FINAL REPORT

1 General Information

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Project number: 271330745

Project title: Population genomics and environmental adaptation in *Drosophila ananassae*

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Name(s) of the cooperation partners: none

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2 Summary

English

Phase 1 of the project therefore used CCRT to explore the genetic basis of cold adaptation. First, we identified candidate genes involved in the physiological response to cold shock by comparing gene expression in cold-tolerant and cold-sensitive lines. Second, we could map three quantitative trait loci (QTL) that together explained a substantial 64% of the variation in CCRT in the ancestral population. Last, molecular genome editing tools based on CRISPR/Cas9 and PiggyBac systems, were established for the first time in *D. ananassae* to enable future functional studies of candidate genes, not only for cold stress tolerance.

Phase 2 extended this work, motivated by debate about whether CCRT alone fully captures ecologically relevant cold tolerance. Further, experiments included cold acclimation and additional phenotypes. The key findings were that cold acclimation improves cold tolerance and dramatically alters gene expression, particularly in ionoregulatory tissues. Importantly, additional cold tolerance phenotypes, such as lethal time and cold shock mortality, were found not to correlate significantly with chill coma recovery time. This highlights that while CCRT is useful, studying multiple phenotypes provides a more complete understanding of cold tolerance. Further genomic analysis linked specific regions influencing CCRT to biological processes like muscle development and metabolism. Overall, this project advanced our knowledge of thermal adaptation in natural populations.

Deutsch

In Phase 1 des Projekts wurde daher die CCRT eingesetzt, um die genetischen Grundlagen der Kälteanpassung zu untersuchen. Zunächst wurden Kandidatengene identifiziert, die an der physiologischen Reaktion auf Kälteschock beteiligt sind, indem die Genexpression in kältetoleranten und kälteempfindlichen Linien verglichen wurde. Drei quantitative Merkmalsloci (QTL) wurden kartiert, die zusammen 64 % der CCRT-Variationen in der angestammten Population erklärten. Erstmals wurden molekulare Genom-Editing-Tools auf der Grundlage von CRISPR/*Cas9*- und PiggyBac-Systemen in *D. ananassae* etabliert, um künftige Funktionsstudien von Kandidatengenen, nicht nur für Kältestresstoleranz, zu ermöglichen.

In Phase 2 wurde mit der Untersuchung weiterer Phänotypen ergänzt, da CCRT allein die ökologisch relevante Kältetoleranz nichtvollständig erfasst. Außerdem wurden Experimente zur Kälteakklimatisierung, sowohl systemisch, als auch in ionoregulatorischen Geweben, durchgeführt. Die wichtigsten Ergebnisse waren, dass die Kälteakklimatisierung die Kältetoleranz verbessert und die Genexpression, insbesondere in ionoregulatorischen Geweben, drastisch verändert. Wichtig ist, dass zusätzliche Kältetoleranz-Phänotypen, wie die Letalzeit und die Kälteschock-Sterblichkeit, nicht signifikant mit der Erholungszeit im Kälteschlaf kor-

relieren. Dies zeigt, dass die CCRT zwar nützlich ist, die Untersuchung mehrerer Phänotypen jedoch ein umfassenderes Verständnis der Kältetoleranz ermöglicht. Weitere genomische Analysen brachten spezifische Regionen, die die CCRT beeinflussen, mit biologischen Prozessen wie Muskelentwicklung und Stoffwechsel in Verbindung. Insgesamt hat dieses Projekt unser Wissen über die thermische Anpassung in natürlichen Populationen erweitert.

3 Progress Report

Background and objectives

Temperature is one of the major factors influencing the geographical distribution and abundance of many animals. As ectothermic organisms, the body temperature of insects mainly follows the external environment and resilience towards thermal extremes is essential for adaptation to new environments such those arising through species expansion or climate change. Over the past 10 years, we established the vinegar fly *Drosophila ananassae* as model organism to elucidate the genetic basis of thermal adaptation. Specifically, the overarching goal of this project was to explore patterns of cold stress tolerance and adaptation in natural populations. *D. ananassae* is a cosmopolitan species of vinegar flies that mainly occurs in tropical and subtropical regions (Das et al, 2004). The species likely originated in Southeast Asia and its migration to other parts of the world seems to follow human routes. Its occurrence in both tropical and temperate regions makes this species a good model to study cold adaptation. Chill coma recovery time (CCRT), the time an insect needs to recover from a chill-induced coma, is a key indicator of cold tolerance that can be easily and reliably measured in the lab. Our preliminary studies found that *D. ananassae* strains from a temperate population (Kathmandu, Nepal) recover more quickly from chill coma than flies from a tropical population (Bangkok, Thailand). Interestingly, there is polymorphism for chill coma recovery time within a putatively ancestral tropical population.Specifically, we characterized the transcriptional response to (a) cold shock and (b) cold acclimation in order to identify candidate genes involved in adaptation to cold in *D. ananassae*.

In the first phase of this project, we performed experiments to test the hypothesis that fewer genes – in comparison to *D. melanogaster* – underly the observed difference in cold shock recovery time in the ancestral *D. ananassae* population from Bangkok, Thailand. This part of the project addressed the following research questions: (1) Which genes are differentially expressed before and after cold shock? (2) Are differences in gene expression between strains with low cold resistance and strains with high cold resistance? (3) What are the specific genetic changes associated with increased cold tolerance? (4) Are there signatures of positive selection in cold tolerance related genes?

First, we did a transcriptome comparison and analyzed differences in gene expression among cold-sensitive and cold-tolerant strains before and after a cold shock. This allowed us to identify genes that play a role in the physiological response to cold in *D. ananassae* (Königer & Grath, 2018). Second, we mapped causal genomic regions that influence the cold tolerance phenotype. The most cold-tolerant and the most cold-sensitive fly strains of the Bangkok population were used to create a panel of recombinant inbred advanced intercross lines (RIAILs) as a mapping population. Molecular markers were established by ddRAD-sequencing, and a hierarchical approach was used for QTL mapping, combining standard interval mapping to identify loci of major effect and a multiple-QTL model to identify interactions among loci and estimate QTL effects. This approach revealed that variation at three genomic regions explained as much as 64% of the phenotypic variation between the most cold-tolerant and the most cold-sensitive strain (Königer et al., 2019). Third, we initiated and tested genetic tools and protocols for the first-time application of genome engineering in *D. ananassae*. The most cold-tolerant and the most cold-sensitive strains from the Bangkok population and an additional strain from the derived Kathmandu population were used to generate transgenic strains with a germline-specific source of *Cas9* (Yılmaz et al., 2023). Further, plasmids for functional knock-outs of cold-tolerance candidate genes were constructed, and preliminary tests for CRISPR/*Cas9*-mediated homology-directed repair were carried out.

In the second phase of this project, we extended our experiments to investigate the biological function of our candidate genes with respect to tissue-specific gene expression and taking into account periods of cold acclimation and additional phenotypes. This research was motivated by debate regarding the mechanisms and generality of the CCRT phenotype alone as indicator for ecologocially relevant cold tolerance (Andersen et al., 2015; Andersen & Overgaard, 2019; Overgaard et al., 2011). Furthermore, it has been observed that cold acclimation triggers substantial changes in the transcriptional response to cold in *D. suzukii* (Enriquez & Colinet, 2019) and reorganizes both the transcriptome and metabolome of whole animals (MacMillan et al., 2016) and the transcriptome of the gut in *D. melanogaster* (MacMillan et al., 2017). Additionally, it has been shown that the regulation of ion and water homeostasis are key physiological features for cold tolerance in *Drosophila* (Terhzaz et al., 2015; Yerushalmi et al., 2018) and *Gryllus* (DesMarteaux et al., 2017) renal and gut tissues.

The experiments outlined above for phase I of this project, had several limitations with respect to the determination which impact on the fly's physiology the identified candidate genes may have. First, we focused only on the the response to cold shock as determined by one specific phenotype, namely chill coma recovery time (CCRT). Second, we only considered whole adult flies and therefore could not directly test whether or not a knock-down or knock-out of our candidate genes in tissues shown as responsible for ion regulation would have an

effect on cold tolerance phenotypes. Finally, as the knock-out of candidate genes in the whole fly was unsuccessful, it is also possible that such a systemic knock-out would actually be lethal. We therefore addressed the following open questions regarding the cold tolerance mechanisms in *Drosophila* in the second phase of the project: (1) What is the transcriptomic response to cold acclimation in ionoregulatory tissues (i.e. hindgut and Malpighian tubules) of *D. ananassae*? (2) What are the genomic changes underlying differences in gene expression between cold-tolerant and cold-sensitive strains? (3) Which additional phenotypes show differences between cold-tolerant and cold-sensitive strains?

Project-specific results and findings (including findings that contradict initial hypotheses)

Overall the project was successful resulting in a number of high profile and internationally recognized papers (see section 4). All manuscripts have undergone, or are currently in the process of peer-review, **ensuring validity or verifiability of research results**. Although specific methodologies were modified as the project progressed (see section 5), there were **no major deviations from the original concept**.

Below I include abstracts of our major findings (full references can be found in section 4):

i) *Transcriptome analysis reveals candidate genes for cold tolerance in Drosophila ananassae* (Königer & Grath, 2018): Coping with daily and seasonal temperature fluctuations is a key adaptive process for species to colonize temperate regions all over the globe. Over the past 18,000 years, the tropical species *Drosophila ananassae* expanded its home range from tropical regions in Southeast Asia to more temperate regions. Phenotypic assays of chill coma recovery time (CCRT) together with previously published population genetic data suggest that only a small number of genes underlie improved cold hardiness in the cold-adapted populations. We used high-throughput RNA sequencing to analyze differential gene expression before and after exposure to a cold shock in cold-tolerant lines (those with fast chill coma recovery, CCR) and cold-sensitive lines (slow CCR) from a population originating from Bangkok, Thailand (the ancestral species range). We identified two candidate genes with a significant interaction between cold tolerance and cold shock treatment: GF14647 and GF15058. Further, our data suggest that selection for increased cold tolerance did not operate through the increased activity of heat shock proteins, but more likely through the stabilization of the actin cytoskeleton and a delayed onset of apoptosis.

ii) *Three quantitative trait loci explain more than 60% of variation for chill coma recovery time in a natural population of Drosophila ananassae* (Königer et al, 2019): Ectothermic species such as insects are particularly vulnerable to climatic fluctuations. Nevertheless, many insects that evolved and diversified in the tropics have successfully colonized temperate regions all over the globe. To shed light on the genetic basis of cold tolerance in such species, we conducted a quantitative trait locus (QTL) mapping experiment for chill coma recovery time (CCRT) in *Drosophila ananassae*, a cosmopolitan species that has expanded its range from tropical to temperate regions. We created a mapping population of recombinant inbred advanced intercross lines (RIAILs) from two founder strains with diverging CCRT phenotypes. The RIAILs were phenotyped for their CCRT and, together with the founder strains, genotyped for polymorphic markers with double-digest restriction site-associated DNA (ddRAD) sequencing. Using a hierarchical mapping approach that combined standard interval mapping and a multiple-QTL model, we mapped three QTL which altogether explained 64% of the phenotypic variance. For two of the identified QTL, we found evidence of epistasis. To narrow down the list of cold tolerance candidate genes, we cross-referenced the QTL intervals with genes that we previously identified as differentially expressed in response to cold in *D. ananassae*, and with thermotolerance candidate genes of *D. melanogaster*. Among the 58 differentially expressed genes that were contained within the QTL, GF15058 showed a significant interaction of the CCRT phenotype and gene expression. Further, we identified the orthologs of four *D. melanogaster* thermotolerance candidate genes, *MtnA*, *klarsicht*, *CG5246* (*D.ana/GF17132*) and *CG10383* (*D.ana/GF14829*) as candidates for cold tolerance in *D. ananassae*.

iii) *Tropical super flies: integrating Cas9 into Drosophila ananassae and its phenotypic effects* (Yılmaz et al., 2023): Ectotherms such as insects are animals whose body temperature largely depends on ambient temperature and temperature variations provide a selection pressure affecting the geographical distribution of these species. However, over the course of evolution, some insect species managed to colonize environments characterized by various temperature ranges. Therefore, insects provide an excellent study system to investigate the basis of adaptation to temperature changes and extremes. We are generally using the vinegar fly *Drosophila ananassae* as a model system to investigate the genetic basis of cold tolerance. This species has expanded from its tropical ancestral range to more temperate regions resulting in a cosmopolitan, domestic distribution. Previously, we identified candidate genes significantly associated with cold tolerance in this species. We now established molecular genetic tools to assess the function of these genes. Using CRISPR/*Cas9* method-

ology for genome editing and the PiggyBac system, the *Cas9* enzyme was successfully integrated into the genome of three fly strains with different levels of cold tolerance. We further report on preliminary findings that the *Cas9* integration itself did not have a consistent effect on tolerance to cold. In conclusion, we offer with our study the molecular tools that allow studying stress-related candidate genes in *D. ananassae* in the future. In addition, we point out and provide guidance on the challenges that come with genome editing in a non-model species.

iv) *Navigating the cold: integrative transcriptome sequencing approach reveals ionoregulatory and whole-body responses to cold acclimation in Drosophila ananassae* (Yılmaz et al., 2025): Understanding how species adapt to changing environments is a major goal in evolutionary biology and can elucidate the impact of climate change. Climate imposes inevitable effects on the geographical distribution of insects as their body temperature primarily depends on the environment. The vinegar fly *Drosophila ananassae* expanded from its tropical ancestral range to more temperate regions, which requires adaptation to colder climates. Transcriptome and genome-wide association studies focusing on the ancestral-range population identified the targets of selection related to ionoregulatory tissues. However, how cosmopolitan *D. ananassae* adapted to colder environments, where low temperatures last longer, is still unknown. Here, we present a study on the effect of long-term cold exposure on *D. ananassae*, examining the gene expression variation in the whole body and the ionoregulatory tissues, namely the hindgut and the Malpighian tubule. To elucidate molecular mechanisms of cold adaptation during species expansion, we included cold-tolerant and cold-sensitive strains from the ancestral species range and cold-tolerant strains from the derived species range. We show that cold acclimation improves cold tolerance and results in differential expression of more than half of the transcriptome in the ionoregulatory tissues and the whole body. Notably, we provide complementary insight into molecular processes at four levels: strains, populations, phenotypes, and tissues. By determining the biochemical pathways of phenotypic plasticity underlying cold tolerance, our results enhance our understanding of how environmental changes affect thermal adaptation in natural populations.

v) *Dissecting cold tolerance in Drosophila ananassae: a multi-phenotypic and bulk segregant analysis* (Yılmaz et al., *in preparation*): As a major element of the environment, temperature impacts the geographical ranges of insects. Therefore, the worldwide distribution of insects follows adaptation to wide ranges of temperature. A population of *Drosophila ananassae* from the ancestral region showed variation in cold tolerance levels. The ancestral Bangkok population also differed from the derived Kathmandu population regarding cold tolerance.

However, the phenotypic variation was only measured by chill coma assays, and no further characterization of the cold tolerance phenotype was performed. Here, we aimed to characterize further the iso-female lines of Bangkok and Kathmandu populations and the recombinant inbred lines generated from the ancestral population using lethal time and cold shock mortality in addition to chill coma recovery time. We showed that cold tolerance phenotypes differ between sexes, the additional phenotypes do not correlate significantly to chill coma recovery time, and some recombinant inbred lines have extreme phenotypes with higher tolerance than the tolerant founder or lower tolerance than the sensitive founder. We performed bulk segregant analysis using the recombinant inbred lines that exhibited extreme chill coma recovery time to identify genomic regions responsible for the phenotype. We identified 16 regions with significant association with the phenotype and showed that the genes in the putative regions were enriched in muscle development, metabolic processes, and cytoskeletal protein binding. Our results provide further evidence for the need for multiple cold tolerance phenotypes and shed light on the genetic architecture of adaptive phenotypic changes in natural populations of *Drosophila*.

Handling of research data

All raw data and analysis scripts have been (or will be) made publicly available on publication, either on public repositories or as supplementary materials. All sequence data was submitted to publicly accessible databases. Data produced by next-generation sequencing was submitted to the NCBI Short Read Archive (SRA) and Gene Expression Omnibus (GEO). Other types of data that do not fit into the structure of the public databases such as phenotypic data were published as supplementary files or, when too extensive, submitted to the Dryad digital repository (http://datadryad.org). These measures ensured their long-term availability to the research community. In accordance with funding provisions, the research data will be archived following publications or project ending, and will be stored for at least 10 years. Data is stored locally on a server at the LMU Munich Biocenter, which is backed up daily to the Leibniz-Rechenzentrum (LRZ) in Munich and stored long-term on magnetic tapes. The data management in this project follows the DFG Guidelines on the Handling of Research Data and the Guidelines for Safeguarding Good Scientific Practice, Avoiding Scientific Misconduct and Dealing with Violations, as well as the Guidelines on the Handling of Research Data of the LMU Munich.

Scientific events, science communication measures

I have presented results generated through the project funding at numerous occasions both within Germany and internationally, including in France, Austria, Switzerland, the

Netherlands, Czech Republic and the United Kingdom. Both myself and individual team members have presented our results at international conferences (e.g. ESEB, EDRC, ISEMPH, PopGroup).

Project developments

As described above, we were able to carry out all of the objectives without any major deviations from our original plans. However, during the course of the project we were able to participate in some additional research projects that were not described in our original proposals. For example, in collaboration with Prof. Dr. John Parsch (LMU Munich) we studied the response to oxidative stress in *Drosophila melanogaster*. Our results suggest that the physiological response to cold and oxidative stress share common biochemical pathways and were published in *G3: Genes|Genomes|Genetics* (Ramnarine et al., 2022). Additionally we participated in the European *Drosophila* consortium (DrosEU) to study the population genetics of *D. melanogaster* over time and across space. Within this framwork, we have been collecting natural *D. melanogaster* populations from across Europe since 2014. Initial population genomics analyses by the consortium revealed patterns of local adaptation (Kapun et al., 2020; 2021). In 2018, the consortium established 168 isofemale lines, which serve as genetically invariable fly lines, representing nine European *D. melanogaster* populations that are currently phenotyped for several life history, physiological, morphology, and behavioural organismal traits, including chill coma recovery time. In cooperation with researchers from DrosEU, I provided a guide to transcriptomic approaches in ecology and evolutionary biology, weighing the benefits and limitations of analysing gene expression in whole bodies, specific tissues, or individual cells (Hoedjes et al., 2024). Given technological advancements, our work addresses the challenge of selecting the most suitable experimental approach, taking into consideration sample preparation, RNA content, and the need for well-annotated genomes, which is a particular concern when studying non-model organisms. We compare bulk RNAseq (whole bodies or tissues) with single-cell RNAseq (scRNAseq) and highlight the advantages and disadvantages of each approach with the goal in offering guidance for researchers to make informed decisions. Crucially, we propose how scRNAseq can enhance existing bulk RNAseq datasets through deconvolution methods, thereby bridging the gap between systemic overviews and cell-type specific information. Ultimately, we advise researchers on selecting the most suitable RNAseq approach based on their specific research goals, model system and prior knowledge, and advocate for an integrated strategy.

Contributing researchers

The major contributors to the published research stemming from this project were the PI and the doctoral students Annabella Königer (funded from part I) and Vera Miyase Yılmaz (funded from part II). Many components of the research projects were carried out by Master's level student researchers and interns, under my supervision. Some of the listed publications involved collaborations with other researchers, including Prof. Dr. John Parsch (LMU Munich) and the DrosEU consortium as described above. In addition, several current and former members of the Grath lab funded by other sources contributed to the published research and appear as co-authors on the papers.

Bibliography

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Yılmaz VM, Bao Z, Grath S: Navigating the Cold: Integrative Transcriptome Sequencing Approach Reveals Ionoregulatory and Whole-Body Responses to Cold Acclimation in *Drosophila ananassae*. *Accepted for publication in Genome Biology and Evolution*. Available under bioRχiv: https://doi.org/10.1101/2024.07.18.604044.

Yılmaz VM, Kara FT, Grath S. Dissecting Cold Tolerance in *Drosophila ananassae*: A Multi-Phenotypic and Bulk Segregant Analysis. *In preparation for submission to G3 Genes|Genomes|Genetics*. Available under bioRχiv: https://www.biorxiv.org/content/10.1101/2025.04.23.650207v1.

4 Published Project Results

4.1 Category A – Articles in peer-reviewed journals, contributions to peer-reviewed conferences or to anthology volumes, and book publications

Directly related publications accepted in peer reviewed journals. Team members directly funded by the project are in bold.

1. Hoedjes KM, Grath S, Posnien N, Ritchie MG, Schlötterer C, Abbott JK, Almudi I, Coronado-Zamora M, Durmaz Mitchell E, Flatt T, Fricke C, Glaser-Schmitt A, González J, Holman L, Kankare M, Lenhart B, Orengo DJ, Snook RR, **Yılmaz VM**, Leeban Y (2024): From whole bodies to single cells: A guide to transcriptomic approaches for ecology and evolutionary biology.*Molecular Ecology*, https://doi.org/10.1111/mec.17382.
2. **Yılmaz VM**, Ramnarine TJS, **Königer A**, Mussgnug S, Grath S (2023): Tropical super flies: Integrating *Cas9*into *Drosophila ananassae*and its phenotypic effects. *Journal of Insect Physiology*, 147:104516. doi: 10.1016/j.jinsphys.2023.104516.
3. **Königer A**, Arif S, Grath S (2019): Three quantitative trait loci explain more than 60% of variation for chill coma recovery time in a natural population of *Drosophila ananassae*.*G3: Genes, Genomes, Genetics*,9(11):3715-3725. doi: 10.1534/g3.119.400453.
4. **Königer A**, Grath S (2018): Transcriptome analysis reveals candidate genes for cold tolerance in *Drosophila ananassae*.*Genes (Basel)*, 9(12):624. doi: 10.3390/genes9120624.
5. Ramnarine TJS, Grath S, Parsch J (2022): Natural variation in the transcriptional response of *Drosophila melanogaster*to oxidative stress. *G3 Genes|Genomes|Genetics*, 12(1), jkab366. doi: 10.1093/g3journal/jkab366.
6. Kapun M, Nunez JCB, Bogaerts-Márquez M, Murga-Moreno J, Paris M, Outten J, Coronado-Zamora M, C, Omar Rota-Stabelli O, García Guerreiro MP, Casillas S, Dorcas J, Orengo DC, Puerma E, Kankare M, Ometto L, Loeschcke V, Onder BS, Abbott JK, Schaeffer SW, Rajpurohit S, Behrman EL, Schou MF, Merritt TJS, Lazzaro BP, Glaser-Schmitt A, Argyridou E, Staubach F, Wang Y, Tauber E, Serga SV, Daniel K. Fabian DK, Kelly A. Dyer KA, Wheat CW, Parsch J, Grath S, Veselinovic MS, Stamenkovic-Radak M, Jelic M, Buendía-Ruíz AJ, Gómez-Julián MJ, Espinosa-Jimenez ML, Gallardo-Jiménez FD, Patenkovic A, Eric K, Tanaskovic M, Ullastres A, Guio L, Merenciano M, Guirao-Rico S, Horváth V, Obbard DJ, Pasyukova E, Alatortsev VE, Vieira CP, Vieira J, Torres JR, Kozeretska I, Maistrenko OM, Montchamp-Moreau C, Mukha DV, Machado HE, Paulo TF, Barbadilla A, Petrov D, Schmidt P, Gonzalez J, Flatt T, Bergland AO (2021): Drosophila Evolution over Space and Time (DEST) – A New Population Genomics Resource. *Molecular Biology and Evolution*, msab259. doi: 10.1093/molbev/msab259.
7. Kapun M, Barrón MG, Staubach F, Obbard DJ, Wiberg RAW, Vieira J, Goubert C, Rota-Stabelli O, Kankare M, Bogaerts-Márquez M, Haudry A, Waidele L, Kozeretska I, Pasyukova EG, Loeschcke V, Pascual M, Vieira CP, Serga S, Montchamp-Moreau C, Abbott J, Gibert P, Porcelli D, Posnien N, Sánchez-Gracia A, Grath S, Sucena É, Bergland AO, Guerreiro MPG, Onder BS, Argyridou E, Guio L, Schou MF, Deplancke B, Vieira C, Ritchie MG, Zwaan BJ, Tauber E, Orengo DJ, Puerma E, Aguadé M, Schmidt P, Parsch J, Betancourt AJ, Flatt T, González J. (2020): Genomic analysis of European *Drosophila melanogaster* populations on a dense spatial scale reveals longitudinal population structure and continent-wide selection. *Molecular Biology and Evolution*,37(9):2661-2678. doi: 10.1093/molbev/msaa120.

4.2 Category B – Any other form of published results

Manuscripts submitted/under review and available on bioRχiv and dissertations

8. **Königer A**: *The molecular basis of cold tolerance in Drosophila ananassae*. [Doctoral dissertation, LMU Munich], https://edoc.ub.uni-muenchen.de/24852/. doi: 10.5282/edoc.24852
9. **Yılmaz VM**, Bao Z, Grath S: Navigating the Cold: Integrative Transcriptome Sequencing Approach Reveals Ionoregulatory and Whole-Body Responses to Cold Acclimation in *Drosophila ananassae*. *Accepted for publication in Genome Biology and Evolution*. Available under bioRχiv: https://doi.org/10.1101/2024.07.18.604044.
10. **Yılmaz VM**, Kara FT, Grath S. Dissecting Cold Tolerance in *Drosophila ananassae*: A Multi-Phenotypic and Bulk Segregant Analysis. *In preparation for submission to G3 Genes|Genomes|Genetics*. Available under bioRχiv: https://www.biorxiv.org/content/10.1101/2025.04.23.650207v1.

Over the course of the funding period I have published a further 13 original manuscripts, invited reviews or book chapters (see https://scholar.google.com/citations?hl=en&user=yxiVf-HQAAAAJ for a full list).

4.3 Patents (applied for and granted)

None.