

ESF Exploratory Workshop on

Understanding The Functional Consequences Of Natural Variation In Ecological Adaptation

Scientific Report

Veterinary University of Vienna
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Convened by:
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1. Executive summary:

It is well established that natural populations harbor huge amounts of molecular variation. The neutral theory of molecular evolution suggested that the effect of most mutations is so small that they can be considered as neutral. Recent evidence, however, demonstrated that a large proportion of natural variation seems to be involved in adaptation to the environment. With the acceleration in the development of sequencing technology we have now access to a huge amount of information on molecular variation. Population genetics has developed powerful tools to identify genes under selection, but the functional validation of such ecologically important variants requires new functional approaches suitable to measure effects that are important in the wild, but difficult to score in the laboratory.

Conversely, there is increasing evidence that natural variation in structural or regulatory genes have major effects, which were only recognized through the comparison of different naturally occurring variants.

The workshop was attended by 30 scientists from 12 different countries. It took place on the 15th and 16th of September, just after the *Drosophila* European Conference and an *Arabidopsis* meeting which allowed several specialists of these model species to take part. The workshop was held at the Veterinary University of Vienna for two intensive days of presentations and discussions. Frequent coffee breaks and two out door dinners gave the opportunity for many interactions. Marek Konarzewski (ESF Standing Committee for Life, Earth and Environmental Sciences) presented in detail the activity and the funding programs of the ESF. The invited speakers covered a broad range of topics and approaches, ranging from theoretical population genetics and quantitative genetics to developmental biology.

The workshop provided a platform for both functional biologists and population geneticists to develop concepts and new tools to study the functional consequences of natural variation, in particular in the context of ecology. Naturalists and population geneticists presented data on the extent of genetic variation, in natural populations or between closely related species for particular adaptive traits (adaptation to photoperiod or environmental temperature, host-parasite interaction, flower-pollinator adaptation). Several talks insisted on the importance of ecological factors (temperature, hosts, diet) involved in the geographical distribution of populations and on the molecular variation at the DNA or expression level. Several examples of gene x environment interaction (temperature, diet) were discussed. Developmental geneticists and populations geneticist described methods to identify the molecular basis of phenotypic difference at the species level or between closely related species. In addition, the effect of demography (extinction, re-colonization) was also shown to be a major factor shaping natural variation at particular loci. Whereas QTL mapping had been used for many years to identify molecular variants with functional implications, innovative and alternative methods were described using chosen populations or species groups. High efficiency meiotic mapping was illustrated by the efficient screening of intragenic recombinants using appropriate markers to identify regulatory mutation involved in species differences. Developmental biologists described new methods to reveal and analyze very small phenotypic differences (intraspecific variation in the spatio-temporal expression of developmental regulatory genes, enhancement of phenotypic differences by genetic and environment manipulation). A major conclusion from several functional biologists is that there is much more functional molecular variation at the species level and between closely related species than expected from the study of a few model species. For example particular regulatory sequences well characterized in *Drosophila melanogaster* (Polycomb response elements) have dramatically diverged in closely related species. Genes involved in pigmentation differences

between closely related species of *Drosophila* map in genomic regions where no gene involved in pigmentation have been identified in *Drosophila melanogaster* despite a hundred years since the isolation of the first pigmentation mutants in this model species. Thus, functional biologist specialist of model species can help to understand the functional implications of molecular variation, but, clearly, the study of natural variation could also teach a lot to functional biologists.

A scientific discussion took place at the end of the workshop. A general conclusion was that there is still a strong need for more ecological data, in particular on wild populations of model species such as *Drosophila melanogaster*.

2. Scientific content of the event:

The scientific presentations were organized in different sessions focusing on particular themes, but most presentations covered several fields and had more general implications. The first part of the day focused on the implications of selection on molecular variation. Joachim Hermisson described the patterns of molecular variation that could be produced by selection under different parameters (recurrent beneficial mutation, dominant or recessive beneficial mutation). Jakob Müller gave a general introduction on heterozygosity and fitness correlations in the wild and illustrated it with a study on blue tits.

In the second part of the day, the talks focused on the global analysis of genetic variation, in particular at the expression level and on the influence of the environment in the distribution of genetic variation. Greg Gibson showed how different diet could affect global gene expression in human populations. Volker Loeschke presented a large set of data on the genetic basis for adaptation of natural populations of *Drosophila melanogaster* to temperature stress. John Parsch illustrated the adaptation of *Drosophila* populations from Europe and Africa at the level of the transcriptome. Karl Schmidt presented a detailed study on the diversification in a multigenic family both at the structural (coding sequence) and expression level driven by positive selection in a group of closely related species of plants.

In the third session, the talks gave examples of successful identification of molecular variants involved in adaptation using candidate gene approaches in non model species chosen for their particular interest to study adaptation. Philip Schlüter presented data on the molecular basis of species specific flower-pollinator interactions in orchids. Christopher Wheat described how balancing selection could maintain variation at two metabolic loci involved in flight performance in the Glanville fritillary butterfly. This species has a well studied metapopulation in Finland characterized by hundreds of small populations and a high turnover. Different alleles are favored for the colonization of empty niches or maintenance of existing populations.

The last part of the day and the first talk of the second day focused on the different methods including QTL analysis to identify natural variants involved in adaptation. Sergey Nuzhdin gave a very stimulating talk and presented innovative methods to identify functional important variants. In particular, he proposed to combine microarrays and sequencing strategy with selection experiments or with the study of particular populations or species groups. Indeed, populations recently formed by admixture or particular species groups generated through hybridization represent particularly suitable models to analyze the functional implications of natural variation. Pär Ingvarsson described data on the molecular basis of adaptation of bud growth and dormancy to geographical variation in photoperiod in the aspen

tree. Thomas Morgan illustrated the successful identification of QTL involved in resistance to temperature stress in *Drosophila*. Ralf Sommer presented recent data on the ecology of *Pristionchus* nematodes. These nematodes live in close association with scarab beetles. The molecular basis of for some of these specific interactions are being successfully unraveled. Lars Steinmetz described a innovative method in yeast that could be applied fruitfully to other model species. Using genomic deficiency or knock out available in a model species , this method allow to identify the molecular basis for a particular ecologically relevant trait differing between this model species/strain and a wild isolate.

All the other talks of the second day focused on the functional implications of differences inside species or between closely related species. Jean David presented many data showing how environmental conditions, in particular temperature, can affect the development of several traits in *Drosophila*, and how populations adapt to environmental conditions. Juliette de Meaux gave a presentation on the molecular basis of the evolution of seed dormancy in *Arabidopsis*. Artyom Kopp illustrated how the knowledge acquired in a model species could be used to analyse the molecular basis of morphological variation in closely related species using pigmentation in different species of *Drosophila* as a meta model. Different genes can be involved in similar morphological differentiation and the molecular bases are not always polygenic, as a given locus can have a major effect. Results from non model *Drosophila* species suggests that we do not know yet all relevant genes involved in major regulatory networks or pigment synthesis pathways. Martin Jaekel showed how natural variation in the spatio temporal regulation of a major developmental regulatory gene could be revealed and analysed in *Drosophila melanogaster* using precise mathematical analysis of in situ hybridization data. Alistair McGregor described how highly efficient meiotic mapping using appropriate markers can be used to identify the molecular bases of morphological differences between species close enough to form fertile hybrids. Jean-Michel Gibert described the analysis of the functional implications of amino acid polymorphisms in a chromatin regulator and the gene x environment (temperature) interaction induced by these polymorphisms. Leonie Ringrose described how regulatory sequences, well characterized in *Drosophila melanogaster* (Polycomb Response Element), could diverge in closely related species and be lost or gain at high frequency.

3. Assesment of the results, contribution to the future direction of the field

This exploratory workshop was successful in bringing together scientists with very different expertise such as functional biologist analyzing particular processes at the molecular level and population geneticists studying variation in the traits controlled by these processes in natural populations. Many of the participants of the workshop did not have the opportunity to meet before. The workshop allowed them to interact in a stimulating way and to discuss future collaborations. A major conclusion is that the emerging field of ecological genetics requires multidisciplinary approaches and collaboration between scientists with different expertise. Particularly promising interactions are those taking place between groups studying a particular process in a model species and researchers interested in ecologically relevant variation in this process in a satellite species. Similarly, the expertise of specialist of selection is very fruitful as selection can, under particular parameter conditions, lead to patterns of molecular variation difficult to recognize as a footprint of selection. Model species can be analyzed at very high precision in the laboratory under controlled conditions. However, the ecology of many model species in the wild is still not well enough understood to fully benefit from all the molecular and functional analysis available to understand the genetic basis of adaptation. For example, the effect of fitness and the precise involvement in adaptation of

particular variable traits (example pigmentation in flies) is still poorly understood, in particular because of their correlation with many other traits. Thus, more study on the extent of molecular but also phenotypic variation in the wild is required. In this respect, the close association between scarab beetles and the new nematode model *Pristionchus* described by Ralf Sommer is certainly a step forward in the right direction.

Research topics that were identified for joint projects are:

Massively parallel sequencing of natural populations

Analysis of the naked region in *Drosophila* (FWF proposal by McAlister)

Analysis of buffering: potential funding agencies: EU, Human frontiers

4. Final Programme

Saturday 15th September:

9:00-9:30: **Registration and Welcome**

Presentation of the European Science Foundation (ESF).

Marek Konarzewski (ESF Standing Committee for Life, Earth and Environmental Sciences).

From molecules to phenotype:

Hitchhiking mapping

9:30-10:00: Joachim Hermisson: "*Soft sweeps and hitchhiking mapping*"

10:00-10:30: Jakob Müller: "*Heterozygosity - fitness correlations in a natural bird population*"

10:30-11:15: General discussion with coffee served

Expression analysis

11:15-11:55: Greg Gibson: "*The Human Transition Project: Effect of Lifestyle on Gene Expression in Moroccan Amazigh*"

11:55-12:35: Volker Loeschcke: "*Environmental stress and adaptation using *Drosophila* as a model – from gene expression to fitness estimation in the wild*"

12:35-13:35: Lunch

13:35-13:55: John Parsch: "*Gene expression variation in African and European populations of *Drosophila melanogaster**"

Interspecific analysis of coding sequences

13:55-14:35: Karl Schmid: "*Regulatory versus structural evolution in a short chain dehydrogenase gene family in Brassicaceae*".

From phenotype to molecules:

Candidate gene approach

14:35-14:55 Philip Schlüter: "*The genetic basis for specific pollinator attraction in the sexually deceptive orchid *Ophrys**"

14:55-15:40: General discussion with coffee served

15:40-16:00: Christopher Wheat " *Identifying and studying candidate genes in the wild: Pgi and Troponin-t affect butterfly flight.* "

QTL mapping

16:00-16:40: Sergey Nuzhdin: "*Genomics of local adaptation*"

16:40-17:00: Pelle Ingvarson: "*The genetic basis of local adaptation in bud phenology across a latitudinal gradient in European aspen (Populus tremula)*".

17:00-17:30: General discussion with coffee served

17:30-18:00 Theodore Morgan: "*The genomics of thermotolerance in Drosophila*"

18:00-18:45 Ralf Sommer: "*Multitrophic interactions of nematodes with beetles and bacteria: A genetic approach to ecology*"

19:30: Dinner at Weinhof Zimmermann, Mitterwurzgasse 20, 1190 Wien.

Sunday 16th September

9:00-9:40: Lars Steinmetz: "*The polygenic basis of complex traits in yeast*"

Functional implications of natural variation and their link to adaptation

9:40-10:20: Jean David: "*Adaptive aspects of phenotypic plasticity: some examples in Drosophila*"

10:20-11:00: Juliette de Meaux: " *Evolution of seed dormancy*"

11:00-11:45: General discussion with coffee served

11:45-12:25: Artyom Kopp: "*Color pattern variation in Drosophila: Genetic basis and functional consequences*"

12:25-13:05: Martin Jaekel: "*Natural variation in the segmentation gene network of D. melanogaster*"

13:05-14:00: Lunch

14:00-14:40: Alistair MacGregor: " *Morphological evolution through multiple mutations at a single gene*"

14:40-15:20: Jean-Michel Gibert: "*Polymorphisms in the Polycomb Group gene *crm* and thermal adaptation in Drosophila.*"

15:20-16:00 Leonie Ringrose: "*Evolutionary Plasticity of Polycomb/Trithorax response elements in Drosophila species*"

16:00-18:00: final discussion: Research agenda and discussion on potential collaborations.

18:30: Dinner at Meierei, prater-haptallee 3.

5. Statistical information on participants

The workshop was attended by 30 participants from 12 countries (Australia, Austria, Denmark, Finland, France, Germany, Italy, Spain, Sweden, Switzerland, United Kingdom, United States of America). There 7 women and 23 men. The age structure was well balanced, participants ranging from PhD students to a Professor Emeritus.

6. Final list of Participants

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