THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF BIOLOGY

OXIDATIVE STRESS ALTERS THE FLIGHT MUSCLE LIPIDOME OF A LOWLAND BUTTERFLY

JULIANNE PEKNY
SPRING 2015

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree
in Biology
with honors in Biology

Reviewed and approved* by the following:

James Marden
Professor of Biology
Thesis Supervisor

Katriona Shea
Alumni Professor in the Biological Sciences
Honors Adviser

* Signatures are on file in the Schreyer Honors College.

ABSTRACT

Among conflicting theories of aging, both the oxidative stress hypothesis and rate of living theory have been strongly supported for decades. Here, we seek to illustrate that these two schools of thought may work in conjunction, with the oxidative stress hypothesis providing the biochemical basis for the rate of living theory. Within a lowland butterfly metapopulation, we observed the differences in the global lipid composition (lipidome) between individuals that were rested, subjected to prolonged flight, and injected with an oxidative stress-causing agent. Between these treatments we found nonrandom discrepancies in the abundance of various specific lipids that serve as biomarkers for oxidative damage. The flight performance of individuals with specific *Sdhd* alleles, a polymorphism previously documented as having an association with tracheal development, oxygen conductance, and colonization success, was observed, with those containing the M allele boasting the highest flight metabolic rates, as well as the most elevated abundances in those lipids associated with oxidative damage. These findings suggest that the observed decline in flight performance (i.e. aging) is highly correlated with those lipids related to oxidative damage, illustrating how the two main theories of aging may work in conjunction. Further, our results prompt future research into how the *Sdhd* polymorphism influences the flight strategy of Glanville fritillaries (*Melitaea cinxia*) in relation to acute and chronic oxidative stress, shaping individual behavior and metapopulation dynamics.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

I'd first like to thank Dr. Jim Marden, who has been an incredibly kind and patient mentor to me over the past 4 years. Without him, this thesis and much of my success so far as a scientist would not have been possible. A huge thank you to the rest of the Marden lab, especially Dr. Howard Fescemeyer, Dr. Emily Hornett, and Dr. Ruud Schilder for all their help, guidance, and abundance of enthusiasm. Thanks also to Dr. Chris Wheat, the Carney family, and all our other friends in Catalonia for their hospitality and helpfulness abroad. To Dr. Phil Smith, thank you so much for all your help in lipidomics, as well as your patience for teaching me to interpret novel data. Finally, thank you to Dr. Katriona Shea for both her time and support while providing me with feedback during the writing process. Work for this thesis was supported by Dr. Marden's NSF grant IOS-1354667.

Chapter 1

Introduction

Despite modern advances in science and technology, the exact mechanics of the aging process are yet unresolved. Among several emergent and conflicting theories of aging [1-5], support for both the rate of living theory and the oxidative stress hypothesis has persisted for decades [6-17]. Here, we investigate the possibility of a relationship between these two ideas through lipid profiling following oxidative stress.

Two postulates define the rate of living theory: (i) the life span of an individual is dependent upon the rate its energy is utilized (metabolic rate), as well as (ii) the amount of energy consumed during its adult life [18]. Similarly, the oxidative stress hypothesis (originally dubbed the free radical hypothesis [19]) proposes that reactive oxygen species (ROS) stimulate molecular oxidative damage, consequently catalyzing aging, characterized by the degradation of physiological function. These two schools of thought are closely linked by the principle that oxidative stress may provide the biochemical basis for the rate of living theory: aerobic cells generate ROS [20] and thus increased energy expenditure may be correlated with the magnitude of potential damage caused by these compounds.

When metabolic rate is sustained at a high level over a prolonged period of time, there is the possibility that the oxygen supply carried to cells will not be sufficient to comply with the increased demand, leading to ischemia [21]. In oxygen-deprived cells, cellular respiration slows, leading to rapid and irreparable damage, as the cell's natural defense against the creation of ROS is overcome [22]. To cease injury, oxygen-carrying blood must be quickly reestablished to the

ischemic area; however, this reestablishment itself can cause further damage in the phenomenon termed “ischemia reperfusion injury” (IRI) [21,23]. Through the reintroduction of blood to compromised ischemic areas, IRI has the potential to not only heighten local damage but to cause systemic injury, as well [24, 25].

Among the many effects of IRI are the generation of ROS, mitochondrial dysfunction and swelling, lipid peroxidation, and cell membrane damage [23], making it an acute illustration of aging based on the rate of living theory and oxidative stress hypothesis in conjunction. In a 2013 study by Marden and colleagues investigating genetic variation in tracheal development and oxygen conductance to flight muscle cells in Glanville fritillary (*Melitaea cinxia*) butterflies, it was found that mitochondrial swelling was more severe in those individuals which utilized their flight muscles more in relation to the oxygen supplied to them [26]. As mitochondria are a source of free radicals during reperfusion, they are likely targets for consequent lipid peroxidation [27,28]. We hypothesized that the increased mitochondrial swelling observed in Glanville fritillaries may indicate reperfusion and its consequent disruption of the mitochondrial membrane, occurring due to forced flight and therefore augmented metabolic rate.

Peroxidation products such as these and other lipid biomarkers have been identified across a variety of taxa, including both humans and insects [29]. This is made possible through the rapidly growing field of lipidomics, in which modern lipid extraction and mass spectrometry techniques are used together to identify and characterize nearly the entire spectrum of cellular lipids, many of which play a critical role in biological function [30]. As the field of lipidomics continues its growth, novel lipid profiles are rapidly discovered in both model [29,31-33] and non-model species [34], increasing the reach and potential of targeting lipids for investigation across multiple disciplines.

In this study, we examined a lowland butterfly, the Glanville fritillary (*Melitaea cinxia*). This species has become a model system for metapopulation dynamics [35], processes that are influenced strongly by the dispersal and colonization of small habitat patches in a heterogeneous landscape. For Glanville fritillaries, only dry meadows with rock outcrops support the larval host plant, causing flight distance for dispersal between suitable habitats to be quite variable[36]. Ecological genomic studies have shown that genetic variation affecting flight metabolic rate is associated with large variation in dispersal in the field, and differential representation and success of different genotypes in newly colonized patches and patches of different size [37-40].

One of these genetic polymorphisms comprises alleles in a regulatory region of the Kreb's cycle gene *succinate dehydrogenase d* (*Sdhd*), which physiological studies revealed encodes one of the four subunits of the enzyme succinate dehydrogenase (SDH; 41). Specifically in Glanville fritillaries, it was found that one *Sdhd* allele in particular coded for reduced SDH activity, increased tracheal development, and better flight metabolic performance [26]. Conversely, in butterflies with less tracheal development, flight metabolic performance declined sharply over time and swollen and disrupted mitochondria were observed within insect flight muscles.

Here, we extend that physiological characterization by determining how short bouts of forced flight affect the global lipid profile of Glanville fritillaries with regard to their *Sdhd* genotype and flight performance. Through the application of three separate treatments prompting a resting metabolic rate, forced flight, and chemically-induced oxidative stress, we investigated the lipid profile of each individual and, as a result, were able to identify potential products of lipid peroxidation in a species not yet examined through a lipidomic lens. In this way, we were able to speculate on the influence of oxidative stress, ROS, and IRI on observed

differences in flight performance and elevated metabolic rate between physiologically distinct individuals.

Chapter 2

Materials and Methods

Collection

Our sample consisted of wild-caught adult Glanville fritillary (*Melitaea cinxia*) butterflies, collected at the beginning of the flying season outside Tavertet, Viladrau, and Rupit, Catalunya (Spain). All individuals appeared to have recently emerged as adults (i.e. fresh colors and scales; no substantial wing wear). They were housed inside mesh cages in a cool, shaded area where they did not fly and were fed a honey water solution twice daily.

Treatment

After one day of rest following capture, butterflies were subjected to a flight test. Each individual was stimulated to fly as continuously as possible over a three-minute period in a 1L jar. Within the jar, CO_2-free air was passed at 0.95 L min^{-1} and either a normoxic or mildly hypoxic (14% oxygen-over-nitrogen mix) environment was maintained over the course of the flight. The order of normoxia or hypoxia first was randomized, with the second flight held the following day in the alternative gas concentration. These data were first analyzed using a cross-over analysis [42] in which butterfly was treated as a random factor, with day, gas, and day*gas as factors. We then included *Sdhd* genotype in the model as an additional factor and also analyzed the data separately for each gas treatment while accounting for history (order of gas

treatments). Immediately following the second flight, each individual was flash frozen in liquid nitrogen.

A second group of randomly chosen butterflies did not undergo flight tests, but rather were rested for 44 hours in cool shade (no flying). Six of these individuals had 2uL of insect saline containing 30mM paraquat injected into the lateral thorax, and 4 hours later they were flash frozen. The remaining individuals were rested in cool, shaded cages and flash frozen after the two-day period. After freezing, all butterflies were maintained in liquid nitrogen during transfer and in storage.

Lipidomics Preparation

Taken from the liquid nitrogen storage, butterflies were immediately placed on dry ice for dissection. To investigate how lipid composition varied with treatment and genotype, samples were homogenized and prepared for lipidomics processing using an established protocol for sample quality optimization [43]. First, the thorax was sliced into two halves using a sagittal section along the dorsal-longitudinal axis. The anterior half of one of these sections (i.e. ~25% of the thorax) was used for lipid analyses. This tissue was weighed to the nearest 0.1mg, placed in a round-bottomed 2ml Eppendorf Safe-Lock microcentrifuge tube and homogenized to a dry powder with a steel bead for 45 seconds at 20Hz on a Retsch MM301 mixer mill in pre-chilled hardware (-20°C) located in a cold room maintained at 5°C. To this still-frozen powder, 2ml of cold 3:3:2 isopropanol:acetonitrile:water solution containing 1 uM chloropropamide (an internal standard) was added, after which the mixtures were homogenized again for 2 minutes at 20Hz on the mixer mill.

The homogenized samples were shaken at 4˚C on a multi-therm shaker (Benchmark Scientific H5000-HC Multi-Therm Shaker) for 20 minutes. Solids were removed by centrifugation at 12000 x G for 20 minutes on an Eppendorf 5402 Refrigerated Centrifuge (4˚C), then the supernatant was transferred to clean, labeled 2ml Eppendorf tubes and stored in the 4˚C refrigerator until further processing. Protein pellets from the homogenized samples were stored in the -80˚C freezer.

At the Metabolomics Core Facility in the Huck Institutes of Life Sciences (University Park, PA) supernatants were dried in a vacuum centrifuge at room temperature, then dissolved in 300uL 50:50 acetonitrile:water solution. 5uL of sample were transferred to autosampler vials; tubes were crimped and stored in the facility's 4˚C refrigerator.

Lipidomics Processing

We next used liquid chromatography-mass spectrometry (LC-MS) analysis to create a lipid profile for each individual, utilizing the methodologies previously employed for investigating lipid composition within insect tissues [44]. Samples (5uL) were run using the HPLC-QTOFMS (Shimadzu Prominence 20-UFLC XR and AB Sciex 5600 TripleTOF) platform equipped with a Waters Acquity CSH C18 column (150 mm × 2.1 mm with a particle size of 1.7 μm; Waters Corporation, Milford, MA, USA). The composition of mobile phase A was 60% acetonitrile and 40% water with 10 mM ammonium formate and 0.1% formic acid. Mobile phase B consisted of 90% isopropanol and 10% acetonitrile with 10 mM ammonium formate and 0.1% formic acid. Flow rate and temperature were maintained at 0.225 ml/min and 55 °C, respectively. Positive and negative ion electrospray ionization mass spectra were acquired over

the mass range 100-1200 Da in IDA (Information Dependent Acquisition) mode, but for the scope of this experiment only the positive ionization data were analyzed. The LC-MS traces were aligned together using MarkerView (AB Sciex) software and analyzed for monoisotopic ions with a column retention time between 1 and 20 minutes.

Genotyping

Each individual was also genotyped for an indel polymorphism in *succinate dehydrogenase D* (*Sdhd*), previously associated with variation in hypoxia-inducible factor (HIF) signaling, tracheal development, and oxygen conductance to the flight muscles in Glanville fritillaries [26]. Total RNA was extracted using Trizol reagent (Life Technologies) from individual flash frozen heads, followed by RNA column purification (RNAeasy, Qiagen) and cDNA synthesis with T7-(dT)24 primers. Fluorescently labeled primers were used to PCR amplify a portion of the 3-UTR containing the indel from cDNA. Fragment analysis was used to associate size of labeled amplicons with the indel allele (deletion [*D*], mini deletion [*M*], insertion [*I*], or extra deletion [*E*]).

Lipidomics Data Processing

From LC-MS, 22,515 peaks were generated for each focal butterfly in positive ionization mode. From those data, we analyzed the 3,485 monoisotopic peaks with retention times between 1 and 20 minutes for which at least one butterfly had a non-zero reading. These peaks were normalized first by the chloropropamide standard, correcting for machine drift over the course of

the numerous samples run. Peak abundance was also normalized by wet tissue mass (g) to yield a measure of lipid abundance per amount of tissue sampled from each individual.

We used Metaboanalyst 3.0 software (www.metaboanalyst.ca) to examine the dataset in its entirety and determine whether there was a clear difference in free lipid composition between treatments. Within this program, we used a mass tolerance of 0.250 (m/z) and retention time tolerance of 5 seconds. Peak abundance was log transformed for comparison and filtered based on interquartile range (IQR).

We wrote a custom analysis script (Igor 6.0; Wavemetrics) that performed a series of Wilcoxon tests (the non-parametric equivalent of a T-test) comparing mean log-transformed (value + 1, to retain zero values) normalized monoisotopic peak heights between the rested and paraquat-injected butterflies. The purpose of this script was to identify a set of lipid species likely created by the paraquat treatment, which is well known to create oxidative damage in living tissue [45]. We wrote similar custom scripts in which the log-transformed lipid peak height (abundance) data were regressed against the ratio of day 2 to day 1 peak metabolic rate in the flight tests.

Preliminary exploration of these data indicated that a large number of lipid species had strong inverse correlations with the ratio of day 2/day 1 performance, but that two individuals had large increases in their day 2 metabolic performance unrelated to their lipid profiles. These two outliers may have been stunned at the time of capture in the field and performed poorly on day 1, but recovered by day 2. The correlation between many lipids and the day 2/day 1 ratio of flight metabolic rate in the remaining 28 individuals was very consistent and robust, suggesting that exclusion of the two outliers was justified and required in order to observe the major features of the data relating lipid profiles to the temporal trend in performance variation.

We used these separate results for the association between particular lipid abundances and i) paraquat treatment, and ii) day 1/day 2 peak FMR, to test the hypothesis that lipids indicative of paraquat-induced oxidative damage are more abundant in butterflies that had greater declines in flight performance. We performed additional analyses to determine if the overlap in lipid species associated with both paraquat and declining peak FMR were likely a collection of random false positives from thousands of tests. Rather than using a Bonferroni [46] approach, which doesn't identify which of the lowest P-value cases are false positives, we instead compared the lowest P-value results from one experiment with those from a second independent experiment. In addition, we examined the distribution of P-values in both experiments and performed a Fisher's exact test on the p-values that were significant in both experiments.

Generating Fragment Spectra

When processing our samples using lipidomics, the data was acquired in Information Dependent Acquisition (IDA) mode. In this mode, abundant lipid species triggered an automatic survey scan for which up to 20 MS/MS product ion spectra were acquired before the next survey scan began. Because the lipids of most interest to us exist in low abundance within our samples, they were not abundant enough to trigger an automatic survey scan in IDA.

In an effort to identify these lipids, samples were prepared again for lipidomics using those six individuals for which our top 13 lipids of interest proved most abundant. These samples were prepared using exactly the same methodologies described in **Lipidomics Preparation**, above, with the complementary anterior section of the thorax used. Samples were processed using the same LC-MS parameters as before, but this time we included the MS/MS

scan in the duty cycle so that even our lipids of interest would generate fragment spectra. These data were further explored using LipidView to isolate secondary spectra for these lipid species.

Chapter 3

Results

Sdhd Genotype Associations with Flight Metabolic Rate

When butterflies were flown in a normoxic atmosphere, total CO_2 emitted during the three minutes of flying did not vary significantly with the order of normoxia or hypoxia atmospheric gas treatment, body mass, or gender. Butterflies carrying one or more copies of the *Sdhd M* allele, shown previously to be associated with greater tracheal elaboration in the flight muscles [26], had greater total CO_2 emission in normoxia (about 30% higher; P=0.02). Peak FMR in normoxia did not vary significantly with any of those factors. In the mildly hypoxic atmosphere (14% O_2), total CO_2 emitted was about 33% higher when the hypoxia flight was performed first (P=0.008), declined with increasing body mass (P=0.05), and fell just above the threshold for significance as higher in butterflies carrying the *Sdhd M* allele (P=0.06). Peak FMR in hypoxia showed much the same associations, varying significantly with treatment order (P=0.01), body mass (P=0.02), *Sdhd M* allele (P=0.04) and was borderline higher in females (P=0.07). Overall, total CO_2 and peak FMR were greatly affected by gas treatment and the presence of an *Sdhd M* allele, but not the day flown, the interaction between day and gas treatment, or gender (Table 1).

Flown butterflies varied in their ability to achieve a comparable peak flight metabolic rate on two consecutive days. Some individuals increased in peak FMR by as much as 70% on the second day, whereas others achieved less than half their day 1 peak FMR on the second day.

There was no significant tendency to increase or decrease in performance over consecutive days, as the mean ratio of day 2/day 1 peak FMR did not differ from 1 (P=0.58).

Table 1. Factors associated with flight metabolic rate (FMR) on consecutive days in (random order) normoxia (21% O_2) and hypoxia (14% O_2). Sample size = 13 females, 17 males. These are the same data reported for Spain butterflies in Marden et al., 2013. I present these data again here because I helped collect these data and my research compared metabolic rates of these butterflies with lipid composition of their flight muscles after the second flight (see below).

a. Basic cross-over analysis in which butterfly was a random factor (i.e. repeated measures on individuals drawn randomly from a population) and we examine the effect of the two treatments and their interaction.

	Source	Nparm	DF	DFDen	F Ratio	Prob > F
Total CO_2 Emitted	Day	1	1	28	0.11	0.74
	Gas	1	1	28	4.23	0.049
	Day*Gas	1	1	28	0.49	0.49
Peak FMR	Day	1	1	28	1.31	0.26
	Gas	1	1	28	2.03	0.17
	Day*Gas	1	1	28	0.018	0.89

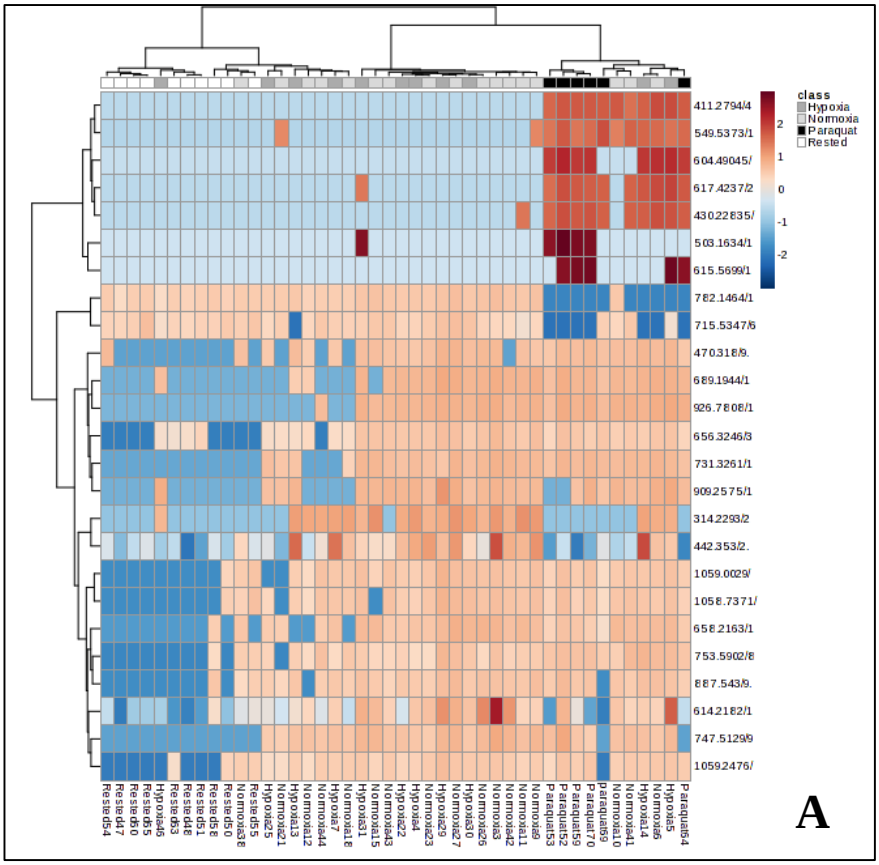
b. Basic cross-over also examining the effects of the presence or absence of the *Sdhd M* allele.

	Source	Nparm	DF	DFDen	F Ratio	Prob > F
Total CO_2 Emitted	Day	1	1	28	0.11	0.74
	Gas	1	1	28	4.23	0.049
	Day*Gas	1	1	27	1.29	0.27
	Sdhd M vs. no *M*	1	1	27	5.50	0.027
Peak FMR	Day	1	1	28	1.31	0.26
	Gas	1	1	28	2.03	0.17
	Day*Gas	1	1	27	0.22	0.64
	Sdhd M vs. no *M*	1	1	27	3.94	0.057

Treatment Effects on Flight Muscle Lipidome

To test the hypothesis that global lipid composition in the flight muscles was affected by the applied treatments (rested, paraquat-injected, flown in hypoxia first, flown in normoxia first), we performed cluster analyses of the entire set of monoisotopic peaks. These analyses (Metaboanalyst 3.0) are illustrated graphically by heatmaps (Figure 1). Individuals from the different treatments generally grouped together, in groupings determined primarily by approximately 25 lipid species. All rested individuals clustered together with two flown individuals also present within their cluster, with low abundance in these 25 peaks (Figure 1a). Butterflies from the paraquat-injected and flown treatments had a higher abundance of these peaks and were intermixed in a cluster distinct from rested butterflies, but not consistently distinguishable from each other.

When the two flown treatments (order of normoxia vs. hypoxia treatment) were examined separately, they clustered into distinct groups, with the exception of one individual (Figure 1b). Here again, about 25 lipid species were responsible for the differentiation. Among those lipids, normoxia-first individuals sometimes showed a higher abundance, whereas, in other cases, hypoxia-first butterflies were higher.



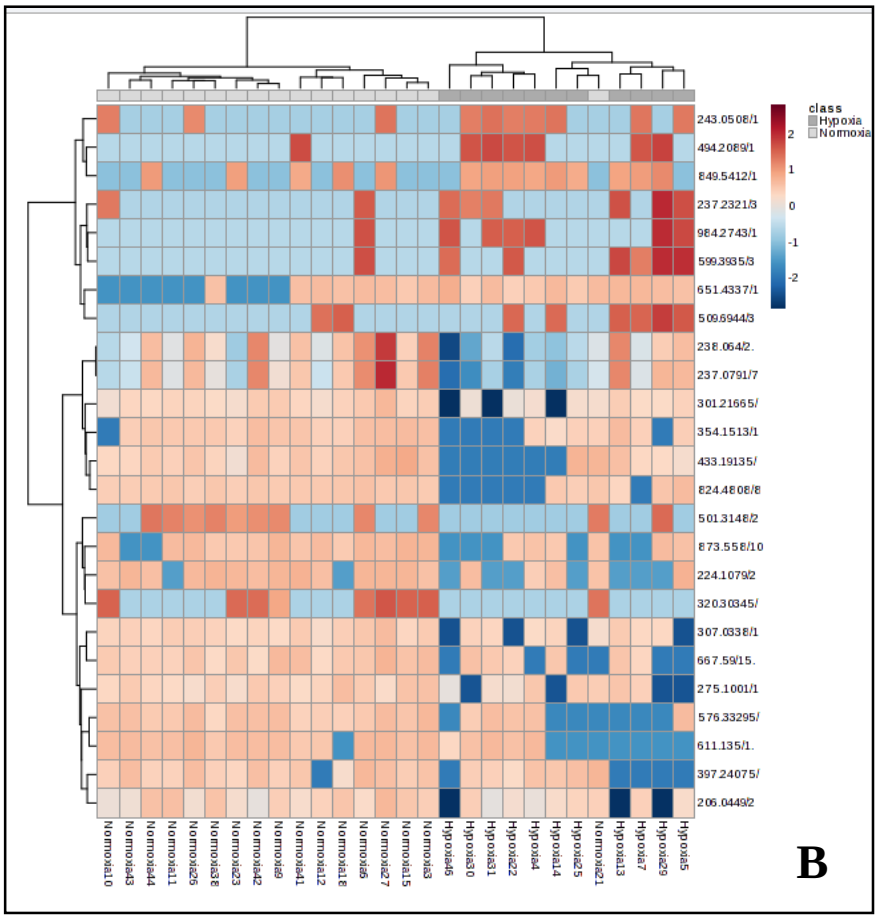


Figure 1. The twenty-five lipid species most different in abundance between (a) individuals of each treatment type (rested, injected with paraquat, flown in normoxia first, flown in hypoxia first) and (b) only those individuals subjected to flight tests, both based on cluster analyses.

Flight Metabolic Rate and Oxidative Damage

To test the hypothesis that individual decreases in flight metabolic performance over consecutive days were related to oxidative damage in flight muscle membranes, we used regression analyses to compare the day 2/day 1 peak FMR with abundance of lipid species that showed the most significant (lowest P value) increase in paraquat-treated butterflies compared to rested butterflies. This hypothesis was strongly supported, as the day 2/day 1 ratio of peak FMR decreased as the abundance of these lipids increased (Figure 2a. $p=0.0008$; 2b. $p<0.0001$). For these same peaks, there was a marked difference in abundance between rested individuals and those injected with paraquat; those rested consistently had a lower abundance (Wilcoxon Ranked-Sum: 2a. $p=0.0003$; 2b. $p=0.0090$). These results were also observed when we used the normalized mean abundance of the top thirteen most significant peaks for day 2/ day 1 peak FMR, where each peak mean abundance was divided by the sample mean for that peak so that peaks had equal weight (Figure 2c: linear regression $p<0.0001$; Wilcoxon Ranked-Sum: $p=0.0042$).

Significant differences in lipid composition between treatments may represent real effects or else false discovery given that we performed 3,485 tests. If these results are dominated by false discovery, the frequency distribution of P-values would be approximately uniform, however we found a very large over-abundance of small P-values, with 19.74% of these values less than 0.05 (Figure 3a).

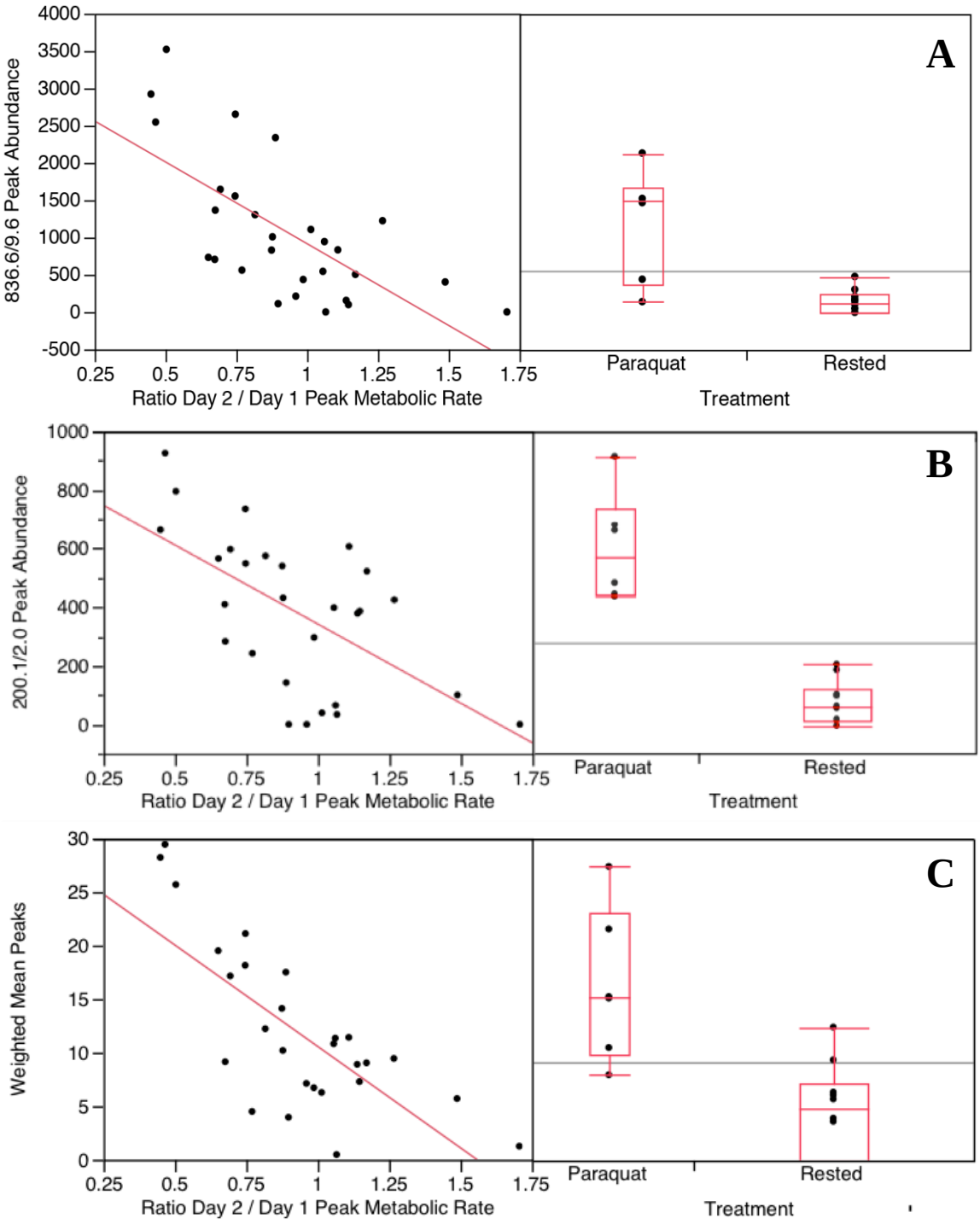


Figure 2. Comparisons of the correlation between peak metabolic rate ratio from day 2 to day 1 against peak abundance and box plots illustrating peak abundance between paraquat-injected and rested individuals for peaks with *m/z* value and retention time (minutes) of (a) 836.6/9.6, (b) 200.1/2.0, and the normalized mean abundance (c).

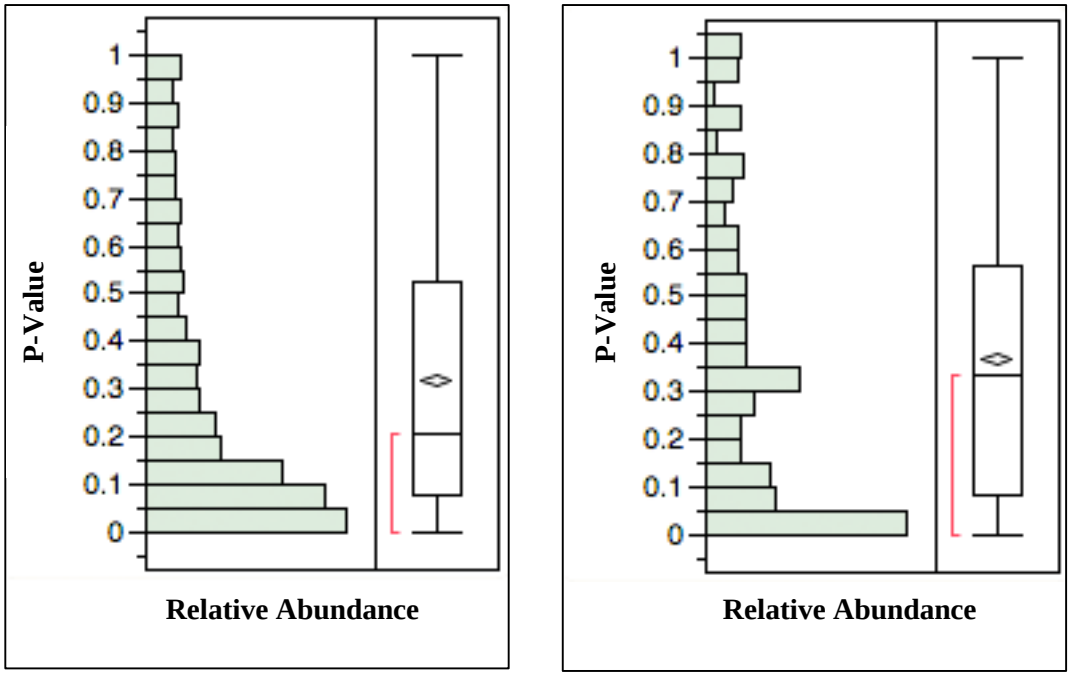


Figure 3. The distribution of P-values generated by (a) the Least Squares Regression for the relationship between peak metabolic rate ratio across both days of flight trials and peak abundance, as well as (b) the Wilcoxon Ranked Sum test between treatment (paraquat-injected or rested) and peak abundance.

Similar to the results for the paraquat treatment, the distribution of P-values relating lipid abundances to flight showed nonuniformity and an over-abundance of small P-values, with 15.23% <0.05 (Figure 3b). These results indicate a large overabundance of low P-value associations with both the paraquat treatment and the flight phenotype, and therefore were not likely to be strictly a collection of false positive associations. We then proceeded to look for individual lipids that had significant association in both experiments (Figure 4), to test the hypothesis that day-to-day decline in flight ability is associated with accumulation of lipids known to be elevated by paraquat (i.e. putative markers of oxidative damage).

Identification of a number of lipid species abundances that vary with treatment or phenotype in two independent experiments also carries the possibility of false positives. In other words, if there is a 5% chance of false positive association in each experiment, what is the

random probability of some of those being significantly associated in both experiments? To examine this question, we used a Fisher's Exact Test to determine the probability of peaks being significant with both paraquat treatment and flight decline. Of 2160 peaks appropriate for analysis by both tests, 517 were significantly ($p<0.05$) related to flight decline, 390 were significantly elevated by paraquat treatment, and 139 were significantly associated with both. There was a very small probability of this large an overlap occurring by chance (Fisher's Exact Test: $p=0.0002$). Furthermore, of the top 100 correlations between lipid abundances and the ratio of day 2/day 1 peak FMR, 99 were negative (higher abundance in butterflies with greater decline) whereas random false positives would yield close to an even mix of positive and negative associations.

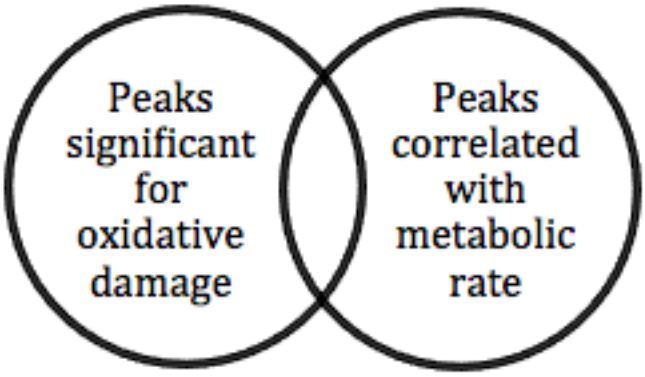


Figure 4. Hypothesis of overlap for individual lipids significantly elevated in paraquat-treated butterflies and in butterflies with the largest decline in metabolic rate between day 1 and day 2.

Flight Metabolic Rate and *Sdhd* on Lipid Profile

Along with a relationship between decline in flight metabolic rate and elevated lipids known to be associated with oxidative damage, we also observed an interaction between this phenomenon and the presence of the *Sdhd M* allele. While the abundance of our lipids of interest, denoted by the normalized mean abundance, was higher dictated primarily by the day 2/day 1 peak FMR ($P<0.0001$), those individuals containing at least one *M* allele tended to have a higher mean peaks value than no-*M* individuals with comparable flight decline (Figure 5; $P=0.0251$).

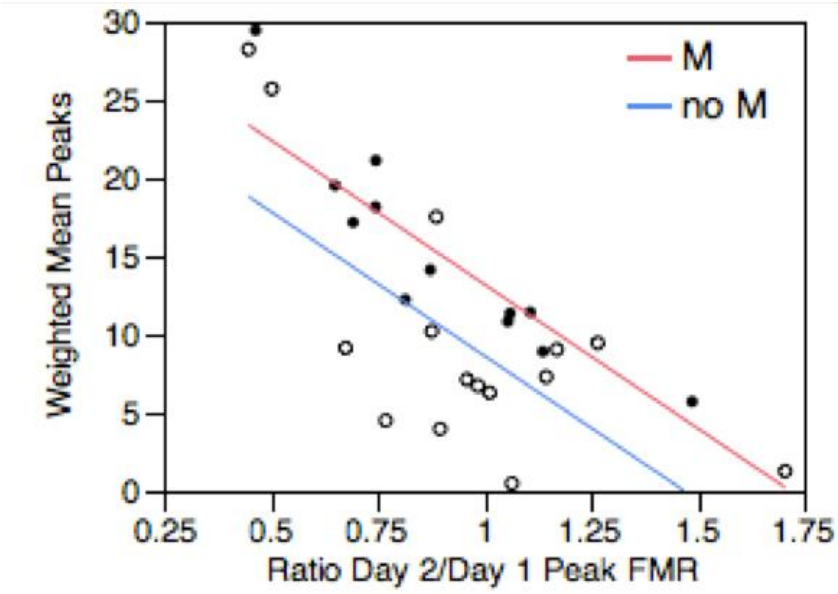


Figure 5. The correlation between the normalized mean abundance for our peaks of interest and the day 2/day 1 peak FMR with regard to the presence or absence of the *Sdhd M* allele.

Fragment Spectra for Lipids of Interest

The fragment composition of each of these target lipids was examined using the tandem MS/MS capability of the instrument (LC-MS/MS; Figure 6). While most lipids species were still too low in abundance to generate a clear spectrum, we were able to isolate quality spectra for three lipids of interest.

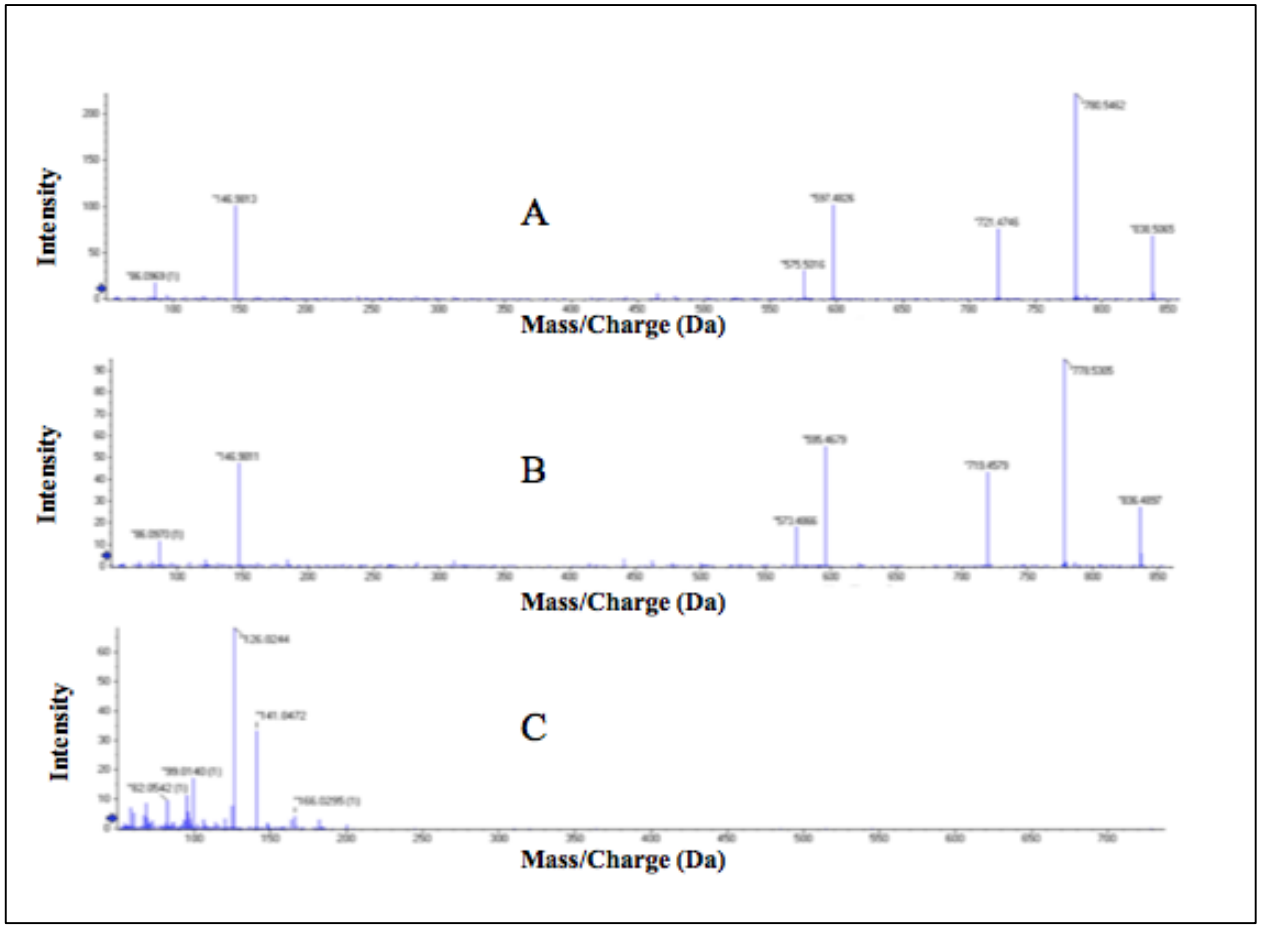


Figure 6. Tandem MS/MS spectrometry revealing the fragment composition for each of the peaks with an *m/z* score in positive ion mode of a) 838..5, b) 836.5, and c) 200.1.

Chapter 4

Discussion

Here, we show that the lipidome of flight muscles in a lowland butterfly changes significantly following oxidative stress. Multiple lipid species proved significantly different between those individuals rested and those subjected to oxidative stress, whether through prolonged flight or paraquat injection. For those individuals flown, there was no obvious trend for an increase or decrease in flight performance between day 1 and day 2; flight performance did not consistently decrease on the second day due to a tired-out individual, nor was the performance on the second day augmented due to acclimation and a full day of rest. Instead, decline in flight performance was correlated with an elevation in those lipids associated with the oxidative damage observed in flight muscles following a paraquat treatment, while rested individuals had a very low abundance or completely lacked these lipids.

Various recent studies have identified strong biomarkers indicating oxidative stress [47-49]. Here, we found that several lipids, quite low in abundance, are also indicative of oxidative damage and may play a larger role than previously credited. The importance of characterizing low-abundant lipid species in a heterogeneous lipidome as biomarkers for disease prediction, detection, and monitoring progression has been shown [50], but unfortunately investigating less abundant lipids makes the identification of each lipid quite difficult, especially in an insect model with very little biomass. Further, although the lipidomics field is fast-growing, many lipid species are still unidentified even in model species, for which the lipid database is growing quickly [29,51]. It was challenging to generate secondary spectra and explore fragment composition for these lipids in low abundance. However, though unable to identify them majority of our lipids of interest within *M. cinxia*, we were able to compare them with the current

literature, finding similarities between our lipids of interest and those identified in *Drosophila* following oxidative damage [29].

Two lipids significant for oxidative stress and also correlated with metabolic rate (m/z scores in positive ion mode of 836.5 and 838.5) did generate clear fragment spectra. From these spectra and the current literature, we were able to speculate on the identity of these peaks, determining that both are sodiated glycerol phosphatidylcholines (i.e. the true identities of these molecules are $[M+Na]^+$=836.5 and $[M+Na]^+$=836.5, respectively). Notably, peaks at *m/z* 86, *m/z*146, [M+Na-205], [M+Na-183] and [M+Na-59] are all indicative of this categorization [52,53] and are likewise all present in the fragment spectra for both $[M+Na]^+$=836.5 and $[M+Na]^+$=836.5 (Figure 6). Although the structures of the fatty acid tails on these lipids are unclear, lipids of corresponding molecular masses and class were generated as models from the LIPID MAPS database (www.lipidmaps.org). These models, shown in Figure 7, below, illustrate the potential, non-sodiated structure of these two categorized lipids, with uncertainty only present in the fatty acid tail structures. As the molecular mass of sodium is roughly 23 Da, the molecular masses of the non-sodiated lipids are 815.5 and 813.5, respectively.

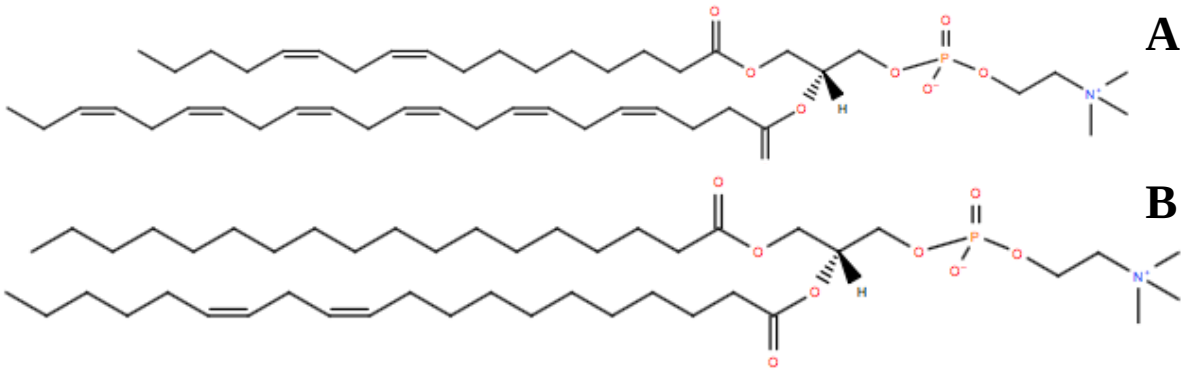


Figure 7. Structures generated through the LIPID MAPS database for lipids of (a) *m/z* 815.5 and (b) *m/z* 813.5

This type of lipid has a strong documented relationship with the platelet-activating factor (PAF), an integral player in several pathological and physiological processes [54]. Above all, PAF functions as an intra- and intercellular mediator in communication, present in the cell in low concentrations under normal conditions. Under oxidative stress, however, high amounts of the platelet-activating factor are released at the outset of reperfusion [54,55]. Therefore, the two lipids identified here have a strong link to reperfusion injury, as their presence indicates the triggering of the platelet-activating factor.

Based on the swollen and disrupted mitochondria previously observed following oxidative stress in Glanville fritillaries [26], the other unidentified lipids of interest shown here could be the peroxidized products of compromised mitochondrial membranes. In previous studies, cardiolipin was identified as the main biological indicator of mitochondrial damage [56,57]. Our peaks could be cardiolipin or cardiolipin byproducts, as we noted before that the lipid database for nonmodel insects is very limited, and the fatty acid structure of cardiolipin varies between taxa [58-60]. Furthermore, while cardiolipin is one of the major targets for oxidative damage, other phospholipids are targeted later as damage continues after the initial impact [58]. Thus, peroxidized cardiolipin may be present in our samples but other oxidation products may have been more significantly different between our treatments.

Additionally, this discrepancy between peroxidation products early and later on following oxidative damage may play a role in the differences between lipidomes observed here in those butterflies flown first in hypoxia or normoxia. When individuals were flown in hypoxia first, total CO_2 emitted by each butterfly was higher in the reduced oxygen environment than in normoxia, indicating that individuals are working harder and potentially leading to increased

oxidative stress and consequent reperfusion. Those butterflies flown in normoxia-first, then, would be flash frozen immediately following a rough flight in hypoxia and exhibit the immediate effects of oxidative damage. On the other hand, those flown in hypoxia first would have more of the peroxidation products found later following oxidative stress. Conversely, hypoxia-first butterflies may have also already begun healing and therefore show less of the peroxidation products (or compounds necessary for the healing process) than those frozen immediately following a flight in hypoxia.

As predicted, the total CO_2 emission during flight for butterflies containing the *Sdhd M* allele was higher in both normoxic and hypoxic environments, and peak FMR was higher in hypoxia. This was expected based on the observed greater tracheal development seen before in these individuals [26] and their consequent augmented oxygen delivery. Unexpectedly, however, it was also observed that this morph had higher levels of the lipids associated with oxidative damage than other individuals experiencing the same amount of flight decline without an *Sdhd M* allele. This suggests that these individuals may accumulate more damage at the same level of decline as other butterflies due to faster reperfusion through their highly branched tracheae. However, it has been observed previously in Glanville fritillaries that the most oxidative damage appears to occur in those individuals containing at least one *D* allele [26]. As such, it is also possible that *M* individuals that were strong fliers the first day and acquired a high amount of damage have less of a decline in flight performance between the first and second day. In this way, individuals containing the *M* allele would have higher damage than lesser fliers but comparable decline ratios and a higher abundance of lipids associated with oxidative stress.

This anomaly lends further curiosity to the mechanisms that maintain intermediate allele frequencies of the *Sdhd* polymorphism across wild Glanville fritillary metapopulations. While

Marden et al. (2013) saw more advanced mitochondrial damage in *D* allele individuals, an earlier study showed better flight performance in those with the *D* allele, but only when flown for a single day and for those who also had a particular *pgi* allele, also highly associated with flight performance, dispersal and longevity [38-40]. These flight tests lasted for ten minutes, as compared to those with a duration of 3 minutes, conducted here. In a metapopulation with such high local extinction and recolonization turnover across a heterogeneous landscape, strong flight performance to access neighboring habitable patches is crucial.

Here, we have only examined the effects of short bouts of flight on each allele type. It is possible that individuals carrying different *Sdhd* allele types have developed different flight strategies (Figure 8) for the long distances between habitable patches as well as short forays in search of food, allowing for this polymorphism to be maintained across the metapopulation. It was previously determined that there is a large overrepresentation of the *Sdhd D* allele in newly formed populations [37], suggesting that these individuals perform particularly well during dispersal between patches. While discrepancies in flight behavior have not been documented in the Glanville fritillary, the adoption of longer and straighter trajectories for flight between habitable patches as compared to short foray loops has been studied in other butterfly models [61].

Stimulating the butterflies within contained jars induces some degree of panic, causing them to fly more quickly and more continuously than they may typically, and simulating an inaccurate representation of one or both allele types' flight strategy. *D* allele individuals, shown to be especially prevalent in new colonies and thus seemingly more fit to disperse, are potentially stronger fliers in terms of endurance (Figure 8a), while *M* allele individuals with more developed tracheae can excel with short bouts of faster flight (Figure 8b). In this situation, *M* allele

individuals would perform "better" in our shorter, more intense respirometry trials while *D* allele individuals would be less suited to this type of flight, and therefore be subjected to worse oxidative stress and consequently higher levels of mitochondrial damage, especially as differences between biomarkers of acute and chronic oxidative stress have been documented [62]. As it stands, it is unclear which allele type is truly at an advantage in terms of flight performance, but the evidence shown here and previously documenting discrepancies between successes in flight trials strongly supports the idea of colonizing *D* allele individuals possessing a higher endurance suited for longer dispersal flights than *M* allele individuals, whose foraging forays help maintain an establish colony. Due to the separate benefits of these flight strategies, the *Sdhd* polymorphism would be maintained, as it has been observed, at intermediate allele frequencies.

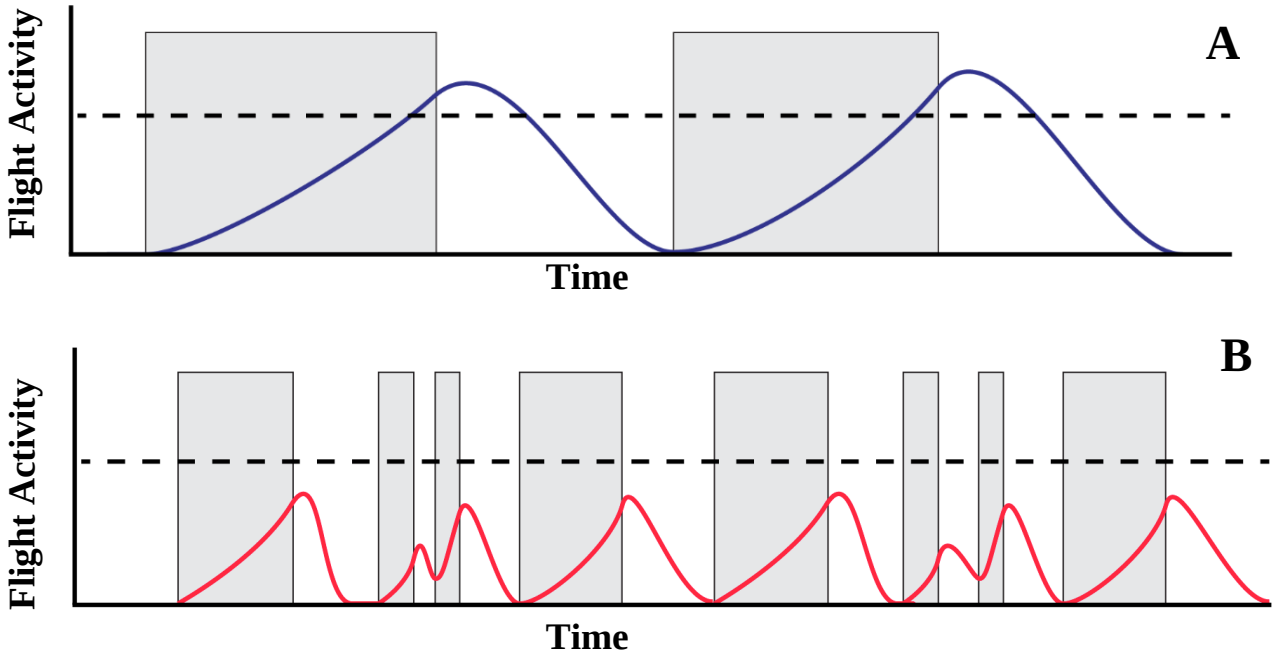


Figure 8. Illustration of potential flight strategies for (a) individuals containing the *D Sdhd* allele and (b) those with *M*, where the gray boxes represent flight activity over time, the colored lines show relative mitochondrial damage accumulation between the two morphs, and the dotted lines are standard across both panels, indicating a level of damage associated with elevated mortality.

Across all *Sdhd* genotypes, individuals with a higher decline in flight performance accumulated more damage in their flight muscles, compromising physiological function (i.e. aging), and lending support to the idea that the rate of living theory and oxidative stress hypothesis work are two complementary and major aspects of the aging process. To further explore this point, future studies must investigate the long-term effects of this type of damage and the extent to which compromises the longevity of an individual. The lipids characterized here as indicators of oxidative stress further emphasize the importance of low-abundance lipids in the lipidome following injury.

Oxidized lipids are beneficial for use as biomarkers for oxidative stress, especially as they have the capacity to be observed *in vivo* and noninvasively [63]. This is enormously useful, as oxidative stress is not catalyzed only by aerobic activity, but also environmental pollution [64,65], brain trauma [44,56], and several illnesses ranging from depression to Alzheimer's disease [48,50,58,66]. These biomarkers will facilitate the prediction, detection, and monitoring of such afflictions and as such also aid in their prevention and eradication. The function of oxidized lipids as biomarkers and their role in the aging process have far-reaching applications stretching between the world of medicine and evolutionary ecology.

BIBLIOGRAPHY

1. Jin K (2010). Modern biological theories of aging. *Aging and Disease 1*(2), 72.
2. Brunk UT and Terman A (2002). The mitochondrial-lysosomal axis theory of aging. *European Journal of Biochemistry, 269(*8), 1996-2002.
3. Harley CB, Vaziri H, Counter CM, Allsopp RC (1992). The telomere hypothesis of cellular aging. *Experimental Gerontology*, *27*(4), 375-382.
4. Walford RL (1969). The immunologic theory of aging. *Immunological Reviews*, *2*(1), 171.
5. Bjorksten J and Tenhu H (1990). The crosslinking theory of aging – Added evidence. *Experimental gerontology, 25*(2), 91-95.
6. Barzilai N, Huffman DM, Muzumdar RH, and Bartke A (2012). The criticial role of metabolic pathways in aging. *Diabetes, 61*(6), 1315-1322.
7. Paradies G, Petrosillo G, Paradies V, Ruggiero FM (2011). Mitochondrial dysfunction in brain aging: role of oxidative stress and cardiolipin. *Neurochemsitry international, 58*(4), 447-457.
8. Brys K, Vanfleteren JR, Braeckman BP (2007). Testing the rate-of-living/oxidative damage theory of aging in the nematode model Caenorhabditis elegans. *Experimental gerontology, 42*(9), 845-851.
9. Feng J, Bussière F, Hekimi S (2001). Mitochondrial Electron Transport Is a Key Determinant of Life Span in Caenorhabditis elegans. *Developmental Cell, 1*(5), 633-644.
10. Hekimi S, Burgess J, Bussière F, Meng Y, Bénard C (2001). Genetics of lifespan in C. elegans: molecular diversity, physiological complexity, mechanistic simplicity. *TRENDS in Genetics*, *17*(12), 712-718.
11. Soha RS, Mockett RJ, Orr WC (2000). Current issues concerning the role of oxidative stress in aging: a perspective. *The molecular genetics of aging*, 45-66.
12. Hand SC and Hardewig I (1996). Downregulation of cellular metabolism during environmental stress: mechanisms and implications. *Annual review of physiology*, *58*(1), 539-563.
13. Sohal RS (1986). The rate of living theory: a contemporary interpretation. *Insect aging*, 23-44.
14. Sohal RS (1982). Oxygen consumption and life span in the adult male housefly, Musca domestica. *Age*, *5*(1), 21-24.
15. Lyman CP, O'Brien RC, Greene GC, Papafrangos ED (1981). Hibernation and longevity in the Turkish hamster Mesocricetus brandti. *Science, 212*(4495), 668-670.
16. Miquel J, Lundgren PR, Bensch KG, Atlan H (1976). Effects of temperature on the life span, vitality and fine structure of Drosophila melanogaster. *Mechanisms of ageing and development*, *5*, 347-370.
17. Loeb J and Northrop JH (1917). *On the influence of food and temperature upon the duration of life.*
18. Pearl R (1928). *The rate of living*. London: University Press.
19. Harman D (1955). *Aging: a theory based on free radical and radiation chemistry*. Berkeley, CA: University of California Radiation Laboratory.
20. Fridovich I (1978). The biology of oxygen radicals. *Science*, *201*(4359), 875-880.
21. Anaya-Prado R, Toledo-Pereyra LH, Lentsch AB, Ward PA (2002). Ischemia/reperfusion injury. *Journal of Surgical Research*, *105*(2), 248-258.

22. Anaya-Prado R and Toledo-Pererya LH (2002). The molecular events underlying Ischemia/reperfusion injury. *Transplantation Proceedings*, *34*(7), 2518-2519.
23. Dorweiler B, Pruefer D, Andrasi TB, Maksan SM, Schmiedt W, Neufang A, Vahl CF (2007). Ischemia-reperfusion injury. *European Journal of Trauma and Emergency Surgery*, *33*(6), 600-612.
24. Seekamp A and Ward PA (1992). Ischemia-reperfusion injury. *Agents and actions. Supplements*, *41*, 137-152.
25. Yassin MMI, Harkin DW, Barros D'Sa AAB, Halliday MI, Rowlands BJ (2002). Lower l imb ischemia-reperfusion injury triggers a systemic inflammatory response and multiple organ dysfunction. *World journal of surgery*, *26*(1), 115-121.
26. Marden JH, Fescemyer HW, Schilder RJ, Doerfler WR, Vera JC, Wheat CW (2013). Genetic variation in HIF signaling underlies quantitative variation in physiological and life-history traits within lowland butterfly populations. *Evolution,* 67, 1105-1115.
27. Petrosillo G, Ruggiero FM, Di Venosa N, Paradies G (2003). Decreased complex III activity in mitochondria isolated from rat heart subjected to ischemia and reperfusion: role of reactive oxygen species and cardiolipin.*The FASEB Journal*, *17*(6), 714-716.
28. Paradies G, Petrosillo G, Pistolese M, Di Venosa N, Federici A, Ruggiero FM (2004). Decrease in mitochondrial complex I activity in ischemic/reperfused rat heart involvement of reactive oxygen species and cardiolipin. *Circulation Research*, *94*(1), 53-59.
29. Narayanaswamy P, Shinde S, Sulc R, Kraut R, Staples G, Thiam CH, Grimm R, Sellergren B, Torta F, Wenk MR (2014). Lipidomic "Deep Profiling": An Enhanced Workflow to Reveal New Molecular Species of Signaling Lipids. *Analytical Chemistry* 86, 3043-3047.
30. Wenk MR (2005). The emerging field of lipidomics. *Nature Reviews Drug Discovery*, *4*, 594-610.
31. Fyrst H, Zhang X, Herr DR, Byun HS, Bittman R, Phan VH, Harris GL, Saba JD (2008). Identification and characterization by electrospray mass spectrometry of endogenous *Drosophila* sphingadienes. *Journal of Lipid Research*, *49*, 597-606.
32. Tortoriello G, Rhodes BP, Takacs SM, Stuart JM, Basnet A, Raboune S, Widlanski TS, Doherty P, Harkany T, Bradshaw HB (2013. Targeted Lipidomics in *Drosophila melanogaster* Identifies Novel 2-Monoacylglycerols and N-acyl Amides. *PLoS ONE 8*(7), e67865.
33. Kambleh MA, Hobania Y, Dow JAT, Watson DG (2008). Metabolomic profiling of drosophila using liquid chromatogapyh Fourier transform mass spectrometry. *FEBS Letters* 2916-2922.
34. Lu N, Wei D, Chen F, Yang ST (2011). Lipidomic profiling and discovery of lipid biomarkers in snow alga *Chlamydomonas nivalis* under salt stress. *European Journal of Lipid Science and Technology 114*(3), 253-265.
35. Ehrlich PR and Hanksi I (Eds.) (2004). *On the wings of checkerspots: a model system for population biology.* Oxford University Press, 2004.
36. Kuussaari M, Nieminen M, and Hanski I (1996). An experimental study of migration in the Glanville fritillary butterfly Melitaea cinxia. *Journal of animal Ecology*, 791-801.
37. Wheat CW, Fescemyer HW, Kvist J, Tas EVA, Vera JC, Frilande MH, Hanski I, Marden JH (2011). Functional genomics of life history variation in a butterfly metapopulation. *Molecular Ecology*, *20*(9), 1813-1828.

38. Haag CR, Saastamoinen M, Marden JH, and Hanski I (2005). A candidate locus for variation in dispersal rate in a butterfly metapopulation. *Proc. Biol. Sci. 272*, 2449–2456.
39. Orsini L, Wheat CW, Haag CR, Kvist J, Frilander MJ, and Hanski I (2009). Fitness differences associated with Pgi SNP genotypes in the Glanville fritillary butterfly (Melitaea cinxia). *J. Evol. Biol. 22*, 367–375.
40. Niitepõld K, Smith AD, Osborne JL, Reynolds DR, Carreck NL, Martin AP, Marden JH, Ovaskainen O, and Hanski I (2009). Flight metabolic rate and Pgi genotype influence butterfly dispersal rate in the field. *Ecology 90*, 2223–2232.
41. Rutter J, Winge DR, Schiffman JD (2010). Succinate dehydrogenase—assembly, regulation, and role in human disease. *Mitochondrion 10*, 393-401.
42. Ratkowsky D, Alldredge R, Evans MA (1992). *Cross-Over experiments: design, analysis and application* (135). CRC Press.
43. Sarafian MH, Gaudin M, Lewis MR, Martin F, Holmes E, Nicholson JK, Dumas M (2014). Objective set of criteria for optimization of sample preparation procedures for ultra-high throughput untargeted blood plasma lipid profiling by ultra performance liquid chromatography-mass spectrometry. *Analytical Chemistry 86*(12), 5766-5774.
44. De Bekker C, Smith PB, Patterson AD, Hughes DP (2013). Metabolomics reveals the heterogeneous secretome of two entomopathogenic fungi to ex vivo cultured insect tissues. *PloS one 8*(8), e70609.
45. Bus JS and Gibson JE (1984). Paraquat: model for oxidant-initiated toxicity. *Environmental Health Perspectives, 55*, 37-46.
46. Cabin RJ, Mitchell RJ (2000). To Bonferroni or Not to Bonferroni: When and How Are the Questions. *Ecological Society of America 81*(3), 246-248.
47. Tyurin VA, Tyurina YY, Borisenko GG, et al. (2000). Oxidative stress following traumatic brain injury in rats: quantitation of biomarkers and detection of free radical intermediates. *J Neurochem* 75, 2178–2189.
48. Korade Z, Xu L, Mirnics K, Porter NA (2013). Lipid Biomarkers of oxidative stress in a genetic mouse model of Smith-Lemli-Opitz syndrome. *Journal of Inherited Metabolic Disease 36*(1), 113-122.
49. Valavanidis A, Vlachogianni T, Fiotakis C (2009). 8-hydroxy-2'-deoxyguanosine (8-OHdG): a critical biomarker of oxidative stress and carcinogenesis. *Journal of environmental science and health Part C, 27*(2), 120-139.
50. Cimino J, Calligaris D, Far J, Dubois D, Blacher S, Sounni NE, Noel A, De Pauw E (2013). Towards Lipidomics of Low-Abundant Species for Exploring Tumor Heterogeneity Guided by High Resolution Mass Spectrometry Imaging. *International Journal of Molecular Science 14*, 24560-24580.
51. Fyrst H, Zhang X, Herr DR, Byun HS, Bittman R, Phan VH, Harris GL, Saba JD (2008). Identification and characterization by electrospray mass spectrometry of endogenous *Drosophila* sphingadienes. *Journal of Lipid Research* 49, 597-606.
52. Domingues P, Domingues MRM, Amado FM, Ferrer-Correia AJ (2001). Characterization of sodiated glycerol phosphatidylcholine phospholipids by mass spectrometry. *Rapid Communications in Mass Spectrometry 15*(10), 799-804.
53. Hsu F and Turk J (2000). Structural determination of sphingomyelin by tandem mass spectrometry with electrospray ionization. *Journal of the American Society for Mass Spectrometry 11*(5), 437-449.

54. Penna C, Bassino E, Alloatti G (2011). Platelet activating factor: the good and the bad in the ischemic/reperfused heart. *Experimentl Biology and Medicine 236*(4), 390-401.
55. Prescott SM, Zimmerman GA, McIntyre TM (1990). Platelet-activating factor. Journal of Biological Chemistry *265*(29), 17381-17384.
56. Bayir H, Tyurin VA, Tyurina YY, Viner R, Ritov V, Amoscato AA, Zhao Q, Zhang QJ, Janesko-Feldman KL, Alexander H, Basova LV, Clark RSB, Kochanek PM, Kagan VE (2007). Selective Early Cardiolipin Peroxidation after Traumatic Brain Injury: An Oxidative Lipidomics Anaylsis. *Annals of Neurology* 62,154-169.
57. Martens J, Keilhoff G, Halangk W, Wartmann T, Gardemann A, Päge I, Schild L (2015). Lipidomic analysis of molecular cardiolipin species in livers exposed to ischemia/reperfusion. *Molecular and Cellular Biochemistry 400*(1-2), 253-263.
58. Schlame M, Shanske S, Doty S, Konig T, Sculco T, Dimauro S, Blanck TJ (1999). Microanalysis of cardiolipin in small biopsies including skeletal muscle from patients with mitochondrial disease. *Journal of Lipid Res* 40, 1585-1592.
59. Kraffe E, Soudant P, Marty Y, Kervarec N, Jehan P (2002). Evidence of a tetradocosahexaenoic cardiolpin in some marine bivalves. *Lipids* 37, 507-514.
60. Schlame M, Ren M, Xu Y, Greenber ML, Haller I (2005). Molecular symmetry in mitochondrial cardiolipins. *Chem. Phys. Lipids* 138, 38-49.
61. Delattre T (2010). Dispersal mood revealed by shifts from routine to direct flights in the meadow brown butterfly Maniola jurtina. *Oikos* 119(12), 1900-1908.
62. Seet RCS, Lee CYJ, Loke WM, Huang SH, Huang H, Looi WF, Chew ES, Quek AML, Lim ECH, Halliwell B (2011). Biomarkers of oxidative damage in cigarette smokers: which biomarkers might reflect acute versus chronic oxidative stress? *Free Radical Biology and Medicine 50*(12), 1787-1793.
63. Meagher EA, FitzGerald GA (2000). Indices of lipid peroxidation in vivo: strengths and limitations. *Free Radic Biol Med* 28, 1745-1750.
64. Valavanidis A, Vlahogianni T, Dassenakis M, Scoullos M (2006). Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. *Ecotoxicology and environmental safety*, *64*(2), 178-189.
65. Li N, Sioutas C, Cho A, Schmitz D, Misra C, Sempf J, Wang M, Oberley T, Froines J, Nel A (2003). Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. *Environmental health perspectives*, *111*(4), 455.
66. Nunomura A, Castellani RJ, Zhu X, Moreira PI, Perry G, Smith MA (2006). Involvement of oxidative stress in alzheimer's disease. *Journal of Neuropathology and Experimental Neurology 65*(7), 631-641.

ACADEMIC **VITA**

Julianne Pekny
Jep5360@psu.edu

EDUCATION

THE PENNSYLVANIA STATE UNIVERSITY
BACHELOR OF SCIENCE IN BIOLOGY, CONCENTRATION IN ECOLOGY
SPANISH MINOR

PROFESSIONAL EXPERIENCE

THE SAN DIEGO ZOO INSTITUTE FOR CONSERVATION RESEARCH
INDEPENDENT RESEARCH FELLOW: MAY 2014-AUGUST 2014

- Collaborated with lead researchers in conservation biology to create a novel protocol for the examination of foraging preference in the endangered Pacific Pocket Mouse, *Perognathus longimembrus pacificus*
- Cared for resident individuals in the captive breeding facility through husbandry, day-to-day care, and enrichment, ultimately aimed at reintroduction
- Live-trapped a variety of small mammals at our field site outside of Julian, CA in a nocturnal desert setting
- Learned the methods of safe handling, identification, and ear tagging
- Presented my findings to a crowd of over 100 San Diego Zoo Global researchers and donors
- Drafting a manuscript for publication with lead mentor, Dr. Debra Shier, proposed for spring 2015

THE ROCKY MOUNTAIN BIOLOGICAL LABORATORY
INDEPENDENT RESEARCH INTERN: JUNE 2013-AUGUST 2013

- Designed and executed an independent project observing parental care strategies in the burying beetle *Nicrophorus investigator* and later presented findings to researchers and the public at a student symposium
- Assembled and monitored live traps, collected and sexed beetles, physically marked individuals for identification, maintained treatments in the laboratory, and mapped research sites using GIS technology
- Aided in side study through setting live traps for small mammals, then handling and marking individuals
- Oversaw the efforts of two graduate field assistants on the project

THE MARDEN PHYSIOLOGICAL ECOLOGY LABORATORY
RESEARCH AND FIELD ASSISTANT: JANUARY 2012-PRESENT

- Full completion of experiment proposed for publication and senior thesis, which examines the effects of augmented metabolic rate and consequent oxidative stress on lipid composition and mitochondrial integrity in the Glanville Fritillary butterfly, *M. cinxia*

- Independently determined metabolic rate of each individual through respirometry trials and the program Igor
- Executed and taught insect dissection, homogenization, and genotyping by qPCR to peers
- Traveled with primary investigator abroad to field sites outside of Barcelona, Spain for a one-month period
- Wild-caught and sexed *M. cinxia* individuals in the field, then maintained in captivity

ADDITIONAL INVOLVEMENT

Green Towers, Evaluate primary literature sources and act as marketing correspondent for this 2014 startup, which strives to bring nature back into homes, offices, and public urban areas

Science Lion Pride, Prospective science student mentor and outreach leader

Penn State Dance Marathon (THON), Committee member, as well as founder of an independent fundraising organization, which has raised over $20,000 for THON since its creation in 2013