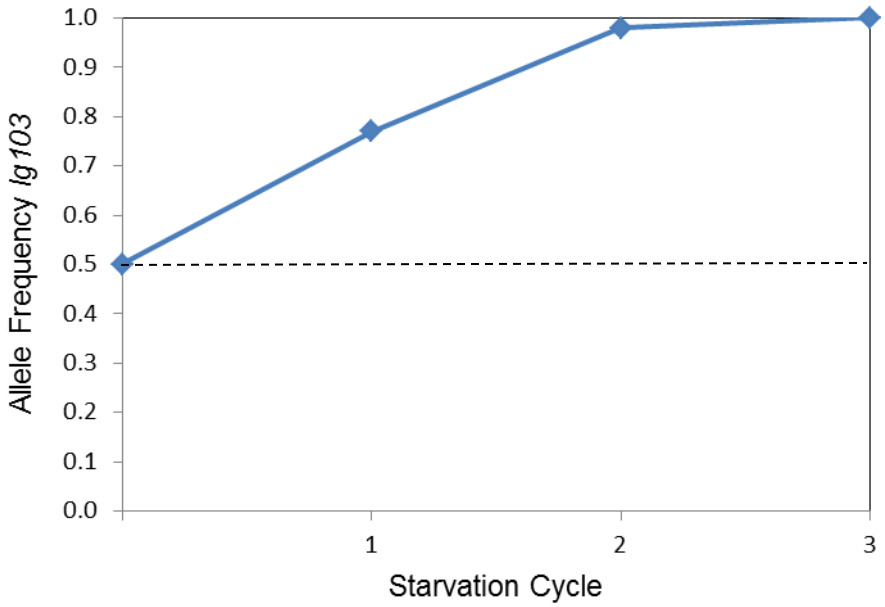


Figure 9. Laboratory natural selection (competition) assay with starvation cycles mimicking conditions in the wild in *pdr-1*(*lg103*). Each starvation cycle represents two reproductive generations. Data presented is from two biological samples. These experiments were conducted with the aid of Susan Angeli, Lithgow laboratory. N=50.

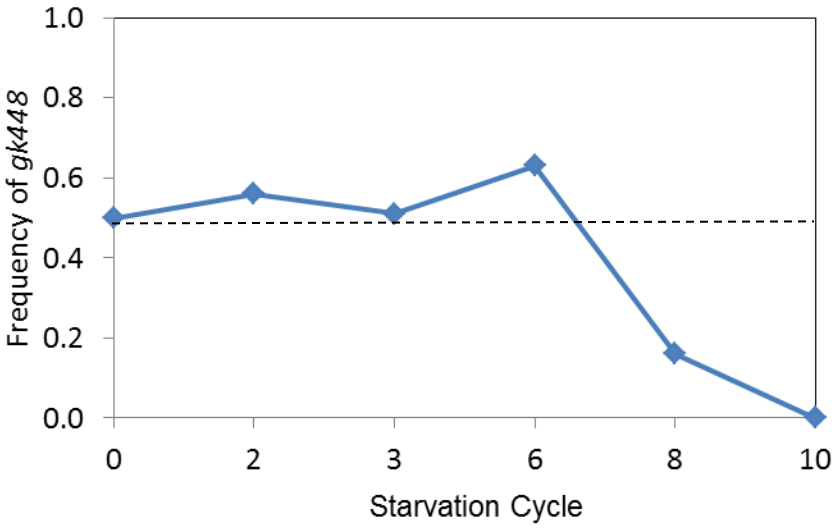


Figure 10. Laboratory natural selection (competition) assay with starvation cycles in pdr-1(*gk448*). Each starvation cycle represents two reproductive generations. Data presented is from two biological samples. These experiments were conducted with the aid of Susan Angeli, Lithgow laboratory. N=50

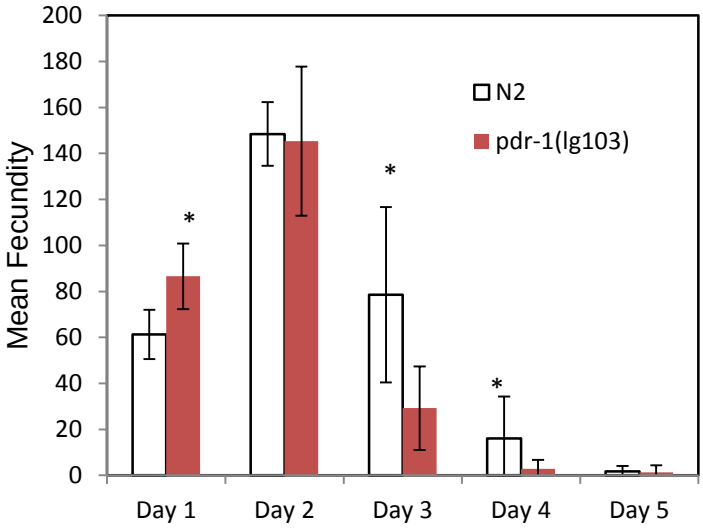


Figure 11. Nutritional stress (Starvation) *pdr-1*(*lg103*) versus wild-type increased fecundity on Day 1. *p<0.05 logrank test. Preliminatry data of one biological replicate. N=10.

After we viewed the natural selection data under nutritional stress conditions, we wanted to see the effect of this stressful condition on broodsize. (Figure 11) On Day 1, the *pdr-1*(*lg103)* worms showed increased fecundity compared to the wild-type worms. However, by Day 3 to Day 5, the wild-type worms were more fecund compared to *pdr-1*(*lg103*). In terms of the early increased reproduction on Day 1, the starvation effect on brood size was similar to early reproductive fitness showed in Figure 1. Preliminary results comparing non starved vs. starved brood size with wild-type and *pdr-1*(*lg103*) showed under starvation conditions, the mutant had increased fecundity on Day 1. Basically, under this stressful condition, they showed to lay more eggs. (Data not shown, non-starvation vs. starvation broodsize)

To determine if the effect of heat stress was different between the wild-type and the *pdr-1* mutants, a thermotolerance assay was conducted.

As shown in Figures 12 and 13, *pdr-1*(*lg103*) and *pdr-1*(*gk448*) showed no significant increase in thermotolerance versus N2 (wild-type) at the maximum survival.

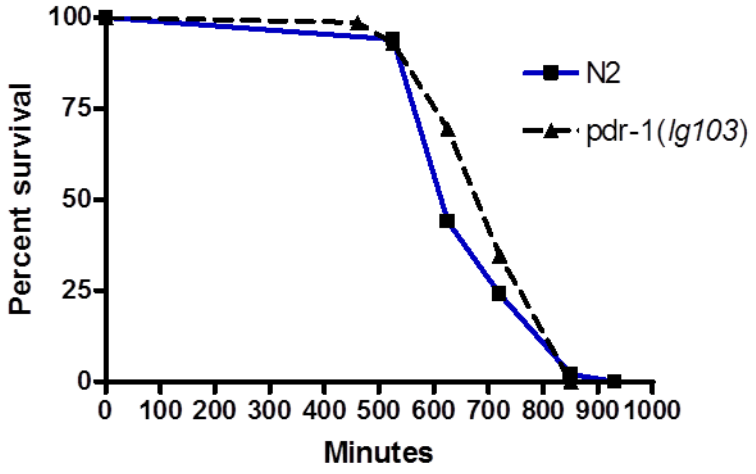


Figure 12. Thermotolerance assay of N2 vs. *pdr-1*(*lg103*) at 35°C. Data shows pdr-1(*lg103*) appears slightly more tolerant to heat stress; however the log-rank test is not significant. Data shows only one biological sample.

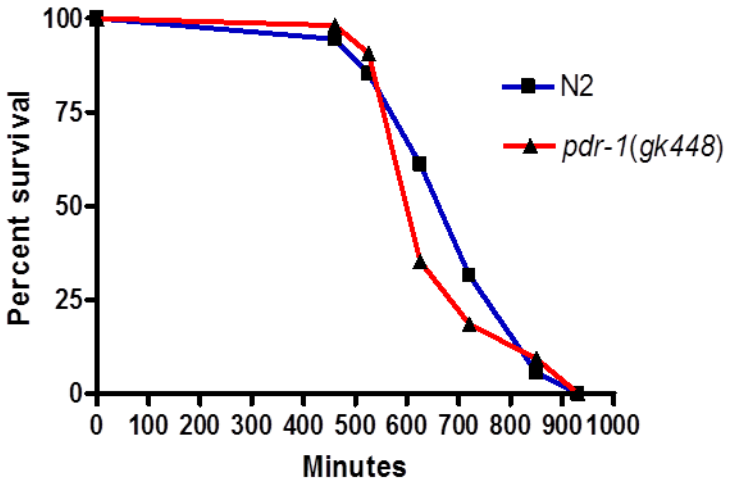
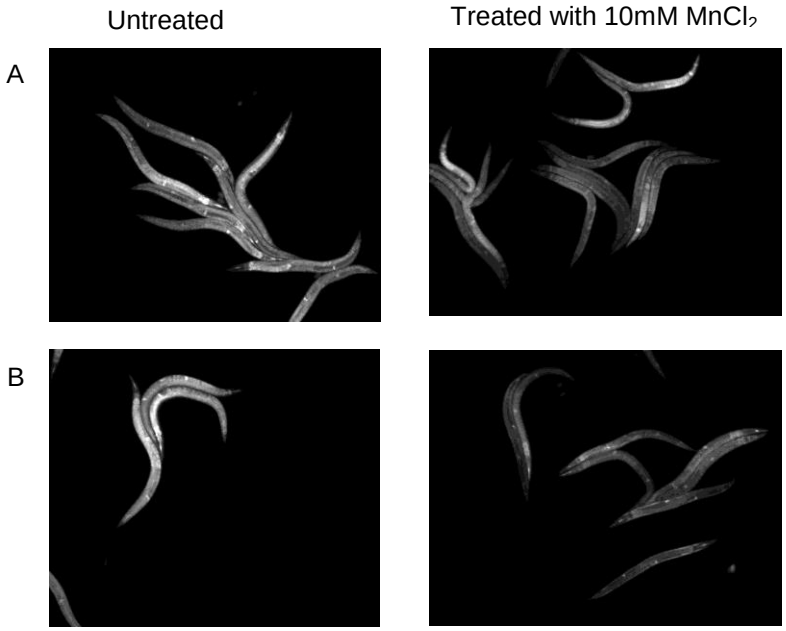


Figure 13 Thermotolerance assay of N2 and pdr-1(*gk448*) during 35°C heat stress. Data shows *pdr-1*(gk448) slightly less heat tolerant. Log-rank test not significant.

Do changes in fecundity in the *pdr-1* mutants track with effects on mitochondrial function?

It has been reported the loss of E3 ligase function has an effect on mitophagy and therefore mitochondrial function (Tanaka, 2010) We wished to determine whether the mutation in *pdr-1* had an effect that might track with fecundity. We exposed worms to TMRM to determine functional mitochondrial membrane potential. *Pdr-1*(*lg103*) was found to have increased TMRM fluorescence, suggesting improved mitochondrial function versus N2 (Figure 14). Although the reasons for this are not clear, this might explain increased fecundity of the mutant (increased mitochondrial function necessary for reproduction). Additionally, when worms were treated with 10mM $MnCl_2$, as expected both N2 and *pdr-1*(*lg103*) showed loss of TMRM fluorescence (lower MMP), however decreases were less in the *pdr-1* mutant (Figure 14).



A) Wild type worms exposed 24hr to TMRM and 10mM $MnCl_2$. Viewed at 20x with fluorescent microscopy.
B) *pdr-1*(*lg103*) exposed 24hr to TMRM and 10mM $MnCl_2$. Viewed at 20x with fluorescent microscopy.

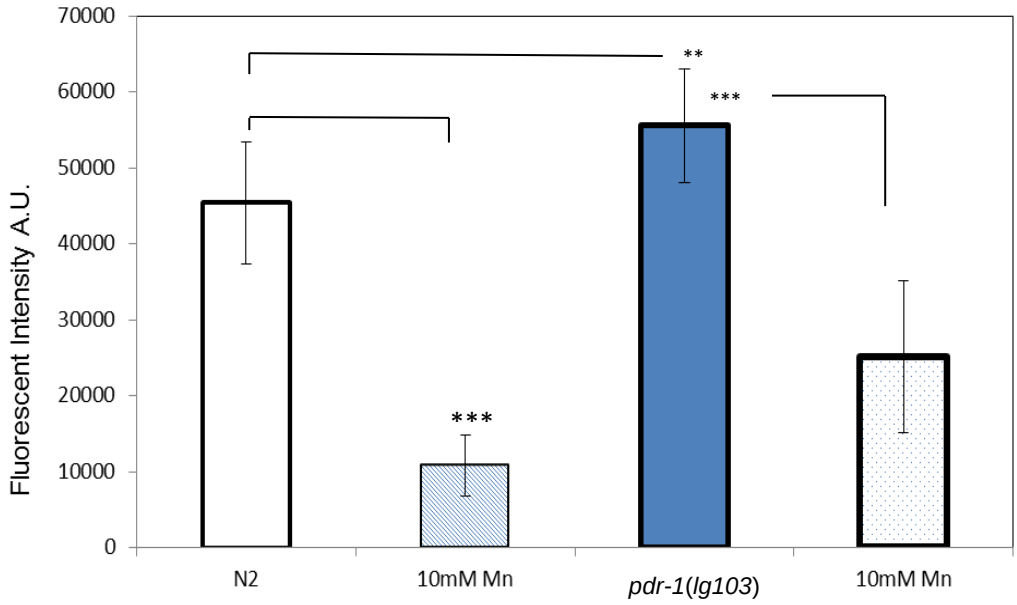
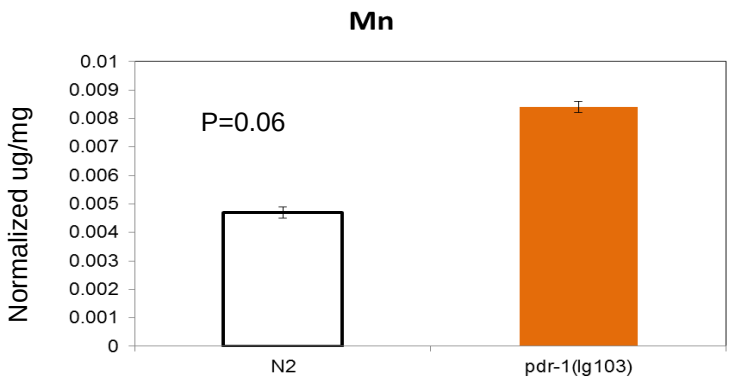


Figure 14. *pdr-1*(*lg103*) displays increased mitochondrial membrane potential, both with and without $MnCl_2$ treatment. TMRE was used to determine differences in MMP between N2 and *pdr-1*(*lg103*). Data shown in biological triplicates. On the left represents N2 with and without 10mM $MnCl_2$ and on the right represent *pdr-1*(*lg103*) same conditions. Student t-test, **p<0.005 and p<0.0005***. This data represents the fluorescent worm images in A) and B).

Do endogenous Mn levels effect reproductive fitness?

Lin et al, 2006, had reported that supplementation with Mn increased total broodsize in wild-type animals by 16%. Additionally, they showed the supplementation also decreased development time or α-time. (Line et al, 2006) This suggested that an increase in endogenous Mn levels in *pdr-1*(*lg103*) larvae as a consequence of loss of parkin E3 ligase activity and elevated DMT-1 levels could be a possible mechanism for the increased reproductive fitness compared to N2 (wild-type).

A



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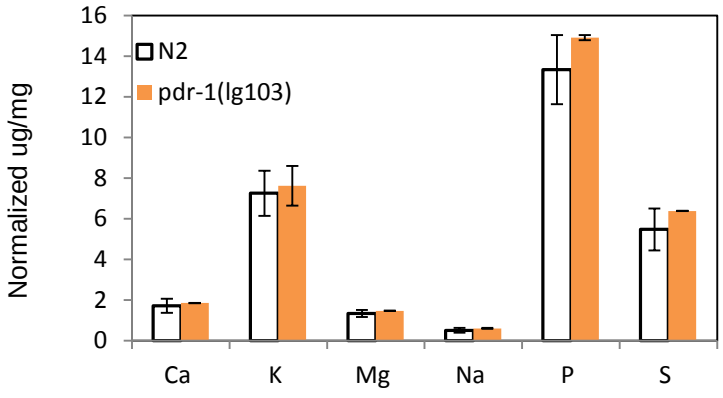


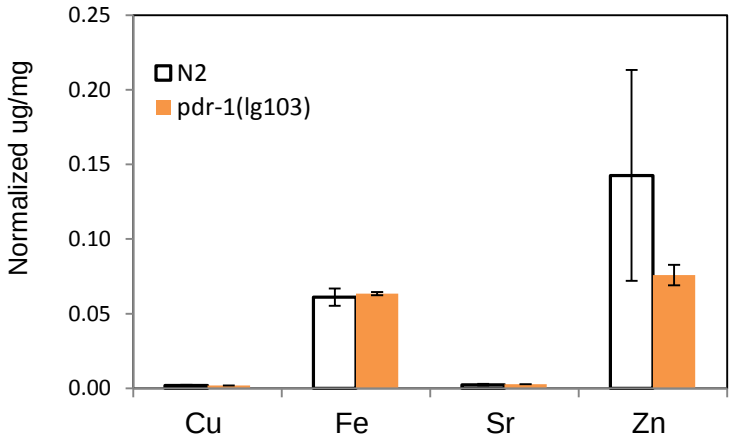


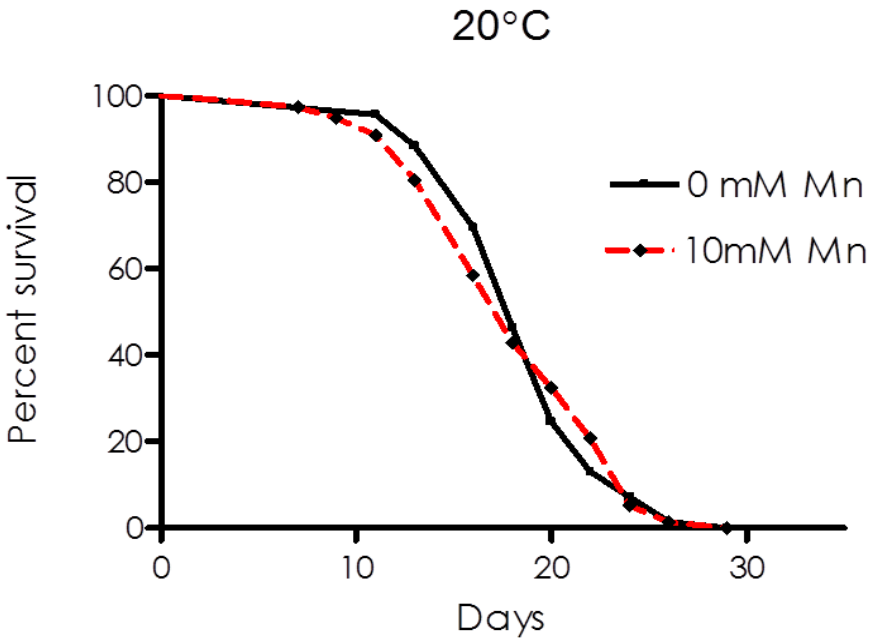
Figure 15. Endogenous element levels in larval *pdr-1*(*lg103*) and N2 worms. (A) Mn levels only, data not significant. (B) Elements with higher endogenous values. (C) Elements with lower endogenous values. Data represents technical triplicates. Other elements analyzed were below detection level.

While results from elemental analysis of *pdr-1* compared to N2 for levels of Mn suggested a trend towards an increase in Mn level, the data was not conclusive, so currently no conclusions can be drawn as to whether *pdr-1* mutants displayed increased endogenous Mn levels which may influence fecundity (Figure 15).

With long term exposure to manganese known to be a risk factor for PD, we assessed how supplementation of Mn had on the lifespan of the *pdr-1* mutant compared to wild-type strain. (Figure 16). While Mn had no impact on maximal lifespan of N2, it did increase maximal lifespan in the *pdr-1*(*lg103*).

The Lithgow laboratory had previously performed dose-response curves of Mn supplementation to determine toxicity. While higher dosages proved toxic, 10 mM $MnCl_2$ demonstrated a putative protective effect in terms of survival. This was therefore the concentrations used in all of our subsequent studies. Lifespan survival curves were conducted with and without supplementation of 10mM $MnCl_2$ to determine the effects of exogenous manganese. The wild-type shows no significant difference between supplementation and without supplementation of $MnCl_2$. In figure 16 (B), *pdr-1*(*lg103*) $MnCl_2$ supplementation shows a significant increase in median lifespan.

A



B

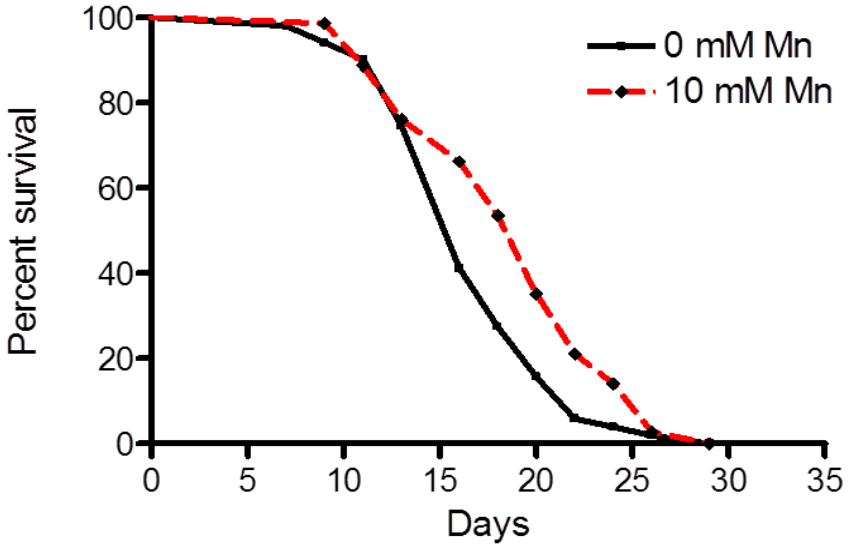


Figure 16. Lifespan survival curve data following supplementation of worms with 10 mM $MnCl_2$ (Susan Angeli, Lithgow and Andersen laboratories). A) N2 shows no significant difference in maximum lifespan between $MnCl_2$ and treatment. Logrank test p=0.9496. B) *pdr-1*(*lg103*) supplemented with 10 mM $MnCl_2$ extends median survival of the mutant. Logrank test *p<0.05 (0.0079).

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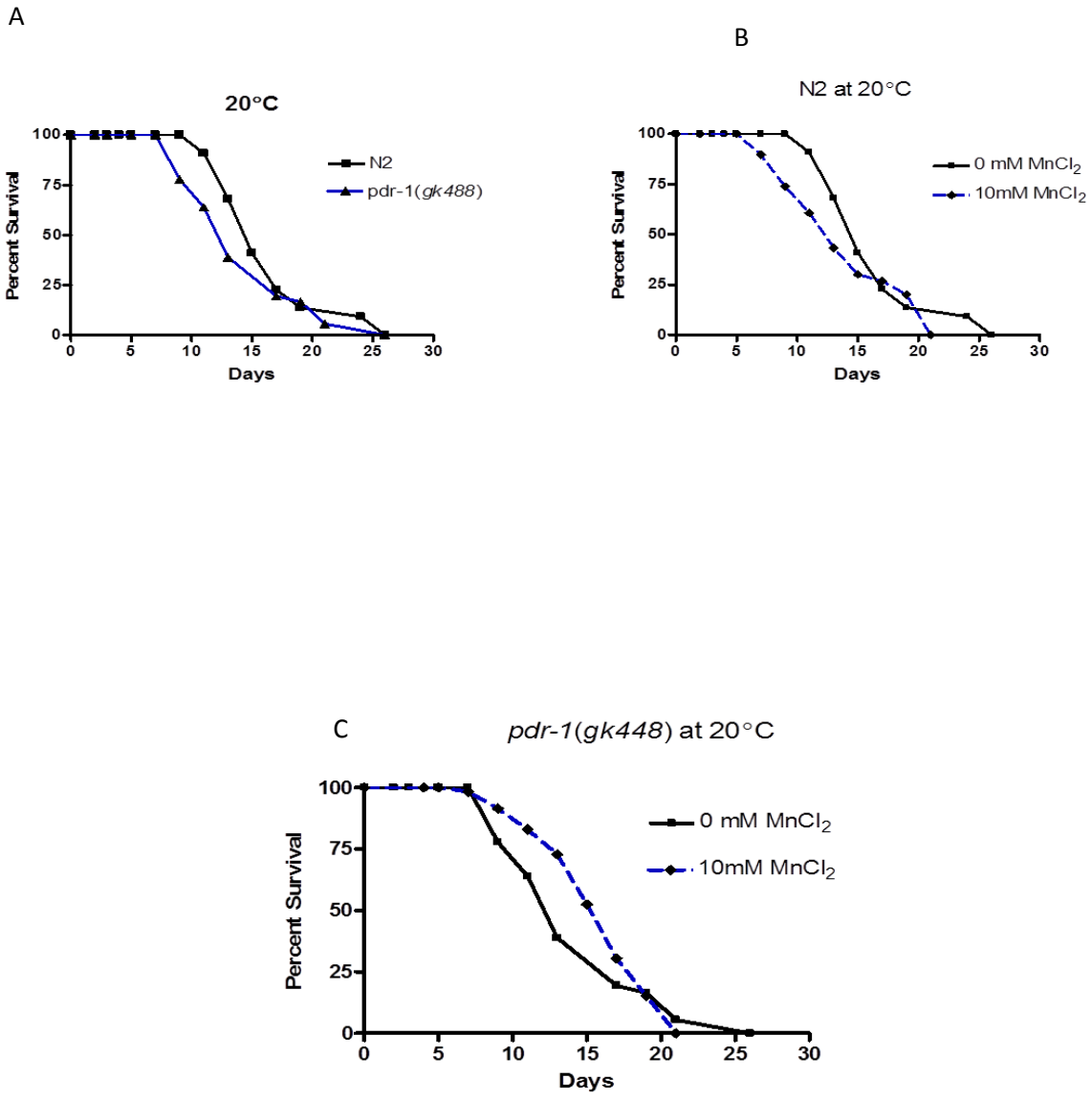

Figure 17. Lifespan survival curves with and without supplementation of $MnCl_2$. A) Data shows at median lifespan *pdr-1*(*gk448*) had decreased survival. Log-rank test shows no significance between the two treatments. B) N2 +/- Mn had significant difference compared to *pdr-1*(*gk448*) +/- Mn. C) *pdr-1*(*gk448*) +/- Mn. Logrank test p=0.392.

I also wanted to determine lifespan with and without manganese exposure for the null allele, *pdr-1*(*gk448*). The lifespan survival curve for *pdr-1*(*gk448*), demonstrated a similar lifespan curve as *pdr-1*(*lg103*) in that both had decreased median lifespan, but there was no difference in the maximum lifespan compared to wild-type. We also exposed *pdr-1*(*gk448*) worms to 10mM $MnCl_2$ to determine the effect of supplementation. *Pdr-1*(gk448) demonstrated a different effect as a consequence of Mn supplementation compared to *pdr-1*(*lg103*) in that maximum survival was 21 days compared to 28 days, respectively.

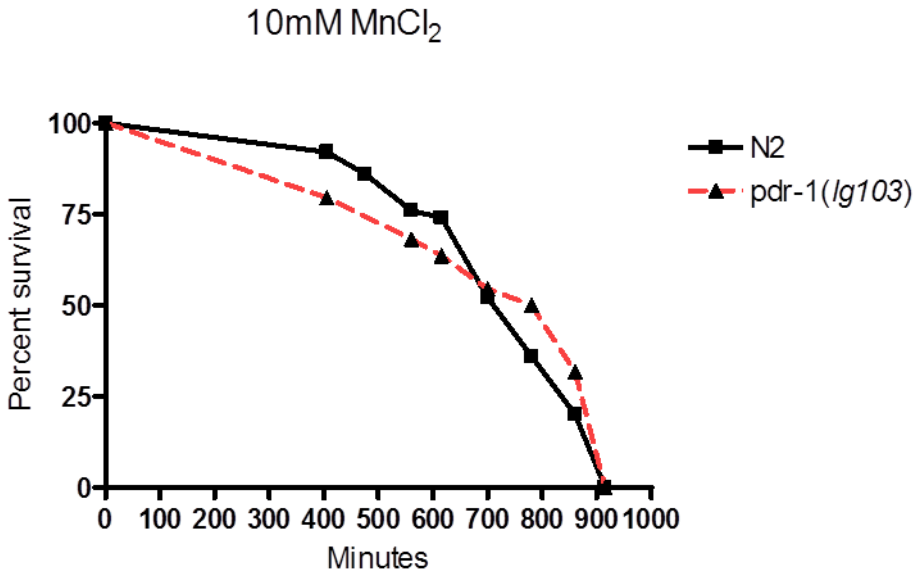

Figure 17. Supplementation with 10 mM $MnCl_2$ showed no differences in thermotolerance between N2 and pdr-1(*lg103*).

Thermotolerance is an assay used to evaluate the response to heat stress. We wanted to determine worms under two stressful conditions, exposure to heat (35°C) coupled with exposure to 10 mM $MnCl_2$.

We wanted to determine if manganese exposure would have a more detrimental effect when coupled with heat stress. As shown in Figure 17 and 18, there was no significant difference between the wild-type and *pdr-1*(*lg103*) or *pdr-1*(*gk448*) under these conditions.

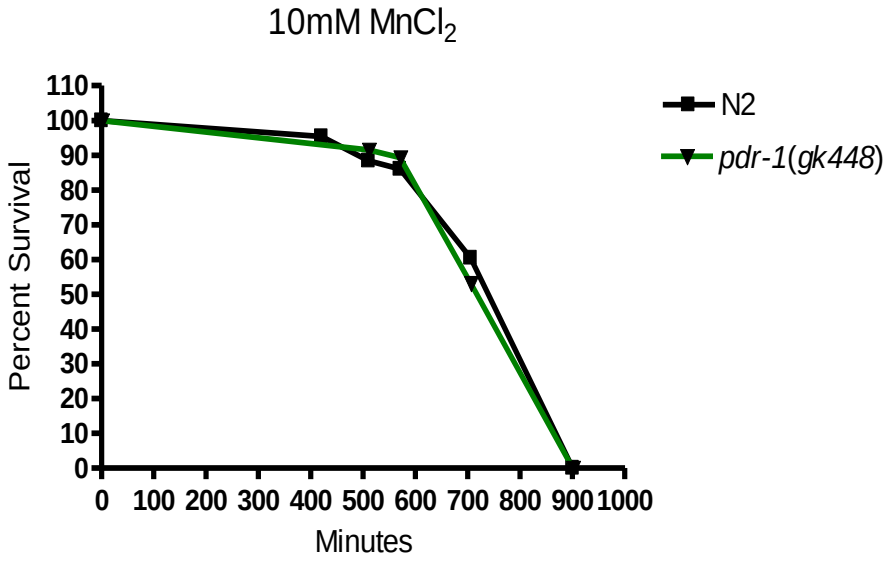

Figure 18. Wild-type and *pdr-1*(*gk448*) showed similar heat stress response. Median survival showed no significant difference

Discussion

pdr-1(lg103) confers increased reproductive fitness

In this thesis, I demonstrated that mutations in the *pdr-1* gene in *C. elegans* akin to those associated with human PD, while resulting in reduced lifespan (late-life affect), are associated with early reproductive fitness. A combination of fertility assays to determine total fecundity, time of first egg lay, and laboratory natural selection experiments suggested that the worm

PARKIN mutant displays antagonistic pleiotropy resulting in early reproductive advantage. This suggests a mechanism by which a late-deleterious allele can be maintained in a population. Based on the brood size studies, *pdr-1(lg103)* demonstrated a phenotype of increased early reproductive fecundity, an important component of Darwinian fitness. While the null allele, *pdr-1*(*gk448*), exhibited an overall total increase fecundity compared to wild-type, the phenotype was different than *pdr-1*(*lg103)*. In both cases, the brood size differences were found not be dependent on the time of first egg lay.

pdr-1 mutant worms median lifespan increased with Mn treatment

Both *pdr-1*(*lg103*) and *pdr-1*(*gk448*) showed a decrease in median lifespan compared to wild-type. However, with supplementation with 10m M $MnCl_2$, the median lifespan of both showed an increase when compared to wild type. With DMT-1 known to be substrate for human parkin, (Roth et al, 2010) the increased median lifespan following manganese supplementation could be due to affects on Mn metabolism that have a beneficial effect on the worms.

It would be interesting to conduct lifespan survival curves on other alleles of the *pdr-1* gene to determine if the phenotypes are different from *pdr-1*(*lg103*). Other deletion alleles have been requested so these experiments can be conducted.

pdr-1(lg103) acts differently than pdr-1(gk448) under heat stress

Frequently lifespan correlates with a stress tolerance phenotype (Walker and Lithgow, 2003). Stress tolerance can be assessed rapidly and therefore we tested for such phenotypes in the *pdr-1*(*lg103*) and *pdr-1*(*gk448*) mutants. We conducted a thermotolerance assay where we shifted the worms from their standard growth temperature of 20°C to 35°C to determine whether mutations in *pdr-1* resulted in heat stress sensitivity. The *pdr-1*(*lg103*) median survival was

increased compared to wild-type under heat stress; whereas, *pdr-1*(*gk448*) showed thermosensitivity.

With supplementation of worms with 10 mM $MnCl_2$ under heat stress, the wild-type and *pdr-1*(*1g103*) displayed similar median survival. Our analysis indicated that *pdr-1*(*lg103*), which results in a partial loss of parkin function, might provide protection against stressful conditions due to some level of residual protein activity. *Pdr-1*(*gk448*) under both heat stress and 10 mM MnCl2 supplementation showed no difference in median or maximum survival when compared to wild-type. This could suggest Mn metabolism increases tolerance to heat stress.

pdr-1(lg103) suggested to confer evolutionary fitness

Antagonistic pleiotropy, described by Williams (1957) and Hamilton (1966), postulated a theory of aging where some alleles act detrimentally at late age but are beneficial at an early age. Consequently, the positive effects of the gene trait would outweigh the negative effects. If pleiotropic alleles are common, this could explain the prevalence of disease-causing alleles in the population. Several disease-causing alleles have been suggested to follow antagonist pleiotropy, including those for Huntington's disease (HD), cystic fibrosis (CF), sickle cell anemia and beta-thalassemia (Carter and Nguyen, 2011). A cohort of HD patients demonstrated a larger family size compared to families without the HD disease allele. The patients had 39% increased offspring compared with unaffected families (Carter and Nguyen, 2011), which would suggest that the HD allele gives rise to an increase in fitness by increase fecundity despite the deleterious effect of the allele later in life. The consequence of a genetic mutation in a population in terms of the evolutionary process depends on both the frequency distribution and the effects on fitness. This may help explain why some diseases still are in the population.

With the laboratory natural selection experiments, we mixed both the wild-type with the *pdr-1* mutants in the same culture plate competing for the same resources. These populations were

cultured over several generations. We found *pdr-1*(*lg103*) displayed an increased fitness over the wild-type including in starvation conditions mimicking those in the wild. In non-starvation conditions, he wild-type worms became extinct by the twelfth generation whereas under starvation conditions, the wild-type became extinct by the sixth generation. A few factors could contribute to more rapid selection under starvation conditions for pdr-1(lg103). It may be that the mutants are laying eggs faster under deprivation of resources or that the developmental time decreases under these conditions. In fact, in preliminary data (not shown) from a fertility assay performed under starvation conditions, *pdr-1*(*lg103*) appeared to lay more eggs on the first day. These results suggest that the trade-off of early fitness is possibly more important rather than lifetime fertility. The data suggests that increased fecundity confers selective advantage, consistent with the pleiotropic effect. Thus, a mutation in a gene that causes familial Parkinson's disease might also be a pleiotropic gene.

In contrast, the null allele, *pdr-1*(*gk448*) displayed a decrease advantage compared with the wild-type in both the laboratory natural selection and starvation natural selection assay. Since, we are selecting the first eggs laid within the population, this could result in a competitive advantage. The broodsize results indicated *pdr-1*(*gk448*) mean fecundity was similar to wild-type. However, as previously discussed, pdr-1(gk448) showed an increased fecundity at day 3. I would speculate that if the natural selection assays differed in the initial day of eggs selected, the wild-type might become extinct rather *pdr-1*(*gk448*). To confirm this scenario, the experiments will be repeated.

Mechanism(s) underlying increased reproductive fitness (Darwinian fitness) in a single allele

Currently, it is unclear why mutations in *pdr-1(lg103)* increase reproductive fitness. Could Manganese (Mn) metabolism play a role in the mechanism of increased reproductive fitness? We hypothesized that the divalent metal transporter (DMT-1) involved in Mn metabolism could be modulating early reproductive fitness (Darwinian fitness) in the worm. Manganese is abundant in the earth's crust and is an essential trace element for many physiological processes, although high levels can become toxic. (Au et al, 2009) Environmental risk factors, including metal exposure, have been postulated to play an important role in PD etiology. It has been shown epidemiologically that families living in rural farm areas or those that are exposed to chronic atmospheric metals including manganese have higher incidents of PD or PD-like symptoms. The name "manganism" has been given to patients whose occupation entails the mining of manganese and in welders chronic exposure to the metal resulting in PD-like symptoms including rigidity, tremors and bradykinesia (Au et al, 2009). Neonatal mice that have been exposed early in life to elevated iron (Fe), another metal transported into the cell via DMT-1, are more susceptible to environmental exposure from chemical toxins including paraquat in older age mice, accelerating SNc dopaminergic neuron loss. It could be speculated other cations; such as, manganese could result in similar results (Peng et al, 2010)

Could there be a link to the expression levels of DMT-1 and reproduction by an unknown pathway? In order to test this, we analyzed endogenous elemental values in L2 and L4 larvae and in day 1 adults via Inductively Coupled Plasma (ICP). L2s displayed the most significance change in Mn and Fe levels. Interestingly, in the first sample set, we saw a significant change in Mn, but there was no significant difference in Fe levels. The second sample set displayed a significant change in the Fe level between wild-type and *pdr-1*(*lg103*) but not Mn.

DMT-1 is highly conserved and three *C. elegans* orthologs, *smf-1*, *smf-2*, and *smf-3,* have been identified. Further experimentation is needed to verify whether DMT-1 or endogenous elemental values effect the reproductive process. This would involve determining elemental values of C. *elegans* homologs *smf-1*, *smf-1*, and *smf-3* mutants both individually and as a triple mutant. This should provide answers as to which metal transporter regulates endogenous elemental content and if manganese levels are increased or decreased with other elements. This information could identify whether a particular transporter in the worm is associated with transport of a specific cation.. Broodsize could also be assessed for each of these homologs to determine their fecundity to see if a particular cation is associated with reproductive fitness.

Based on Lin et al, broodsize was increased by 16% in wild type worms following supplementation with 0.5 mM $MnS0_{4,}$ and, additionally, development time was decreased (Lin et al, 2006). I was not able to recapitulate the same results (preliminary data not shown), in fact, we observed decreased broodsize and mean fecundity in the presence of Mn supplementation. However, I used $MnCl_2$ supplementation in my initial experiments. The difference the anion in $MnSO_4$ and $MnCl_2$ might be a factor in the worm's ability to uptake one or the other. A repeat of the broodsize experiments using $MnSO_4$ is currently underway.

Mitochondrial affects of parkin mutation on mitophagy may play a role in the mechanism underlying Darwinian fitness

Mitochondria are the powerhouse of the cell. Decreases in mitochondrial ATP production can be caused by inhibition of the electron transport chain, also resulting in the production of toxic reactive oxygen species (ROS) (Exner et al, 2012) Loss of parkin function results in reduced mitophagy and increased levels of (damaged) mitochondrial within the cell. Mitochondrial biogenesis is also decreased with the loss of parkin function (Dawson and Dawson, 2010).

Could a reduction in mitochondrial turnover during reproduction, a time of high metabolic demand, be beneficial or contribute to the increase in early fitness? In the early life phase before significant mitochondrial damage has occurred, could this result in a higher number of functional mitochondria?

The *PINK1 gene,* another loss of function mutant that causes juvenile autosomal recessive PD, encodes a mitochondrially targeted Ser/Thr kinase (Whitworth and Pallanck, 2009). It has been shown that *PINK1* signals PARKIN to translocate to misfolded proteins in particular damaged mitochondria for degradation or mitophagy. This involves moving from the cytoplasm to the outer membrane of the mitochondria. Whitworth and Pallanck, showed in flies that *PINK1* acts upstream to PARKIN in a common pathway which regulates mitochondrial fission-fusion and mitophagy (Whitworth and Pallanck, 2010) Evidence using *Drosophila PINK1* and PARKIN mutants demonstrate that *PINK1* acts upstream in that overexpression of PARKIN has the ability to compensate for the loss of *PINK1*, but not the reverse. Additionally, PINK1 overexpression was suppressed by a PARKIN null mutation (Whitworth and Pallanck, 2009).

Based on this information, we could extrapolate that increased numbers of mitochondria during periods of high metabolic demand such as early reproduction is beneficial, however, increased metal exposure, such as manganese could reduce this activity. PARKIN has been confirmed by others in mammalian systems to selective drive mitochondrial turnover including depolarized or damaged mitochondria (Peng et al, 2010). We observed an increase in TMRM expression indicating an increase in membrane potential in *pdr-1*(*lg103*) compared to wild-type, which may indicate an accumulation of partially functional mitochondria. *pdr-1*(*lg103*) treated with 10 mM $MnCl_2$ also displayed an increase in membrane potential versus wildtype,, although in Mn-treated *pdr-1*(*lg103*) mitochondrial membrane potential is decreased when compared to untreated. Recently, PINK has been demonstrated to phosphorylate mitofusin 2 within the outer mitochondrial membrane in response to depolarization, allowing recruitment of parkin (Science

2013). Our data showed an increase in Δψm which could suggest low levels of PINK1 thus low recruitment of parkin to damaged mitochondria. However, as parkin is mutated, even if recruited to the mitochondria, it would not result in induction of mitophagy. Increased TMRM expression of the *pdr-1*(*lg103*) may rather imply that there are a higher number of mitochondria that may be damaged but not completely dysfunctional. This is particularly true during the developmental period where high metabolic demands are present and where there has been less time for damage to occur compared to in aged animals.

To confirm these results and to determine the ratio of active versus inactive mitochondria we plan to expose the worms to JC-1(5,5',66'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolycarbocyanide iodide), a stain that targets mitochondria activity (Iser et al, 2005). By understanding the mechanism of the quality control system of damaged mitochondria, we might be able to link the machinery of this system to increased Darwinian fitness in *C. elegans.*

Conclusions

PARKIN plays an important role in the pathogenesis of PD. PARKIN mutations result in juvenile familial PD, but parkin's function in mitophagy may have important implications for sporadic pathology caused by combined genetic alterations and environmental factors. Although parkin mutations result in reduced lifespan, they are maintained within the human population. This suggests that there may be antagonistic pleiotropy in which mutations confer benefits early in the lifespan while causing detrimental effects with age. To test this possibility, mutations in specific *pdr-1* alleles in *C. elegans* were assessed. We demonstrate that the partial loss of function in the *pdr-1*(*lg103)* mutant demonstrated a phenotype suggested to confer a selective advantage on reproduction whereas, the complete loss of E3 ligase function allele, *pdr-1*(*gk448)*, displayed a different phenotype. My work suggests that particular mutations in the PARK2 gene may have a pleiotropic affect on the population. Previous data suggests that parkin plays a role in mitophagy of dysfunction mitochondria and in mitochondrial biogenesis. It may be that increased numbers of mitochondria due to reduced mitochondrial turnover during the metabolically active developmental period coupled with lower levels of damage may result in increased beneficial affects.. This was found to be enhanced by Mn supplementation. This work demonstrates that ongoing research is needed to further understand how mutations in genes causing neurological diseases may be maintained in the population and specifically whether this involves antagonistic pleiotropy.

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