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Physiological and genetical adaptation to temperature in fish populations

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Abstract

The physiological and genetical adaptation of fishes to environmental temperatures has been studied by analyzing data concerning: (i) the oxygen binding properties of haemoglobin recorded during growth experiments on Atlantic cod, and (ii) the primary structure of haemoglobin (Hb) and lactate dehydrogenase (LDH) of several fish species living in polar and temperate areas. The results on the oxygen binding properties of cod's haemoglobin indicate that for this species a temperature of around 12°C is the most favourable one, irrespective of the haemoglobin genotype, and are in line with recent evidence challenging the existence of significant evolutionary differences between cod stocks in North Atlantic. The primary structures of both Hb and LDH from species living under temperate environments show a higher variability as compared to that from polar species, although the difference in the recurrent patterns of hydrophobicity between the two areas is much larger for Hb. These results highlight the dominant role of physiological and genetical factors in shaping the adaptation to temperature at the individual and at the species level, respectively.

Keywords: Gadus morhua; Temperature effects; Fish haemoglobin; Acclimation,recurrence quantification analysis

1. Introduction

The Atlantic cod populations settled along the Atlantic coast of Norway and in the Baltic and North Seas since a long time are known to show a polymorphic Hb-I with the genotypes Hb-I(1/1), Hb-I(2/2) and Hb-I(1/2) (Sick, 1965; Frydenberg et al., 1965). An increased frequency of the Hb-I (1/1) allele following the North–South cline (see Fig. 1), has been well documented and interpreted as the result of a temperature-induced genetical differentiation. In the last decade, however, studies on population dynamics based on mitochondrial DNA suggested that phenotypically distinguishable differences in cod stocks could be attributed to selective forces acting more at the physiological than at the genetical level (Árnason et al., 2000 and references therein). Among such forces temperature is of obvious importance since all key metabolic enzymes, particularly those linked to

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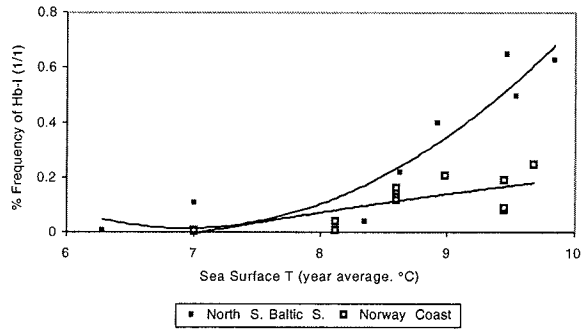

Fig. 1. Effect of the environmental temperature on the Hb (1/1) allele distribution in Atlantic cods. The data on the Hb-I allele are from Frydenberg et al. (1965) and Sick (1965) those on the sea surface temperature from the Global Ice and Sea Surface Temperature (GISST) data base (http://dss.ucar.edu/datasets/ds277.2/).

oxygen metabolism, are expected to respond to temperature variability (Pörtner et al., 2001 and references therein). Thus, the idea that environmental temperature constitute a primary factor in the aggregation of individuals physiologically more than genetically homogeneous, is now challenging the classical view which assigns a prominent role to the genetical component of temperature adaptation. To provide a contribution to this controversial issue, we used data concerning the growth performance and the functional properties of Hb-I from Atlantic cods acclimated at different temperatures and measured under a variety of conditions. The analysis was carried out by a combination of standard statistical methods.

We also took advantage of the availability of the primary structures of haemoglobin (Hb) and lactate dehydrogenase (LDH) from Antarctic fishes (notothenioids), in order to compare them with those of species living in temperate seas. The very low temperature of polar areas should operate as a dominant selection factor on the organisms living at extreme latitudes as a consequence of the constraints imposed on all metabolic activities (Pörtner et al., 2001). As for haemoglobin sequences, our aim was to investigate their variability under conditions in which the abundance of dissolved oxygen obscures the role of oxygen carriers. As for LDH sequences, a key enzyme in anaerobic metabolism, studying its variability is interesting per se, as well as for the background that a protein not involved in the aerobic metabolism may provide to the haemoglobin results.

The comparison of protein sequences from polar and temperate species was initially carried out by a standard best-alignment algorithm (Thompson et al., 1997) and the observed differences in the homology patterns were checked and qualitatively confirmed by a recurrence quantification analysis (RQA) (Webber and Zbilut, 1994), after coding the primary structures in terms of hydrophobicity profiles (Kyte and Doolitle, 1982). Since, due to its crucial role in protein folding, using hydrophobicity (instead of a literal code) focuses on amino acid substitutions related to substantial conformational changes, these results point to a possible dynamical (functional) interpretation of the observed structural differences.

2. Materials and methods

2.1. Physiological data

Growth and oxygen binding data were recorded using a batch of 126 Atlantic cods (growth exp. II) divided in two groups raised at 4°C and 12°C in the Institute of Marine Research (Bergen, NO), and a batch of 44 North Sea cods (growth exp. I) raised at four (4°C, 8°C, 12°C, and 15°C) temperatures at the Alfred Wegener Institute (Bremerhaven, FRG) for a 3 months period between 1999 and 2000. Only the blood samples of 16 individuals from growth experiment II met the quality and quantity requirements of the physiological measurements needed for the present work. A further growth experiment over 272 fishes acclimated at temperatures = 8°C, 12°C and 15°C was carried out in the Institute of Marine Research (Bergen) and gave highly consistent results (not analyzed here). In all cases blood was sampled from anesthetized (Metacainum, 70–90 mg/l) fish by cardiac puncture, and a fraction used for genetical characterization of each specimen by the distribution of Hb components through gel electrophoresis and isoelectric focusing (Sick, 1965). After washing and stripping of the

herythrocytes as described by Brix et al. (1998), haemoglobin (0.200–0.675 mg/ml) was equilibrated with 100 mM Hepes buffer + 100 mM NaCl at pH 7.5 and 8.0. A sample of 0.5 ml was then transferred to a modified glass tonometer thermostatted to 4°C and 12°C for equilibration with gas mixtures and analyzed at 541, 560 and 576 nm in a thermostatted scanning photometer (Beckman model 25) at the desired temperature according to Brix et al. (1998). A detailed account of the growth experiments and of the data collection procedures can be found elsewhere (Brix et al., submitted for publication, and references therein).

The functional features of haemoglobin were characterized by the oxygen affinity (P_{50}) and the cooperativity index (n_{50}) at half saturation with oxygen ($\bar{Y} = 50\%$), defined as

$$P = \ln(pO_2), \quad n = \frac{d(\ln[\bar{Y}(1-\bar{Y})])}{d\ln(pO_2)},$$

where pO_2 is the oxygen partial pressure and $\bar{Y} = (HbO_2)/(Hb + HbO_2)$ is the fractional saturation with oxygen. These parameters are considered faithful indicators of the global functional properties of haemoglobin and, in particular, the cooperativity index (n) is a measure of the sigmoidal shape of the binding curve and estimates the functional interaction of the ligand binding sites (Wyman and Gill, 1989).

2.2. Protein primary structures

The primary structures analyzed in this paper (Table 4) were collected from the SWISS PROT data bank (www.fishbank.org), and represent information available up to December 2001 on haemoglobins and LDHs from fishes living in polar and temperate areas.

2.3. Statistical analysis

2.3.1. Physiological data

Physiological data were submitted to inferential tests based on ANOVA and contingency tables. The choice of ANOVA was dictated by the need of taking into account at the same time the effect of different experimental conditions as genotype, temperature and so forth. In the case of multivariate dependent data (growth experiments), the model of choice was repeated measures analysis of variance in which the growth curves of single fishes were separately taken into account in order to analyze the significance of both acclimation temperature and genotype, together with the possible presence of nonlinear (multiplicative) interactions. In the case of oxygen binding data, a two-way general linear model was adopted.

Contingency tables are generally used to analyze nominal variables by examining the distribution of a "response" variable (Y) as conditioned by the level of a "factor" (X). In the present case, Y is the cluster assigned by a K-means algorithm (Everitt, 1980) to each individual on the basis of its physiological or growth performance, and X is the level corresponding to the acclimation temperature or to the genotype. The data were summarized by frequency counts for the response rates of the Y levels for each level of X, and appropriate chi-square tests were used to verify the null hypothesis, namely that the distribution of units into clusters is not related to X.

2.3.2. Protein primary structures

Since homology scores are computed for pairs of proteins, the lack of independence of these data inhibits the use of inferential statistics and we had to rely on simple descriptive statistics (mean pairwise homologies and variation coefficients).

As for % Determinism scores measured by RQA on hydrophobicity profiles, the possibility to rely on an independent score for each primary structure, allowed us to apply also Student's t-tests.

2.4. Recurrence quantification analysis

RQA is a nonlinear graphical technique useful to detect subtle although significant features in ordered numerical series (Eckmann et al., 1987) which, in the case of proteins, can be easily applied to the hydrophobicity profiles of the primary structures, namely to the ordered series of hydrophobicity values of the amino acid residues (Kyte and Doolitle, 1982) constituting the primary structure. For a protein of length L, one obtains first an "embedding matrix", whose rows are contiguous stretches of residues of given length (embedding dimension, ED)

overlapped by one element, and then a squared $(L-\mathrm{ED})*(L-\mathrm{ED})$ matrix of Euclidean distances between any pair of rows of the embedding matrix. From the distance matrix, a recurrence plot of the same size is immediately derived by darkening points whose coordinates correspond to rows in the embedding matrix (and hence to locations in the primary structure) having distance values lower than a predefined threshold (Radius) in the hydrophobicity space (see Fig. 5). The features of the distance function make the plot symmetric, with a darkened main diagonal marking the identity line. Among the numerical descriptors of recurrence plots introduced by Webber and Zbilut (1994) and used in fields ranging from molecular dynamics to physiology (Faure and Korn, 1997; Manetti et al., 1999), the so called % Determinism, showed able to pick peculiar features of specific protein classes (Zbilut et al., 1998; Zbilut et al., 2000; Giuliani et al., 2000). % Determinism is defined as the fraction of recurrent points adjacent to each other and forming lines of given minimal length (Line) in the direction of the main diagonal. In the case of proteins, it represents a measure of the degree of order of the hydrophobicity distribution along the primary structures. The RQA parameters used in the present work to calculate % Determinism, namely ED = 5, Radius = 3, and Line = 4, were empirically found as a good compromise upon consideration of the length of the proteins at hand and of sensitivity issues.

2.5. Software tools

The homology between primary structures was calculated by the CLUSTAL X program (Thompson et al., 1997). RQA was performed by the freeware made available by one of the authors at http://homepages.luc.edu/~cwebber.

The statistical packages used throughout this work were from the SAS Institute Inc., NC, USA.

3. Results

3.1. Physiological data

Fig. 2 shows the average increase in length measured over individual cods acclimated at four different temperatures (growth exp. I). The weight data collected under identical conditions produced essentially identical trends (not shown). Table 1 illustrates the effects of genotype/acclimation temperature on weight (and length) increase, both in terms of total growth (between-subjects effects) and of growth rate (within-subjects effects). Notice that in no case the genotype effect reaches statistical significance, while a marked effect of the acclimation temperature is evident both in term of fish size (between-subjects effects) and growth rate (within-subject effects).

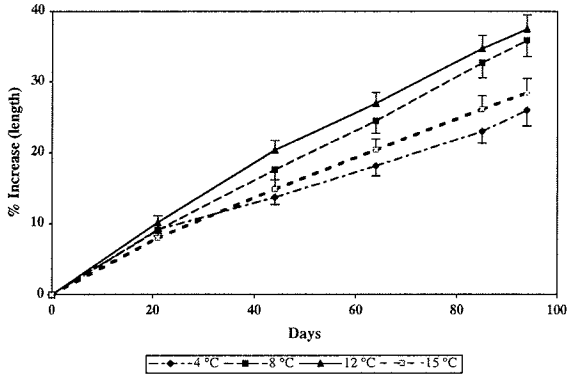

Fig. 2. Effect of the acclimation temperature on the average growth performance of Atlantic cod. Averages refer to 7, 11, 13 and 13 individuals acclimated at 4°C, 8°C, 12°C and 15°C, respectively. Vertical bar indicate standard errors.

Fig. 3 shows the distribution of individual North Sea cods acclimated at 4°C and 12°C (growth exp. II) in the space of the main functional parameters of haemoglobin, namely P_{50}, the oxygen affinity at 50% saturation, and n_{50}, the cooperativity index, measured at physiological pH. Essentially similar results were obtained at pH = 8.0 (not shown). To quantify the correlation among haemoglobin genotype, acclimation, and haemoglobin functional features, individual fishes were distributed into two classes, on the basis of an unsupervised clustering algorithm (K-means) acting over both P_{50} and n_{50}. By means of contingency tables, the distributions obtained by the unsupervised algorithm were tested for congruence with those "a priori" defined by haemoglobin genotype and acclimation temperatures. Under all conditions the unsupervised clustering based upon physiological parameters reproduced

Table 1
Sources of variability in growth data of Atlantic cods

	Weight	Length
(A) *Between subjects*		
Genotype	0.19 (0.66)	0.28 (0.60)
Acclimation	*11.71 (0.0001)*	*11.23 (0.0001)*
Genotype*Acclimation	1.51 (0.23)	1.54 (0.22)
(B) *Within subject*		
Time	*260.62 (0.0001)*	*780.83 (0.0001)*
Time*Genotype	0.11 (0.98)	0.19 (0.94)
Time*Acclimation	*19.75 (0.0001)*	*13.92 (0.0001)*
Time*Acclimation*Genotype	1.70 (0.07)	1.07 (0.39)

The table reports the F-values for the different effects and, in parenthesis, the corresponding p-values. Panel (A) refers to the averages shown in Fig. 2. In panel (B) the growth performance of individual fishes (statistical units) have been considered in terms of repeated measures (over the same statistical unit) at different times. Significant values ($p<0.05$) are in italic.

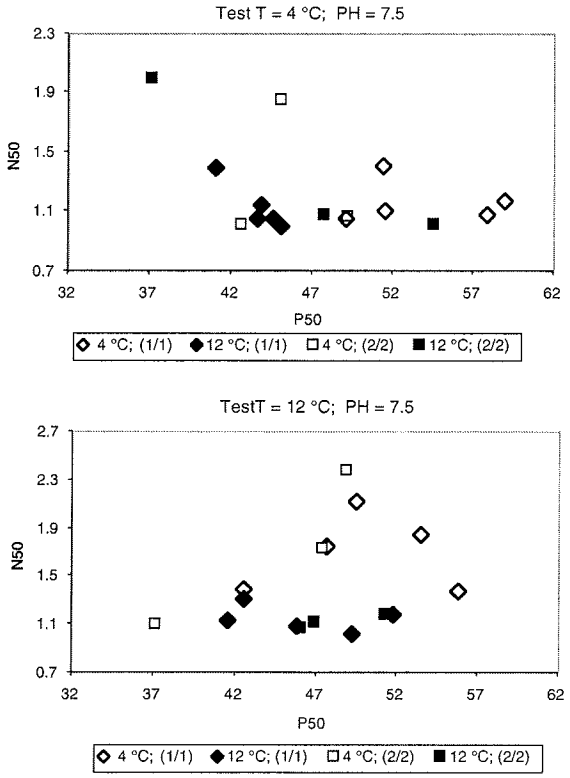

Fig. 3. Acclimation effects on the distribution of individual Atlantic cods based on oxygen binding parameters of haemoglobin. Open and closed and symbols refer to a total of 16 individuals raised in growth experiment II and acclimated at $T=4°C$ and 12°C, respectively. Their blood samples were tested at $T=4°C$ (upper panel) and 12°C (bottom panel). Squares and diamonds refer to 2/2 and 1/1 Hb-I genotypes (see the text for further explanation).

much better the acclimation groups than the genotypes (Table 2), indicating that the physiological adaptation is more relevant than the genetical one.

The latter result was reinforced by the application of a two-way analysis of variance to P_{50} and n_{50} values measured under all conditions ($T=4°C$, 12°C; pH = 7.5, 8.0). Table 3 shows that the genotype effect too reached statistical significance for P_{50} and n_{50} (in terms of interaction with acclimation temperature and by itself), although of much smaller magnitude than acclimation. At the test temperature = 12°C a large difference in statistical significance between acclimation and genotype effects is confirmed. With the only exception of a marginal significance ($p<0.04$) for the genotype*acclimation interaction as for P_{50}, however, the test temperature = 4°C showed no significant difference between genotype and acclimation.

3.2. Protein sequence data

The distribution of pairwise homologies of all the fish species listed in Table 4 is reported in Fig. 4A and illustrates the difference between polar and temperate species. While acclimation effects recorded in the lab concern responses to the environment on a relatively short time scale (duration of the growth experiments), comparing protein sequences of species living in different natural environments provides a much wider temporal and spatial perspective: in this case, the observed differences accumulate in millions years on the basis of mutation/selection mechanisms as well as of purely random genetical drift.

Table 5 shows that, for both Hb and LDH, the average pairwise homology between sequences of polar fishes is pretty higher than that of fishes living in temperate areas, while the variability of the pairwise homology is much larger in the temperate area. These differences are well summarized by the variation coefficient (variance/mean) also reported in Table 5. The increased

Table 2
Contingency analysis of physiological parameters with acclimation temperatures and genotype in individual Atlantic cods

Parameters for unsupervised clustering (experimental settings)	Congruence with acclimation T	Congruence with genotype
P_{50}, n_{50} (pH = 7.5, 8.0; T = 4°C)	0.0005 (*)	0.789 (*)
	0.002 (**)	0.790 (**)
P_{50}, n_{50} (pH = 7.5, 8.0; T = 12°C)	0.050 (*)	0.163 (*)
	0.078 (**)	0.255 (**)

An unsupervised clustering (K-means) of individual fishes was driven by haemoglobin functional parameters (P_{50}, n_{50}) measured at both pH = 7.5 and pH = 8.0 for each of two temperatures (4°C and 12°C). The results were tested for congruence with the a-priori grouping of fishes based upon their acclimation temperatures (T = 4°C and 12°C) and genotypes (Hb-I(1/1), Hb-I(2/2) and Hb-I(1/2)). Columns 2 and 3 contain the p-values of two Chi-square tests: the Likelihood Ratio (*) and the Pearson test (**).

Table 3
Source of variability in the oxygen binding parameters of cod's Haemoglobin

	PH = 7.5		PH = 8.0	
	P_{50}	n_{50}	P_{50}	n_{50}
(A) *Test temperature=12° C*				
Genotype	1.03 (0.33)	*5.13 (0.04)*	*8.00 (0.01)*	0.02 (0.88)
Acclimation	0.10 (0.76)	*6.71 (0.02)*	*15.82 (0.002)*	*11.35 (0.005)*
Genotype*Acclimation	3.20 (0.10)	*7.64 (0.02)*	0.23 (0.64)	0.01 (0.91)
(B) *Test temperature=4° C*				
Genotype	1.20 (0.29)	1.42 (0.26)	0.72 (0.41)	1.63 (0.22)
Acclimation	3.76 (0.07)	0.00 (0.96)	3.59 (0.08)	4.72 (0.05)
Genotype*Acclimation	*5.22 (0.04)*	0.06 (0.80)	0.10 (0.76)	0.83 (0.38)

The data refer to the same individuals shown in Fig. 3. The table reports the F-values for each factor and combination of factors and, in parenthesis, the corresponding p-values. Significant values ($p < 0.05$) are in italic.

homology/decreased diversity is more evident in haemoglobin (aerobic metabolism) than in LDH (anaerobic metabolism), indicating a stronger effect of temperature on the oxygen transport system than on a key enzyme of the anaerobic metabolism. These results show: (i) that extreme temperature conditions reduce the room for random genetical drift in polar species with respect to the temperate ones and (ii) the existence of a genetical component in temperature adaptation of fish species shape the limited variability of polar species.

Sequence comparison studies, however, while allowing for a general appreciation of the amount of genetical variability, do not give direct insight into the functional consequences of such variability in terms of proteins structure and function. Since RQA based descriptions of proteins are strictly related to important functional properties like relative stability and structural order (Giuliani et al., 2002), we complemented the sequence comparison approach by studying the distribution of hydrophobicity along the primary structures, as provided by the main RQA index, % Determinism. Fig. 4B show that the difference between polar and temperate areas lies, for both Hb and LDH, in a higher variability in the temperate area, indicating the existence of stricter constraints on the distribution of hydrophobicity patches in the primary structure of polar proteins. At difference with LDH, however, polar haemoglobins also show a significantly higher mean Determinism than the temperate ones (47.62 vs. 38.74, t-statistic = 2.54, $p < 0.03$). Although a detailed interpretation of these data seems premature, it is worth stressing that a similar analysis carried out

Table 4
Protein sequences of fish species considered in this work

Polar	Temperate
Haemoglobin (alpha chain)	
Notothenia coriiceps (P10777)	*Salmo salar* (P11251)
Cygnodraco mawsonii (P23016)	*Nothotenia angustata* (P29624)
Gymnodraco acuticeps (P29623)	*Anguilla anguilla* (P80945)
Pagothenia bernachii (P80043)	*Cyprinus carpio* (P02016)
	Salmo gairdnerii (P02019)
	Squalus acanthya (P07408))
	Gadus morhua (O42425)
Lactate dehydrogenase	
Chaenocephalus aceratus (AAC63277)	*Dissostichus mawsoni* (AAC63285)
Champsocephalus gunnari (AAC63282)	*Eleginops maclovinus* (AAC63283)
Chionodraco rastrospinosus (AAC63287)	*Fundulus heteroclitus* (AAC06176)
Gobionotothen gibberifrons (AAC63281)	*Paranotothenia magellanica* (AAC63284)
Harpagifer antarticus (AAC63278)	*Petromyzon marinus* (P33571)
Lepinodonotothen nudifrons (AAC63286)	*Sphyraena argentea* (U8000)
Notothenia coriiceps (AAC63280)	*Squalus acanthias* (P00341)
Parachaenichthys charcoti (AAC63279)	

In parenthesis the code of the corresponding primary structure in the SWISS Prot (P/U/O) or GENE (AAC) Data Banks are provided.

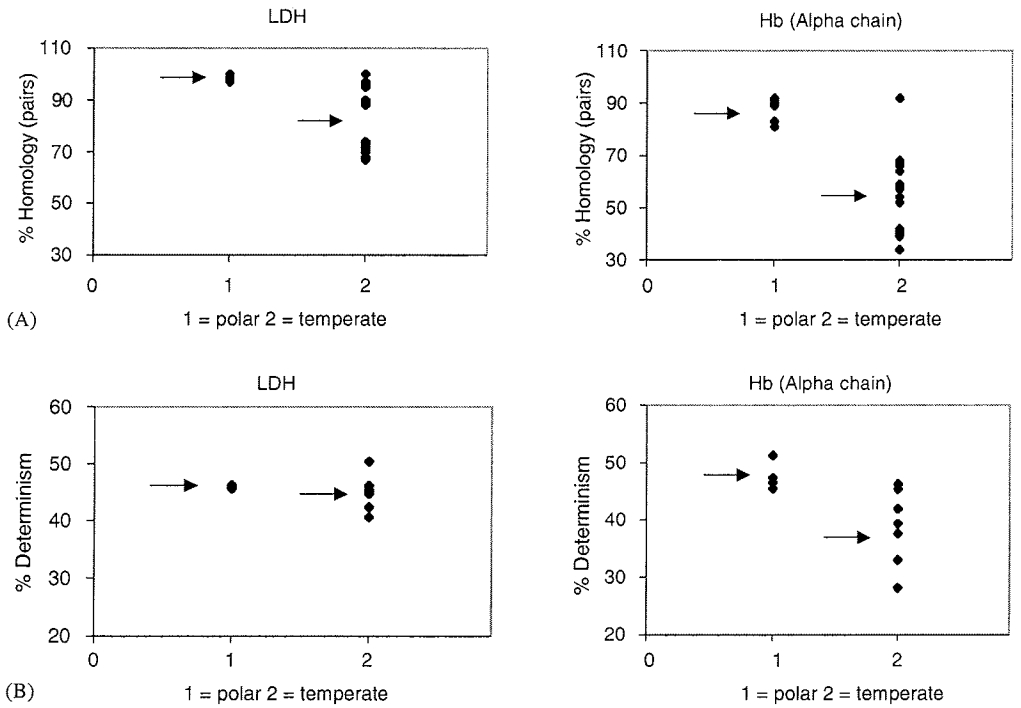

Fig. 4. Structural variability in the primary structure of fish proteins. (A) Pairwise homologies calculatd by a best alignment (CLUSTAL) algorithm. (B) % Determinism in recurrence plots (see Fig. 5). All the sequences listed in Table 4 have been analyzed by best alignment and RQA algorithms, and the results reported in the upper and bottom panels, respectively. The arrows indicate mean values. The actual figures of the mean values, together with the variation coefficients, are listed in Table 5.

Table 5
Variability in fish haemoglobins and lactate dehydrogenases

	Mean pairwise homology	Mean Determinism
Hb (α-chain)		
Polar	87.67 (0.24)	47.62 (0.14)
Temperate	55.48 (3.21)	38.74 (1.12)
LDH		
Polar	98.46 (0.04)	45.97 (0.001)
Temperate	79.03 (1.57)	45.16 (0.12)

The table refers to data (reported in Fig. 4) collected for the species in Table 4. Pairwise homology and % Determinism were calculated as explained in Section 2. The variance/mean (variation coefficient) values are in parenthesis.

over more than a thousand heterogenous proteins (to be submitted) highlighted a strong positive correlation between the amount of % Determinism and the propensity to undergo both intermolecular (aggregation) and intramolecular (folding) protein–protein interactions. Curiously enough, this tendency is paralleled by an increase in structural disorder and flexibility (native misfolding (Dunker et al., 2002) of the corresponding 3D structure. This could suggest a higher flexibility of polar haemoglobins with respect to the temperate ones.

4. Discussion

The first conclusion emerging from the growth data over Atlantic cods analyzed in this work is that 12°C is by far the most favourable temperature, as compared to both colder and warmer ones, irrespective of the Hb genotype (Fig. 2 and Table 2). Concerning the functional features of Hb from individuals acclimated at 12°C and 4°C, both n_{50} and P_{50}, when measured at 12°C, showed a highly significant difference between the two acclimation temperatures (Table 3A), especially at pH = 8.0 ($p < 0.002$ for P_{50}, $p < 0.005$ for n_{50}). At the same test temperature (12°C) and at pH = 7.5, also the genotype effect reached statistical significance, albeit of much lesser magnitude than the acclimation, for n_{50} and P_{50}. At the test temperature = 4°C (Table 3B) no significant effect of either acclimation or genotype (independent of each other) could be detected. The situation may be rationalized upon considering the average enthalpies of oxygen binding calculated over the very same data by the Van 't Hoff equation at 50% saturation (Brix et al., submitted for publication). At pH 7.5 the enthalpy values are of opposite sign at the two acclimation temperatures, namely 1.53 and 0.71 KJ/mol for the 1/1 and 2/2 genotypes, respectively, at acclimation $T = 4$°C, and -1.11 and -0.65 KJ/mol at acclimation $T = 12$°C. Assuming, on the basis of Fig. 2, $T = 12$°C as the optimal growth condition for Atlantic cods, it becomes clear that the haemoglobin of cods acclimated at 4°C shows a higher affinity for (and hence a facilitated uploading of) oxygen at higher temperatures ($\Delta H > 0$), while the affinity of cods acclimated at 12°C would decrease at higher temperatures ($\Delta H < 0$). Since the same trend (although to a different extent) is common to both genotypes, this is highly suggestive of a physiological adaptation. Thus, at the level of a single, "temperate" species, *Gadus morhua*, it seems fair to state that our data indicate an individual, physiological adaptation mainly based on not-genetical effects, with a statistically much less significant contribution of the haemoglobin genotype.

Concerning the comparison of protein sequences from several species living in polar and temperate environments, our main results were: (i) the higher variability of the primary structures of both Hb a-chains and LDH among temperate species, and (ii) the higher value of the mean pairwise homology observed in the polar species (Fig. 4A). While the former result may be intuitively linked to the more stringent selection constraints imposed by the very low temperatures of the polar regions, this is not so for the latter result. Moreover, as for haemoglobins, it cannot be easily reconciled with the idea that the high level of dissolved oxygen in the polar seas should obscure the metabolic importance of the oxygen carrier, and hence reduce the selective power of mutations. Bargelloni et al. (1998), however, in their careful analysis of the haemoglobin loci in notothenioids, found an unexpectedly high value of the KA/KS ratio (where KA = nonsynonymous

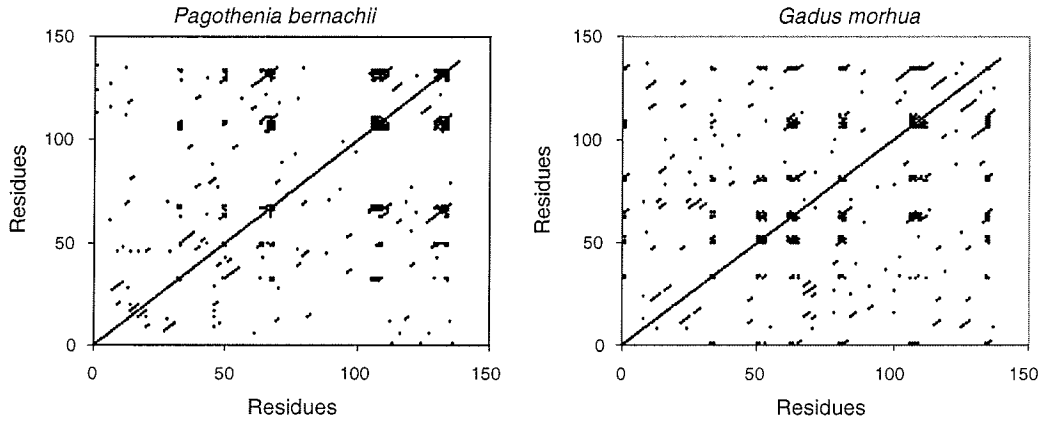

Fig. 5. Typical recurrence plots from the hydrophobicity profiles of the haemoglobin α-chain in a polar (left) and temperate (right) species. % Determinism was 46.5 and 39.3 in the left and right panel, respectively. All recurrence plots in this work have been calculated as explained in the Methods section, using ED = 5 and Line = 4 in the RQA.

and KS = synonymous substitutions): this finding was related to vestigial remains of variability phenomena dating back to the time when notothenioid ancestors used to live in temperate environments. This type of explanation might also be used for the large homology observed in the haemoglobin sequences, provided that one accepts, as a corollary, that the only haemoglobin component nowadays surviving in notothenioids blood, besides a vestigial role as oxygen carrier, is endowed with functions highly dependent on temperature and demanding some finely tuned (and conserved) structural features. In any case, the different degree of protein homology between polar and temperate species points to the genetical adaptation of these species to environmental temperature.

The observed differences in the amount of genetical variability between polar and temperate species provided by homology data are paralleled by differences in the "function-oriented" structural description of protein molecules provided by % Determinism, thus opening the way to a mechanistic interpretation at a molecular level. In order to further exploit this approach, fish LDH seems an ideal system, due to the presence in a number of distinct fish families of variable locations affecting the catalytic properties of fully conserved active sites (Fields and Somero, 1998). Moreover, recent results on the relation between the sequence complexity and the conformational flexibility of proteins (Chung-Jung et al., 2001) allow some speculations concerning polar haemoglobins. The essence of these ideas (Dunker et al., 2002, Giuliani et al., 2002) is that a given % Determinism measured over the hydrophobicity profile (Fig. 5) is (somehow counter-intuitively) an index of molecular flexibility and that conserved deterministic patterns related to the hydrophobic core, as in the case of polar haemoglobins, could indicate a peculiar type of molecular disorder facilitating functionally relevant conformational transitions.

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