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ESF workshop
Genetics of thermal adaptation in ectotherms

Organizers: Dr. Jan Kammenga & Prof. Jacintha Ellers
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Summary

It is well documented that global climate change has resulted in clearly visible ecological changes in many species. However, knowledge about the genetic adaptation to changing ambient temperatures is scant. This is particularly important for most species on earth, which are ectotherms and depend on external temperatures for maintaining their body heat. The aim of this workshop was to explore the underlying genetic mechanisms and genomic adaptation to changing temperature conditions. The diverse array of invited speakers and participants provided a multidimensional perspective on the genetic control of evolutionary trajectories of ectotherms in a changing global climate. More than 15 oral presentations, a debate session on the most urgent questions in thermal biology and how to address them, as well as challenging discussions have led to several main findings and insights resulting from this meeting. Among others, these concern methodological issues to assess the genetics of thermal performance, identification of gaps in our knowledge on thermal adaptation, and the need for a concerted effort of thermal biologists to use their research to advise governments on global warming issues. The new insights resulting from this workshop will spark novel collaborative research into the evolution of thermal adaptation.

Scientific content

The meeting starts with an introductory round, in which each participant states their specialization and research interests. Dr. Kammenga explains the background and purpose of the meeting, after which the first oral presentation starts.

Dr. Balanya addresses latitudinal clines in chromosomal rearrangements in the fruitfly *Drosophila subobscura*. The adaptive nature of these clines in inversion polymorphisms is evidenced by the fact that the same clines have been established in recently colonized American areas. Because the frequency of the inversions changes fast, a comparison of the frequency of inversion polymorphisms in current samples with those several decades ago correlates with global warming. There is a general increase in the frequency of "warm"-adapted inversions. An experimental evolution experiment in the laboratory with several generations at constant, different thermal regimes shows changes in inversion frequency but not in the direction expected.

The discussion focuses on the adequacy of constant temperature regimes in laboratory experiments. One should be very careful to "translate" laboratory results obtained under constant conditions to natural situations. Possibly a caged transplant experiment could resolve the role of temperature in the establishment of these clines. Also, the question arises why the clines are less strong in America. Perhaps this is due to a lack of genetic variation, due to the recent demographical history of the American populations. Finally, the fitness effects of the inversions are discussed, and the potential presence of candidate genes in the inversions. Indeed many heat shock genes are clustered inside the inversions.

The next speaker is **Dr. Schulte** on genomics of thermal adaptation in fishes. She presents recent results on metabolic rate as a mechanistic link between oxygen supply and thermal tolerance in killifish. Northern populations have a higher metabolic rate as expected and a higher COX and CS activity. An interesting point is that Dr. Schulte has used a heterospecific (i.e across different species) microarray to detect temperature-responsive candidate genes. This seems a promising approach despite the relatively long evolutionary history between the two species. Further work will also focus on the sticklebacks as models for thermal adaptation.

The discussion addresses the extent of genetic differences between northern and southern populations of killifish. The plasticity induced by temperature is many times larger than the genetic differentiation in thermal biology. Yet there are significant population genetic differences between the populations. Also, the pro's and cons of cross-specific microarrays are discussed. A potential pitfall is that specific groups of genes are more conserved and therefore show up on the microarray analysis, while the more versatile genes may be the more important for local adaptation.

Dr. Jansen presents his research on thermal adaptation in the marine invertebrates *Mytilus* and *Macoma*. These species have different clades of populations along the European coast and are highly plastic in their thermal responses. The relationship between metabolic rate and temperature depends on the season or the experimental thermal environment. The question is if thermal environment is a limiting function? The research presented suggests that rather temperature allows a high metabolic rate but other factors determine if this high rate is realized.

Dr. Fischer studies thermal adaptation along an altitudinal cline in butterflies. Various proxies for thermal tolerance, such as heat knock-down temperature and chill coma recovery, differed between high and low altitude populations. The main determinant of the genetic differentiation was the enzyme PGI. Dr. Fischer also showed that there is a difference in plasticity between altitudes, with high altitudes having a reduced plasticity.

In the discussion the meaning of the different proxies for thermotolerance are reviewed. And the generality of the key position of the PGI-enzyme in thermal performance is discussed.

Dr. Laurila gives a presentation on latitudinal variation in life-history in frogs. Large scale climatic variation has resulted in genetic divergence in life-history traits, with countergradient variation causing increased growth rates towards the higher latitudes. This has resulted in an adaptive genetic cline driven mostly by season length. But also at the local scale significant differences in thermal performance can exist. Closed canopy ponds harbour frog populations that have a faster development rate and growth rate. The question remains why these populations do not spread southwards despite their favourable life-history? Potentially there are trade-offs with predation rate or other, still unknown, costs.

Dr. Ellers speaks about the evolution of thermal reaction norms in a terrestrial springtail. Temperature-induced plasticity is a genetic trait in itself and should be studied to understand performance of organisms under fluctuating thermal regimes. Local adaptation in thermal responses of growth rate was found between forests and heath habitats, with stronger thermal responses in thermally stable habitats. Also, there is genetic variation in thermal reaction norms, and the plasticity of some traits is genetically correlated. Gene expression analysis was used to identify candidate genes.

Dr. Bijlsma presents work on thermal load and adaptation. Many mutations only show phenotypic deviation in some environments but are neutral in others. This could lead to conditional expression of fitness in relation to temperature. Particularly, in fragmented habitats such mutants may be present in high frequency in the population due to genetic drift and may hamper adaptation. Fixation of alleles under permissive conditions can cause deleterious effects when environment changes.

In the discussion the balance between positive purging effects of inbreeding and negative effects of fixation by inbreeding is considered.

For the next activity on the programme, the participants are split up into three groups. Each group will **brainstorm** about the ultimate thermal adaptation experiment and focus on issues such as what are the most pressing questions and how can they be solved? What is needed in terms of expertise and resources?

After dinner the workgroups report back about the outcome of their respective sessions. There was general consensus among the different groups that thermal adaptation should be studied across all levels of biological organisation, from the gene to the ecosystem. A promising way forward identified by the groups was that research should be carried out from a phylogenetic perspective in order to address the adaptive potential toward thermal change. To facilitate this comparison, it should include model as wel as non-model species looking at the ecology, genetics and evolution across taxa.

The second day starts with a lecture by **Dr. Loeschcke** on the heat hardening response and its ecological consequences. In *Drosophila* the costs and benefits of heat hardening take place at different timescales. Selection experiments show correlated responses between different types of stress resistance. A comparative gene expression study shows that starvation and longevity resistance in particular, select for down regulation of a great variety of genes, while heat resistance causes mainly up regulation. Most importantly, when tested in the field, costs and benefits of heat acclimatization become apparent. The ability to forage successfully under different thermal regimes is genetically based and heritable.

In the discussion the question arises if field experiments magnify differences that were also observed in laboratory experiments or if they show opposite results? Indeed, new fitness effects of heat acclimatization are found, which emphasizes the need for multiple assays when testing for cost and benefits of thermal traits.

Dr. Lucassen speaks about oxygen-limited thermal tolerance in arctic fish. Hypoxia can be caused by thermal limits on ventilation. Using a comparative approach with cold-adapted and eurytherm fish species, he studies changes at the molecular level at the onset of hypoxia, to find the signals for thermal adaptation. The number and functional properties of mitochondria change with temperature, and significant difference in mitochondrial activity are found between cold-adapted and eurytherm fish.

Next, **four PhD-students** present their research projects and shortly summarize their results so far. Their projects (some just started, others half way) are briefly discussed and suggestions are made to possibly improve or strengthen the projects.

Dr. Köhler presents a mechanistic study of thermal dynamics in Mediterranean snails. The snails have various adaptations to cope with the hot environment (>37C). Behavioural orientation to the sun, as well as colour variation of the shell affects the thermodynamics of heat input vs evaporation. Heat shock proteins are hypothesized to play an important role in maintenance of shell colour variation, through the possible capacitor action of Hsp's. Hsp's regulate signal transduction under normal conditions, but under stress this regulation is decreased and hidden genetic variability may become apparent. Experiments on the relationship between shell colour variation and Hsp expression under different temperatures are the first field tests of this hypothesis and the results are consistent with theory.

Dr. Viney presents results on the genetics of life history of the nematode *C. elegans* under environmental stress. Significant genetic variation exists among strains in the response to environmental conditions such as food availability. Phenotypic plasticity of dauer larvae development is related to population growth rate. A QTL analysis shows that there are two QTLs for these traits of which one is associated with both traits but harbours separate loci affecting dauer larva development and growth rate. This analysis has uncovered new genes involved in dauer larva development.

Dr. Teotonio talks about reverse evolution experiments in *Drosophila* selection lines. Bringing these lines under control conditions shows a fast reversion of fitness back to control levels within 50 generations. The evolutionary rate is not determined by loss of genetic variation, because there is no increase in the rate of fitness gain if replicates are crossed. Genotype analysis of all populations using 50 SNP's shows strong linkage disequilibrium. Moreover, control and experimental evolution lines do not differ in gene diversity, only in gene frequency. Therefore adaptation has occured from standing variation, rather than involving new mutations.

The discussion addresses the lack of powerful statistical models to detect deviation from the null model of gene frequency changes by drift.

The final speaker is **Dr. Kammenga** who talks about transcriptional regulation of life-history trade-offs. In nearly all organisms there is a trade-off between life span and offspring production. Using well-genotyped RIL's of *C. elegans*, he shows that this trade-off has a genetic basis and there are shared genes underlying it. Genome-wide transcript levels were measured just before reproduction started, which demonstrated that a limited number of genes regulated the trade-off between life span and offspring production. These genes include several autophagy genes.

During the discussion the question arises if the genes identified in *C. elegans* can also be found in other organisms. No comparative genome search for these specific genes in other model organisms such as *Drosophila* or humans has been carried out yet, but it is also not certain if exactly the same genes will be present in other species. Perhaps only the mechanism is similar but not the exact genes.

Result and impact of the workshop

The workshop ends with a synthesis of the results and insights from the meeting. The points brought forward during the brainstorm session and the discussion following each talk are summarized and put in a broader context. Each participant has noted the specific aspects that he/she found important for future research. The following paragraph summarizes these main findings and the impact they have on the future direction of the field.

1. The importance of applying relevant thermal regimes in laboratory experiments. During the workshop the complexity of thermal environments has once again become apparent. One of the prime difficulties in thermal research is to create a simplified standard environment in the lab that still captures the most essential features of the natural thermal regime. There is no unique solution to this problem. There is agreement as to the need for field validation of laboratory experiments, and the use of multiple proxies for thermal performance.

2. The huge impact of plastic responses to temperature relative to genetic differentiation. From a theoretical point of view genetic differentiation is superfluous if strong plastic responses exist. Why do we find such an enormous plasticity in thermal performance? There is an urgent need to understand the selective forces driving plastic responses to temperature and to generalize these. For example, are constant environments indeed selecting for less plasticity and what are the ecological consequences? However, the exact method of temperature change and thermal acclimatization determines the pastic response, so there is a need for standardized methodology in order to be able to compare species responses.

3. The necessity for collaboration among research groups. It is extremely valuable to understand not only the evolutionary dynamics of thermal adaptation but also the underlying physiological and molecular mechanisms. It is hardly possible to address the whole cascade of adaptation at different biological levels in one research group because of the large variety of techniques involved. A willingness to go beyond one's own study system and to apply techniques cross-species through collaborative research projects should facilitate the integration of proximate and ultimate aspects of thermal adaptation.

4. The behavioural aspect of thermal adaptation is largely unknown. Obviously, animals do not sit around and wait for the temperature to reach their tolerance limits. In extremely hot conditions we hardly see the animals exposed. However, there is a severe lack of knowledge on behavioural regulation of thermal exposure. Unless we are able to detect the temperatures that ectotherms truly experience in the field, we do not know the thermal selection pressures, nor can we estimate the costs and benefits associated with heat avoidance behaviour

5. The lack of general theory to generate predictions for thermal adaptation. Unlike other areas of evolutionary ecology, there has been no general theoretical framework to understand the evolution of thermal adaptation. Various aspects of thermal biology have been studied in isolation. An integrative approach in combination with the appropriate analytical tools is needed to predict and test thermal evolution.

6. *The need for a concerted effort of thermal biologists to advise governments on global warming issues.* Thermal biologists have much of the knowledge to make statements on the vulnerability of species and ecosystems to global warming. They should play an active role in increasing the visibility and accessibility of their research to advise governments on global warming policies to protect threatened species.

Programme "Genetics of thermal adaptation in ectotherms" Wageningen, The Netherlands

Sunday, October 5 2008

9.00	Introduction of all participants
9.30 – 10.10 Joan Balanya	Genetic adaptation to climate change in *Drosophila*, focus on latitudinal clines and genetic polymorphisms.
10.10 – 10.50 Patricia Schulte	Genomics (and genetics) of thermal adaptation in fishes: killifish and stickleback as examples.
10.50 – 11.20	Coffee
11.20 - 12.00 Jeroen Jansen	Temperature adaptation of *Mytilus* en *Macoma*.
12.00 – 12.40 Klaus Fischer	Thermal adaptation in the butterfly *Lycaena tityrus*.
12.40 – 13.40	Lunch
13.40 – 14.20 Anssi Laurila	Adaptation along latitudinal gradients in frogs, focus on spatial and temporal variation in selective pressures.
14.20 – 15.00 Jacintha Ellers	Genetic variation in thermal reaction norms in soil invertebrates, focus on local adaptation of temperature responses and the underlying mechanisms.
15.00 – 15.30	Tea
15.30 – 17.00	Split up session: design your ultimate temperature adaptation experiment ! What are the most pressing questions and how can these be solved? What is required in terms of expertise and resources?
Diner 18.30	
20.00	Presentation of split up groups.

Monday, October 6 2008

9.00– 9.40 Volker Loeschcke	Ecology and genetics of thermal adaptation in Drosophila, focus on molecular mechanisms and adaptive value of stress tolerance.
9.40 – 10.20 Magnus Lucassen	Thermal limits and adaptation in marine ectotherms, focus on physiological acclimation of underlying metabolic processes.
10.20 – 10.40	Coffee
10.40 – 11.20	Short PhD research presentation by: Alexandra Zürn, Ana Viñuela, Coby van Dooremalen & Kirsten Jalvingh
11.20 – 12.00 Heinz Köhler	Biochemical, cytological, behavioural and fluidic heat response strategies in Mediterranean snails and possible implications on morphological variability
12.00 – 12.40 Mark Viney	The genetics of life-history traits of *C. elegans* under environmental stress
12.40 – 13.40	Lunch
13.40 – 14.20 Henrique Teotonio	Quantitative genetics of adaptive evolution, focus on integrating variation at the phenotypic level with variation at the genotypic level.
14.20 – 15.00 Jan Kammenga	Genetic architecture of temperature-induced phenotypic plasticity in life-history traits in *Caenorhabditis elegans*, focus on genetical genomics and gene expression plasticity.
15.00 – 15.30	Tea
15.30 – 17.00	Synthesis