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Population genetics of brown crabs: Local thermal adaptation and its implications on host-pathogen interactions

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Background

Climate projections for the North Sea predict an increase in annual mean temperature of 1.5-3°C towards the end of this century (IPCC 2007) and similar predictions had been made for other regions of the European area. The survival of marine crustacean species will depend e.g. on the degree of their adaptive plasticity to future climate conditions designating the resilience of crustacean populations and thus the distribution and survival of species.

Today populations of the brown crab (*Cancer pagurus*) inhabit preferably hard bottomed habitats at the European coasts from the Mediterranean to the Sub-arctic, facing a wide range of temperature conditions. A temperature dependent pelagic larval phase (Nichols et al. 1982) of 40-140 days and migrations of the female adults to specific spawning areas suggest genetically connected populations on a small scale (compare Ungfors et al. 2009). In contrast brown crab populations are

known to be very sensitive to wrong temperature conditions. As an effect of suppressed physiology and immunity by shifts in temperature conditions in relation to normal local water temperatures they often reveal an increased level of diseases (Chualain et al. 2009).

We will analyse genetic population structure among European populations of *Cancer pagurus* and search for differences in temperature tolerance in crabs from different regions. Due to the wide range in temperature tolerance among populations in contrast to the sensitivity of local populations, we hypothesize that on a large scale populations of brown crabs are not in panmixie with each other revealing different adaptations on local temperature regimes and pathogen challenge.

Purpose of the visit

The purpose of the visit was to collect samples and information of the local population of *Cancer pagurus* in Bødo (Norway) to analyze genetic and phenotypic variation of different European populations in relation to temperature and common diseases. The samples and data collected during the visit gave me the opportunity to compare this "cold adapted" population of *Cancer pagurus* which never faces water temperatures as high as 15°C during seasons (Nielsen & Falck 2006) with other European populations of *Cancer pagurus* for example at Helgoland with water temperatures ranging from as low as 4°C to conditions even higher than 15°C (own observation).

Work carried out during the visit

Sampling of wild population

Cancer pagurus was collected in the bight of Mørkved near Bødo either during diving operations or with traps at the field station. To analyze and compare the genetic population structure of the population of Bødo with other populations among Europe, I collected DNA samples from 71 animals for genetic analysis. To find out whether genetic differences correlate with phenotypic variability I noted eight different morphometric characters per individual and made standardized photos of the carapace for landmark-based geometric analyses.

Temperature experiments

In order to find out what effect chronic temperature stress has on disease susceptibility of *Cancer pagurus* I ran a temperature experiment (slow increase to 15.5°C), and one experiment as a control

(ambient temperature; 7.5°C) and in addition a fast heat shock experiment (15.5°C). Experimental animals were acclimated to laboratory conditions at least for two weeks in ambient seawater prior to the experiments. The experiments were run in a semiclosed recirculation system. During the experiments animals were kept in 20l plastic boxes in aerated and recirculated seawater of controlled temperature (max 12 animals/1m³). 28 animals were exposed to a slow gradually temperature increase (approx 0.7°C/h) from 7.5°C to 15.5°C, 4 animals each were sampled after 10h, 40h, 120h, 175h and 240h. For 8 animals temperature was decreased after 120h to ambient temperature (7.5°C) and were sampled after 0h and 170h reacclimatization. According to this sampling regime 4 animals as technical controls were run for each sampling point (10h, 40h, 120h, 175h, 240h and reaclimatization for 0h and 170h; 28 animals in total). Additionally 8 animals were sampled without any prior treatment as controls.

In order to substract heat shock reactions from immunological reactions 8 animals were exposed to a fast heat shock and put directly in 15.5°C being acclimatisated to 7.5°C and were sampled after 10h and 40h exposed to 15.5°C.

Prior to each experiment I recorded the disease prevalence by pictures of the carapace (according to Vogan & Rowley 2002) and the ventral side of the animal. After the experimental treatment animals were killed, the disease prevalence of each specimen was documented by pictures, and tissue samples were taken and fixed in RNAlater to determine gene expression in the hemolymph, hepatopancreas and muscle tissue. Additionally, DNA samples of the biofilm and the hemolymph were taken to determine internal as well as external bacterial load as an indicator for disease prevalence. In addition hepatopancreas and hemolymph of some animals were fixed in formalin for histological analyses.

Slow temperature increase to 26°C was conducted with 2 animals. The experiment was aborted due to 100% mortality. For this experiment behavioural observations were conducted during the experiment.

Biofilm sampling

In order to determine the normal biofilm of *Cancer pagurus* in the local embayment of Bødo and to compare it with other biofilm formations among Europe I sampled the biofilm community on 10 different patches (approx. 2 cm diameter) on the outer shell of *Cancer pagurus* kept under ambient conditions. More controls were taken from neutral surfaces in the aquaria.

Collected samples and preliminary results

Wild population

I collected DNA samples for microsatellite and SNP analysis from 71 adult *Cancer pagurus* within the size range of 109-199cm carapace width. Preliminary analysis of microsatellite DNA for three different markers (Cpag 15, Cpag 1b9 and Cpag 5d8; McKoewn & Shaw 2008) comparing Norway to German and French populations of *Cancer pagurus* showed significant differences for one locus between sites (Table 1). More markers will be analysed soon.

Table 1. Preliminary results on genetic differences of the population of *Cancer pagurus* in Bødo compared to local populations at Helgoland (Germany) and Roscoff (France) based on three microsatellite DNA loci (Cpag 15, Cpag 1b9 and Cpag 5d8; asterisk indicates significant relations: $p<0.05$ from 500 bootstrap calculated in DEMEtics; Jueterbock et al. 2010).

Norway	Helgoland		Roscoff	
	Dest	Gst.est	Dest	Gst.est
Cpag 15	0.027*	0.014*	0.001	-0.001
Cpag 1b9	0.002	0.001	-0.001	-0.000
Cpag 5d8	-0.006	-0.000	0.037	0.002

I found a different prevalence of shell disease on the carapace of adult *Cancer pagurus* from Helgoland compared to Norway. Mean shell disease prevalence was higher in the Helgoland population during the summer at high temperature conditions and significantly lower in autumn at Bødo during conditions of low temperature (Mann Whitney U-test; $p<0.05$).

(a) Helgoland (b) Bodo

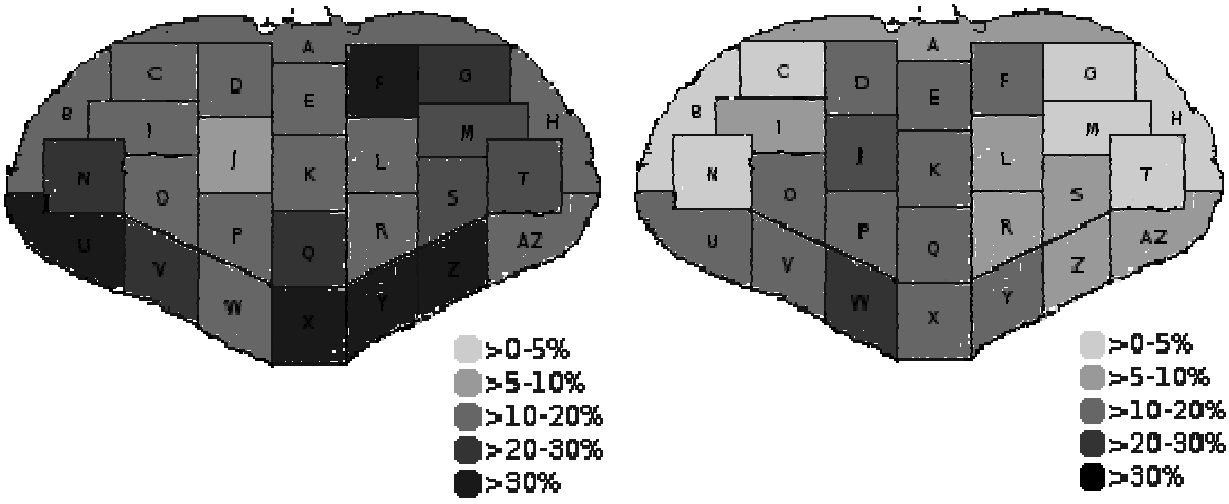

Figure 1. Prevalence of shell disease on carapaces of *Cancer pagurus* at Helgoland (a) and Bodo (b). Grey intensity of each scale shows occurrence of shell disease lesions in different regions (A-AZ) of carapaces (Helgoland N=36; Bodo N=54).

Temperature experiments

From animals of the temperature experiments I collected RNA samples to determine the gene expression level from three different tissues; muscle tissue (N=72), hepatopancreas (N=72) and hemolymph (N=72). I collected 216 RNA samples in total from all experiments which will be analyzed during the next months. Parallel to the sampling of the host reaction on experimental temperature I sampled the bacterial community of the biofilm and hemolymph of the host (total N=144). Despite the unnatural temperature conditions during the experiments all specimen (N=28) survived the slow acclimatization to 15.5°C. I could not observe any difference in shell disease prevalence on the carapace by comparing pictures made prior and after experimental treatment. Surprisingly, I did not observe any sign of pink crab disease which I usually found in *Cancer pagurus* of Helgoland during the summer.

The test trial with the temperature increase to 26°C was lethal for *Cancer pagurus* (N=2) and was terminated to avoid further sacrifices.

Biofilm sampling

I collected 80 biofilm DNA-samples of untreated animals as well as 140 biofilm samples of *Cancer pagurus* kept under different temperature conditions during the experiments. Samples will be analyzed with PCR-SSCP to closer determine variability and temperature dependence of the bacterial biofilm of *Cancer pagurus*.

Planned publications and cooperations

Planned Cooperations

The visit at Bodo gave me a great opportunity to discuss my work with different very experienced scientists in the field of population genetics, marine ecology and physiology in particular with Prof. Galice Hoarau and Prof. Mark Powell.

We planto continue our cooperative research on a new SNP discovery technique using ABI Solid machine. For this purpose, I collected samples from the local population of *Cancer pagurus.* We plan to collaborate by joining forces in bioinformatics by useing informatics resources from the University of Oldenburg and the Høgskolen of Bødo. This cooperation will give us the opportunity to integrate SNP analyses for *Cancer pagurus* using next generation sequencing into our project and will help to closer determine the population structure of *Cancer pagurus* as well as using SNP informations to learn more about the genetic basis of thermal adaptation of *Cancer pagurus*.

In addition, I discussed with Prof. Galice Hoarau and Prof. Mark Powell to closer determine

temperature effects on *Cancer pagurus* in Bødo not only in terms of genetic adaptation but also for physiological consequences. We will continue our discussion about future experiments depending on the results of the gene expression experiments.

Planned publications

The samples collected in Bødo will be integrated in at least 2 publications:

The first will deal with the population structure of *Cancer pagurus* among Europe shown by SNP and microsatellite DNA analysis.I plan to submit the manuscript to Molecular Ecology

The second will compare internal as well as external bacterial communities of *Cancer pagurus* between different local European populations with emphasis on local temperature conditions and disease status of the hosts. I plan to submit the manuscript to Journal of Experimental Biology

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