The Journal of Experimental Biology 210, 1847-1857
Published by The Company of Biologists 2007
doi:10.1242/jeb.002717

Commentary

Molecular and cellular studies in evolutionary physiology of natural vertebrate populations: influences of individual variation and genetic components on sampling and measurements

Mikko Nikinmaa* and Wolfgang Waser

Centre of Excellence in Evolutionary Genetics and Physiology, Department of Biology, FI-20014 Turku, Finland

*Author for correspondence (e-mail: miknik@utu.fi)

Accepted 12 March 2007

Summary

Studies combining ecological, genetic and physiological approaches are needed in evolutionary biology. Although the combination of approaches has been emphasized, such studies have been rare with regard to molecular and cellular studies on natural vertebrate populations. The major reasons for this are that the generation time of vertebrates is long and it is difficult to find a molecular or cell physiological measurement that is both relevant for the fitness of the population and can be repeated an adequate number of times to enable estimations of individual variability. The paucity of suitable physiological parameters is partly due to the fact that most physiological studies have not been directed towards understanding the behaviour of populations but towards understanding the basic mechanisms of the function of individuals. Also, physiological measurements that appear most relevant from the point of view of evolutionary studies are often integrative functions, composed of the function of many genes. When dissecting the integrative functions into components, it is often observed that the same integrative response can be achieved *via* different routes, i.e. changes in the responses of different genes. To enable cellular and molecular physiological studies to be increasingly combined with ecological and genetic studies, it is important that such studies include and report individual variability and that the sample size is increased. In addition, more sophisticated statistical methods should be used than is traditionally done, and when the function of most genes in the integrative response are not known, techniques such as QTL mapping should be used. Hitherto in vertebrates, the methodology has mainly been used in production biology (e.g. meat or milk production). With regard to combining genomic and physiological studies, one must bear in mind that the massive datasets associated with genomic studies need to be further enlarged to enable estimates of individual variation. It is also important to remember that microarray and proteomic data give the levels of mRNA and proteins, respectively. Since the function of the protein can be regulated independently of its transcription or its level in the cell, direct physiological measurements are also needed if estimations of protein activity in the individuals of a population are wanted.

Key words: evolution, genomics, natural populations, QTL mapping, selection.

Introduction

Evolutionary response to natural selection requires heritable genetic variation in the trait subject to selection. Most traits – both structural and functional – in natural populations are heritable. Many of the traits affecting the mean fitness of a given population are physiological responses of individuals, occurring as a result of gene expression at specific times of development or as a result of specific environmental influences. Thus, whenever responses of populations to environmental factors are evaluated, evolutionary biology calls for studies assessing physiological responses, their regulation and variability within populations (see Arnold, 1983; Bennett, 1987), to complement quantitative genetic studies and studies assessing genetic variation in neutral molecular markers. While such studies belong to the realm of comparative physiology, physiological studies, especially those including cellular and molecular components, have rarely incorporated ecological or genetic approaches, although the use of physiological studies in evolutionary biology has repeatedly been emphasized (e.g. Arnold, 1983; Bennett, 1987; Garland and Carter, 1994; Bennett and Lenski, 1999; Feder et al., 2000; Irschick and Garland, 2001; Feder, 2002; Loeschke et al., 2004). Although the presence and importance of individual variation in physiological traits within animal populations has been pointed

out (Arnold, 1983; Bennett, 1987), this individual variability has remained ‘an underutilized resource’ [as given in the original title of Bennett (Bennett, 1987)]. The aim of the present contribution is to illuminate some problems and present some ideas associated with integrating comparative and environmental physiological studies of vertebrates at molecular and cellular levels with genetic and ecological studies of natural populations to further advance evolutionary biology.

Organisms with a short generation time, such as bacteria (e.g. Bennett and Lenski, 1999) and *Drosophila* (e.g. Feder et al., 2002), are often used to study the combination of physiological responses (traits), their genetic variation and evolvability, since the responses of multiple generations to selective forces (e.g. environmental conditions) can be followed in selection experiments relatively easily and rapidly. However, even though the use of vertebrates in evolutionary physiological studies is hampered by the fact that their generation times are long, making it difficult to follow the heritability of responses across generations, there are some reasons, why vertebrate studies are important. First, much of the ecological and evolutionary literature is on vertebrates, and therefore it is helpful if, in addition to studies on invertebrates with short generation times, studies on vertebrates are carried out so that the conclusions based on invertebrates can be related to vertebrate systems. Second, vertebrates are much more visible than invertebrates, whereby they appear more often in public conservation interests. Third, some vertebrates are economically important or used in production biology, both in agri- and aquaculture. Fourth, mammalian studies are considered to be especially relevant for human systems. Notably, medical studies are the best source of genetic information on vertebrates. Apart from medical studies, there are very few functional studies (especially at the cellular and molecular level) on individual genetic variation that have been frequently cited, even within a single generation of a population, although individual variability is important for any population response. This is probably because many of the vertebrate studies with information about differences between individuals are on non-mammalian animals such as lizards and snakes (e.g. Bennett, 1980; Arnold, 1983). Notably, however, Garland’s group have subjected mice to controlled treadmill exercise over many generations, and have followed the performance of animals, focusing additionally on several components of muscle function (Dumke et al., 2001; Gomes et al., 2004; Bronikowski et al., 2006; Garland and Kelly, 2006). Examples of cellular and molecular studies on non-mammalian vertebrates that have considered interindividual differences include those of Crawford’s group, who have studied the evolution of gene expression in *Fundulus heteroclitus* (Whitehead and Crawford, 2006a; Whitehead and Crawford, 2006b).

Why are studies of individual variation in cellular and molecular physiology of vertebrates rare?

There are two major reasons why data reporting individual variation in cellular and molecular physiological responses are scarce. First, in physiological studies concerned with elucidating basic mechanisms of function, variation complicates interpretations, and is thus unwanted. As pointed out by Bennett (Bennett, 1987), however, extreme values of any measured parameter should be as real as those near the mean, and can be important whenever a population responds to a change in environmental conditions. Importantly, the response must be such that the difference between individuals is greater than the variation of the response within an individual (for details, see Bennett, 1987). This causes a problem whenever only one sample can be taken from an animal, since intra-individual variability cannot then be studied.

One way of diminishing individual variation is the use of inbreeding. Inbred strains of, e.g. mice and rats, are extensively used. For example, the web site http://www.informatics.jax.org lists more than 400 inbred strains of mice and more than 200 inbred strains of rats. Since these strains have been selected and bred to express a variety of phenotypes, they are well suited for research in basic functionality. However, some of their phenotypic variability may never be naturally found. Alternatively, very specific human cell lines can be used in the functional studies. In evolutionary studies, the use of several inbred lines (lines started simultaneously from an outbred population) can also be a powerful method. Differences between lines are genetic, whereas all variation within lines must be environmental. Inbreeding is also an important tool in QTL (quantitative trait locus) mapping with natural populations (Slate, 2005). Since natural populations are outbred, however, they are characterized by individual variability. Such individual variability is important in any evolutionary study on natural populations (e.g. Arnold, 1983; Bennett, 1987), and also when evaluating environmental risks caused by contaminants. The variation of acute toxicity of dioxin to inbred rat strains by more than 1000-fold is an example of how different the responses of inbred strains of animals to contaminants can be (Pohjanvirta and Tuomisto, 1994; Tuomisto et al., 1999).

Secondly, to address the genetic basis of individual variation in the physiological properties of a population properly (here, the genetic basis refers to heritability of physiological responses), large sample sizes are needed; in the worst case, i.e. if the heritability of response is low, hundreds of individuals may need to be analyzed. It is very difficult to find a relevant physiological measurement for which this could be accomplished in a reasonable time. On the higher integrative level of organs or whole animals, repeating an experiment several hundred times seems hardly possible, so that often one decides the measurement on the basis of what can be done, without knowing the functional significance or the genetic basis of the response. If the trait is not or only weakly selected for, this has little effect on the conclusions reached. However, in the case of strong selection, knowing the fitness consequences of the measured property is helpful for evaluating whether that property is selected for or not selected for, but covaries with a strongly selected trait. In addition, the properties measured are often subjective, which may lead to

erroneous conclusions, if there is a difference in how the study object and the experimenter sense the property. As an example, the visual cues important for birds are different from those of man. Birds have the ability to detect ultraviolet light (Bennett and Cuthill, 1994; Goldsmith, 1994). UV vision has, consequently, been shown to be important in seeking of prey by predators (Viitala et al., 1995), sexual selection (Siitari et al., 2002) and foraging (Siitari et al., 1999). This example shows that once physiological and ecological approaches have been suitably integrated, evolutionary explanations for a property (in this case for UV vision in birds) can be found.

Physiological measurements on integrative functions are most often used in ecology and evolutionary biology, but are themselves the result of the function of many genes

One of the problems associated with physiological evolutionary studies is that it becomes increasingly difficult to associate the changes observed in complex systems with specific variation in molecular function and gene expression. A change observed in a complex system may be a result of different molecular and genetic responses in different cases, since most phenotypic traits are polygenic. This problem is clearly laid out in ecotoxicological literature: it is relatively simple to associate a toxicant with its molecular action, but much more difficult to show that a specific molecular response (biomarker response) would be behind an ecosystem level response to a pollutant (e.g. Walker et al., 2006).

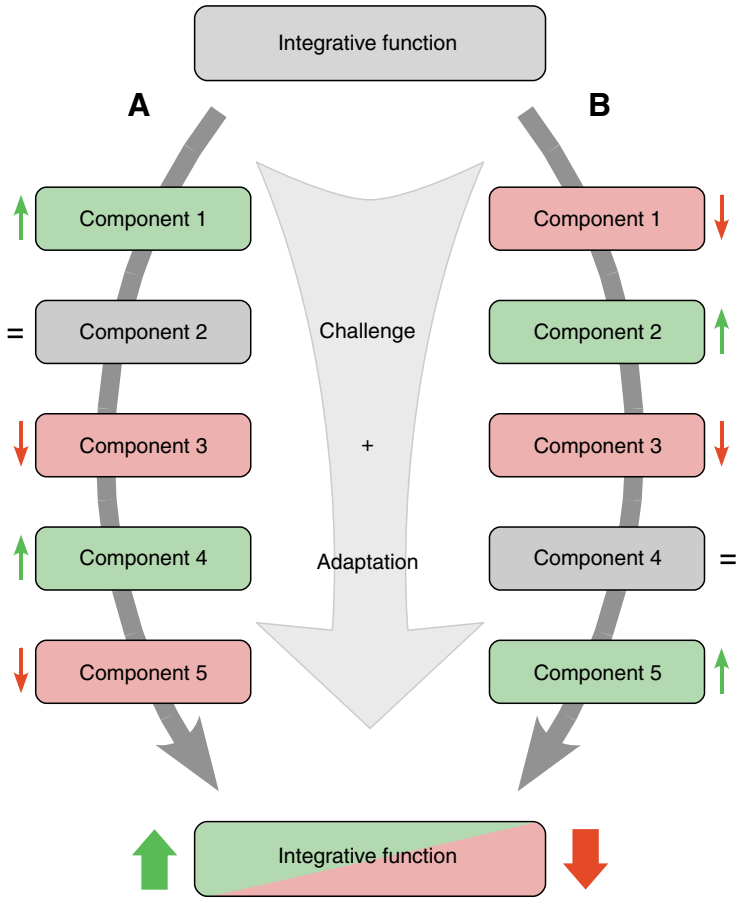

Fig. 1. The same response of an integrative function, consisting of several components (polygenic), to an environmental change can be obtained, even if the different components (genes) respond to the challenge differently.

One of the physiological measurements much studied in evolutionary context is the (standard) metabolic rate. One reason for this is that physiological ecology has traditionally been specifically focussed on energy allocation. Furthermore, in ectothermic animals the standard metabolic rate appears to be related to Darwinian fitness (e.g. Nespolo et al., 2003). Also, individual variation in standard metabolic rates of ectothermic vertebrates has been studied in some detail (e.g. Pough and Andrews, 1984; Steyermark, 2002; Steyermark et al., 2005). The standard metabolic rate is an integrative function that combines membrane and cellular functions from different tissues with different metabolic rates, in the absence of visible muscle work and food processing, and at the thermoneutral zone for endotherms (Rolfe and Brown, 1997). Thus, although in many instances metabolic rate is a highly useful measurement, e.g. when studying the energetics of ecosystems, it combines the function of many metabolic pathways (and many genes). As such, it cannot therefore give information about the evolution of genes involved in the responses leading to changes in metabolism. In evolutionary studies, however, it is often pointed out that the integrative functions can be strongly selected for, but each of the components forming the integrative response will be less selected for (e.g. Garland and Kelly, 2006). In part this is because the same performance/response (under given conditions) can be obtained with several different changes in the components leading to the performance/response, i.e. the geometry of the genetic changes can be different (see Fig. 1). Many useful characteristics of complex traits can be evaluated, e.g. by QTL mapping (Slate, 2005), but as stated by Clark et al. (Clark et al., 2006), the molecular mechanisms behind complex traits quite often remain elusive. A detailed understanding of the mechanism requires (1) that QTL mapping can identify the areas of the genome that are involved in the responses, (2) that detailed genomic studies identify the (normally many) genes that are involved in the QTL, and (3) that physiological studies to show how the gene products function in different environments or during different life stages (see also Erickson et al., 2004; Slate, 2005; Zeng, 2005).

Detailed studies by Oleksiak and coworkers (Oleksiak et al., 2001; Oleksiak et al., 2002; Oleksiak et al., 2005) have dissected the energetics of the killifish *Fundulus heteroclitus* heart into several components. Their experiments combined genomic (cDNA microarray) with more traditional approaches, including detailed statistical analyses. The results

show that there is large between-individual variation in mRNA levels of a number of genes associated with cardiac metabolism in *F. heteroclitus* populations. When functional differences in cardiac metabolism were investigated (Oleksiak et al., 2005), fishes fell in three groups having different aspects of cardiac function – or enzymes of energy production (glycolytic, Krebs cycle or oxidative phosphorylation) – showing clearly discernible differences in the mRNA levels between the groups. The three different aspects of energy utilization in the heart have different influences on the physiology of individuals under different conditions, e.g. temperature and oxygenation. The metabolic differences may also affect the reproductive success of individuals in different environments, but common garden experiments (studies where known populations are subjected to environmental changes in a controlled fashion) are required to assess this. This being the case, it becomes very important that (1) the data on physiological responses are gathered from the same individuals that are being used for genetic studies, (2) the same function is assessed both in the laboratory and in the field, in order to take possible differences between laboratory and field responses into account (e.g. Irschick, 2003), and (3) a more sophisticated statistical treatment of data is used than has traditionally been the case for physiological studies. It is important when considering evolutionary responses that phylogeny is properly taken into account in the data analysis (e.g. Garland et al., 2005).

Important new insights into the process of evolution can be provided by combining physiological responses at the cellular level, their effects on fitness, and their possible effects on the population

The above example shows that an integrative function, such as the metabolic rate, should be dissected into more detailed components if its functional correspondence to gene expression and evolution is sought. In natural populations of vertebrates, physiological function, its inheritance and its possible effects on the evolution of populations have been documented most extensively for the lactate dehydrogenase (LDH) enzyme isoforms of the killifish *Fundulus heteroclitus*, a fish species living at a wide variety of temperatures along the east coast of North America (e.g. Powers et al., 1991). A reason for studies on LDH in this context is that it is an enzyme involved in energy metabolism, which is highly temperature-sensitive. In this species, the proportions of two heart-type LDH enzyme isozymes (which have different temperature-dependencies of properties) show marked variation across the latitudinal range inhabited by the species. The rate of heart-type LDH transcription in populations living at higher latitudes was greater than the transcription rate of the enzyme at lower latitudes (Crawford and Powers, 1992). One point raised in this study was that it appears likely that regulatory sequences controlling the expression of genes may be important in the evolution of physiological traits. In fact, it is possible that many of the environmental effects that are seen as phenotypic plasticity, and traditionally not considered heritable, are the result of gene regulation at control sites outside the expressed genes, as has been shown recently for some traits associated with domestication in maize (Clark et al., 2006) (Fig. 2).

The proportions of the heart-type enzyme isoforms affect physiological traits such as red cell ATP concentration, with a consequence that haemoglobin–oxygen affinity is significantly affected (Powers et al., 1979). It was further suggested that several characteristics affecting the success of populations, such as adult swimming speed, time taken to hatching, developmental rate and survival at high temperatures may be associated with this difference (Powers et al., 1991). It should be noted that, while the above example is the most complete one for vertebrates, there are several investigations using other types of organisms, ranging from bacteria to invertebrates such as *Caenorhabditis elegans* and *Drosophila* species (e.g. Feder et al., 2000; Montooth et al., 2003; Melvin and Ballard, 2006), which have combined physiological and genetic studies. Notably, quantitative genetics has been used to help dissect *Drosophila* metabolic energy production into its components (Montooth et al., 2003).

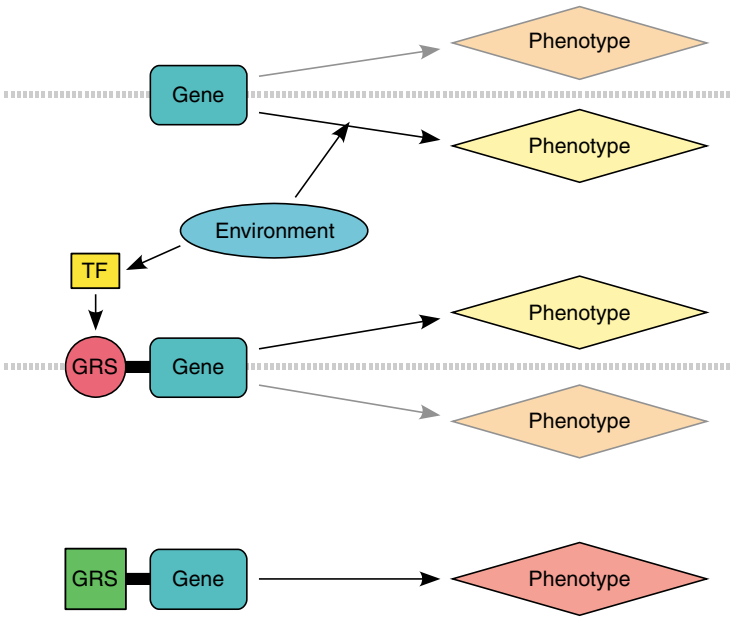

Fig. 2. Environmental effects on the genotype can be of genetic origin. If environment affects the level and consequent binding of a transcription factor (TF) to the gene regulatory sequence (GRS), for example, then different phenotypes are observed depending on the environmentally induced differences in the induction of the gene. If the structure of the gene regulatory sequence changes, binding of the transcription factor can be affected, leading to changes in gene expression and consequent changes in the phenotype.

While large datasets are necessarily generated in comparative functional genomics studies, addressing the role of individual variation in evolutionary genomic studies requires that datasets are further enlarged using several biological replicates

From the preceding sections it is clear that one of the major difficulties in utilizing physiological measurements at the cellular and molecular levels in vertebrates in evolutionary studies is the difficulty of finding a relevant measurement that could be repeated an adequate number of times to achieve a reasonable estimate of individual variation and its heritability. It is worth noting that genomics studies necessarily involve large datasets. For example, in the cDNA microarray studies on mudsuckers, *Gillichthys* (Gracey et al., 2001), zebrafish (Ton et al., 2002), the killifish *Fundulus heteroclitus* (Oleksiak et al., 2002) and rainbow trout (Koskinen et al., 2004; von Schalburg et al., 2005), expression of thousands of genes at mRNA level was investigated. To make it easier to manage the data analysis, general workload and economic constraints of the studies, a commonly used solution to limit the size of dataset was to study either only a few individuals or their pools. Early microarray studies especially used data from only one individual/pool per treatment, and even now it is common to only use 3–4 individuals/pools in 'biological replication'. For example, in the study of Koskinen et al. (Koskinen et al., 2004), four individuals were pooled to obtain the microarray results. If virtually all individuals respond to an environmental change (e.g. a change in temperature) in the same way, it is probable that differences in gene expression associated with the environmental change would be seen even when very low numbers of individuals are used – maybe even when information is available on one individual predating the change and on another after the change. If, however, there is much variation between individuals, the likelihood of detecting the response to an environmental change, and the possibility of estimating the occurrence of the response in different animals with data obtained from only a few individuals diminishes markedly (Fig. 3). Consequently, an increase in resolution of response at the individual level leads to a decrease in the likelihood of observing the response at the population level, even though heritable variation within a population is needed for evolutionary changes. The point that biological replication is very important in any environmental and evolutionary use of microarray studies has already been noted (Gracey and Cossins, 2003; Cossins and Crawford, 2005). The use of cDNA microarrays in evolutionary biology, and the requirements for both technical and biological replication, have recently been reviewed (Whitehead and Crawford, 2006b).

There is one profound difference between the cDNA microarray and candidate gene approaches to study genetic responses to environmental changes. Whereas the candidate gene approach is hypothesis-driven and requires an *a priori* idea of which genes may be important in the environmental response, genomic screening offers the possibility that a change in mRNA level coding for unexpected proteins, for example, is detected (see also Cossins et al., 2006). The approaches can be combined, however. cDNA microarray work may give a possible set of candidate genes, the expression of which can then be studied in more detail. As an example, Derome et al. (Derome et al., 2006) used cDNA microarray methodology to study two sympatric whitefish ecotypes, and found candidate genes associated with swimming activity and energy metabolism. While cDNA microarray studies, i.e. transcript profiling, are best carried out on model species with completely sequenced genomes, lack of sequence information is not a complete barrier to progress (Gracey et al., 2001; Gracey and Cossins, 2003). Also, recent work suggests that cDNA microarray methodology can be used to help assess whether population differences are caused by genetic drift or selection (Whitehead and Crawford, 2006a).

Evaluating responses to environmental changes requires direct physiological measurements in addition to genomic studies

It should always be remembered that cDNA microarray studies show changes in the levels of mRNA. In many cases, such changes indicate changes in the total activity of the

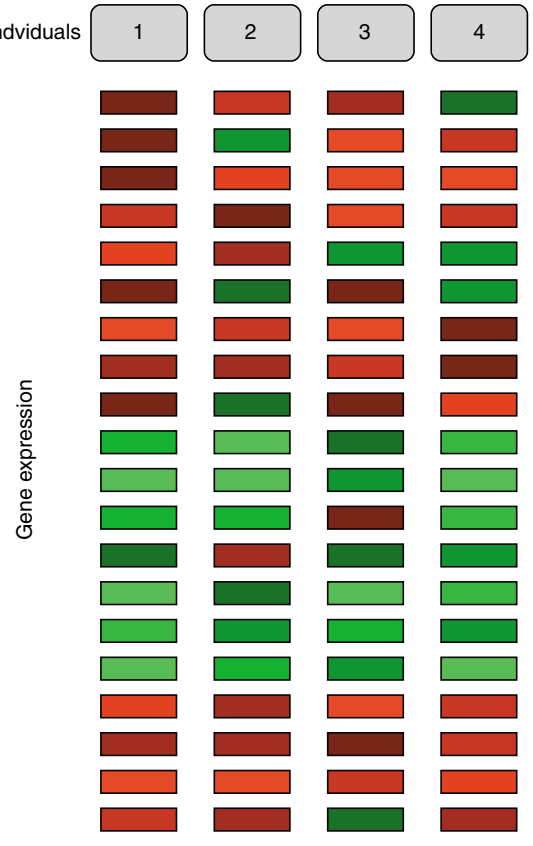

Fig. 3. If the mRNA levels of different individuals respond differently to an environmental change (as given in the figure by different hues of red and green), and the number of analyses from different individuals is not adequate, then a response that is not clearly seen in virtually all individuals may remain undetected,.

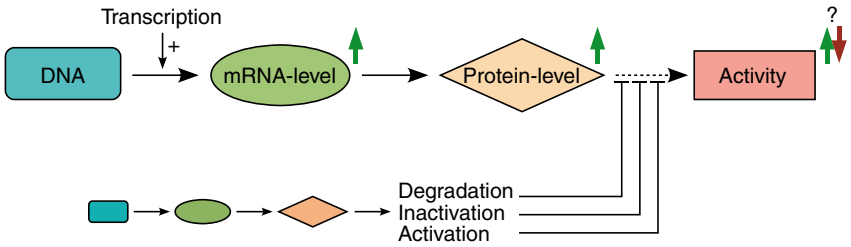

Fig. 4. Increases in mRNA or protein levels do not necessarily indicate increased protein activity. To be certain of a change in the activity of the protein, it must be measured directly under conditions similar to those in the organism.

proteins (enzymes, transporters etc.) coded for. However, this need not always be the case: if the activity of the protein is modified independently from transcription, e.g. by alternative splicing, phosphorylation–dephosphorylation reactions, changes in stability, or allosteric binding of regulator molecules, no changes in mRNA levels may be observed, even when the organism responds appropriately (and the response is genetically regulated) to an environmental change (for a review, see Feder and Walser, 2005). For example, transcriptional regulation by the hypoxia-inducible factor 1α does not require oxygen-dependent changes in the transcription of the factor, but oxygen-dependent changes in the stability of the protein, achieved enzymatically (Ivan et al., 2001; Jaakkola et al., 2001; Kaelin, 2002). Limitations also apply to proteomic studies. While one may know that the amount of protein changes as a response to an environmental factor, one only knows how the activity of the protein (e.g. enzyme) changes if its activity in pertinent conditions has been determined. Thus, after one has seen a change in either the transcript or protein level, it becomes important that the activity of the protein in relevant conditions is measured (Fig. 4). Furthermore, evolutionary changes may appear faster in gene regulatory sequences of the genome than in the protein-coding sequences (McDonald et al., 1977; Huynen and Bork, 1998), which further emphasizes the possibility that individual variation at the gene regulatory sequences generates some of the phenotypic differences, induced by environment, for which a genetic component has not been found. At present the evolution of transcriptional regulation in eukaryotes is poorly understood (Wray et al., 2003).

Note that although meaningful genetic and physiological comparisons of different populations can only be done after the populations are acclimatized to similar conditions, when possibly confounding environmental effects are removed from the study by prior acclimatization of different populations to constant conditions, any (genetic) differences in the regulation of the responses to the environmental cue are also removed (Whitehead and Crawford, 2006a). The above discussion is also important for studies of candidate genes. When a candidate gene that may be important in a given response to the environment is found, a study will be much strengthened if the function of the gene, including the effect of the studied environmental change on it, can be included in the results.

An important component of an individual's fitness is its ability to meet the challenges set by the prevailing environmental conditions. While adaptive phenotypic plasticity has been the focus of many evolutionary studies during the past two decades (for reviews, see Thompson, 1991; Hoffmann et al., 1995; Hoffmann and Merila, 1999; Pigliucci, 2003; Moller and Merila, 2004; Shine, 2005; Fordyce, 2006), the work will benefit immensely if studies on the genetics and physiology of this plasticity are addressed in the same study, e.g. if the question about the relative importance of the control regions of genes in affecting the individual variation in physiological traits as a response to environmental changes can be explored.

Quantitative genetics methods may help in dissecting integrative physiological traits into their genetic components

As indicated above, most physiological variation that has been studied from an evolutionary point of view – or interindividual variability in general – is a complex result of many genes functioning and being regulated at different levels. In most cases the observed variation in a property is quantitative [i.e. the variation is continuous (see Mackay, 2001)]. With the help of the genomic data and QTL mapping, the genetic variation in traits (e.g. in the amount of milk produced) can be localized to specific areas in the genome, and candidate genes involved in producing the trait may be found. This approach, combining research from several traditionally separate areas, has been discussed in detail elsewhere (Vasemagi and Primmer, 2005). Animal QTL mapping has mainly been used by animal breeders (e.g. Gao et al., 2006; Kucerova et al., 2006) and researchers working with *Drosophila* (Mackay, 2001). Since QTL mapping has been used in production biology in particular, the methodology has mostly concentrated on traits that are important in animal husbandry, such as growth, temperature tolerance and immune responses (Moen et al., 2004; Perry et al., 2005; Reid et al., 2005). So far the methods have only seldom been applied to natural populations, because their effective implementation requires either controlled crosses or data from long pedigrees (e.g. Slate, 2005), which are rarely available for wild populations (Kruuk, 2004). However, it is increasingly apparent that QTL mapping and associated studies are also very useful in evolutionary research with natural populations (Orr, 1998; Foster and Baker, 2004; Erickson et al., 2004). The studies may gain more when physiological traits, different from those important in production biology but possibly important in evolutionary and ecological contexts, can be included in the analysis. A good example of combining quantitative genetics and physiological measurements involves dissecting the metabolic flux into its components (Bost et al., 1999; Montooth

et al., 2003). In ecological and evolutionary studies with vertebrates, quantitative genetics and physiological measurements could be combined in studies with the three-spined stickleback *Gasterosteus aculeatus*. This species possesses the characteristics of an ideal model for QTL mapping, because it shows extensive phenotypic divergence including well-documented history of parallel episodes of population divergence due to natural selection, extreme phenotypes can be crossed to obtain a large number of fertile offspring, and a large library of genetic markers (Foster and Baker, 2004) and the genome sequence are available. Notably, a linkage and QTL map (with more than 200 microsatellites) has already been published for this species (Peichel et al., 2001). Similarly, because of their use in aquaculture, rainbow trout and some other salmonids have been studied in some detail using QTL methodology, and have many characteristics where QTL studies, physiological measurements and evolutionary studies could be combined (Jackson et al., 1998; Martyniuk et al., 2003; O'Malley et al., 2003). Again, it is very important that an extensive sample set is used, since an accurate estimation of differentiation between populations (as given by Q_{ST} index), for example, typically requires more than ten populations (O'Hara and Merila, 2005).

Searching for cellular and molecular physiological parameters in vertebrates that can be studied from an evolutionary angle, including estimation of individual variation

Being able to associate the response (phenotype) with its heritability (genotype) is necessary if a physiological function is to be valuable for evolutionary studies, so it is important that the genotype–phenotype relationship be understood and studied. Thus, research emphasis should, in our opinion, be placed on systems where it is possible to relate the two. This suggestion is not opposite to the Krogh principle (i.e. that for every function there is a species in which it is best studied), but expands it (i.e. for every function and its heritability there is a species in which the interrelationship is best studied). It is our opinion that comparative physiology as a field has progressed sufficiently to enable integration with evolutionary biology. Notably, studies on an individual's adjustments to environmental changes, which have been a very important component of studies in comparative physiology, deserve continued emphasis, since they help both to define phenotypic plasticity and to evaluate which functions and regulatory pathways can be utilized in evolutionary phenomena such as local adaptations.

Whenever one tries to show that a physiological response is important evolutionarily, the best possible scenario for a response is that its change can be directly associated to a gene. While there are many reports about this in biomedical literature, especially for monogenic diseases (e.g. Giallourakis et al., 2005), studies that combine genetic and cellular and molecular physiological data in an environmental context are rare. This also applies to studies on vertebrates reporting variability between individuals in cellular and molecular physiological responses to environmental factors.

As in other fields, the usual practise of studies in comparative and environmental physiology is to report means and standard deviations (or standard errors of the mean) for evaluated parameters, whereby the central tendency is highly emphasized [for a more detailed discussion, see Bennett (Bennett, 1987)]. However, as indicated by studies even with zebrafish, variability between individuals and different strains increases when one is studying animals other than the traditional experimental animals (Guryev et al., 2006). The presence of large variability is often mentioned in studies with fish (e.g. Roesner et al., 2006). In view of this, as was pointed out (Bennett, 1987), one important aspect of studies in evolutionary physiology is to remain open to functional differences between individuals as a source of possibilities for genetic local adaptations. While individual differences are clearly observed in integrative functions such as performance (e.g. Bennett, 1987; Kingsolver and Huey, 2003; Arnold, 2003; Huey et al., 2003), they also occur even when the response is the result of the function of a limited number of genes, i.e. at the cellular and molecular levels. Such functional inter-individual differences are shown below, with three examples mainly from our own work on fish.

First, and the clearest reported case, is glucose transport across the erythrocyte membrane of some fishes. Tse and Young (Tse and Young, 1990) reported that for *Anguilla japonica* erythrocytes, specific cytochalasin B-sensitive glucose transport across erythrocyte membrane varied from 0 to 20 mmol l cells^{-1} h^{-1} (at 20°C in the presence of 5 mmol l^{-1} extracellular glucose; data from 50 fish). Similarly, glucose transport across *Cyprinus carpio* erythrocyte membrane varied from 0.08 to 1.0 mmol l cells^{-1} h^{-1} [at 20°C in the presence of 3 mmol l^{-1} extracellular glucose; data from 8 fish (Tiihonen et al., 1995)]. Both studies tried to relate the variability to factors commonly associated with changes in glucose transport or utilization, e.g. cellular ATP concentration or fish mass, but did not find any correlation. In both cases, the most likely explanation for variability was genetic variation in the studied individuals (Tse and Young, 1990). The variability of glucose transport across the membrane between individuals will be important in terms of cellular energy production, since glucose availability may be one of the factors affecting the use of this substrate in energy production, at least in erythrocytes (Nikinmaa and Tiihonen, 1994). Further, it is probable that such variation can be pinpointed to a single or few genes.

Second, because of the properties of water, fishes encounter hypoxic conditions, especially in the freshwater environment (e.g. Nikinmaa, 2002). In hypoxic conditions, several genes are induced, and are under transcriptional regulation by hypoxia-inducible factor 1α (Semenza, 2000; Wenger, 2002; Nikinmaa and Rees, 2005). One peculiar feature of some teleost fishes is that there is marked variability in the presence of hypoxia-inducible factor 1α in normoxic conditions (Fig. 5). This partially coincides with mass variations between individual crucian carp (Sollid et al., 2006), but most of the variation

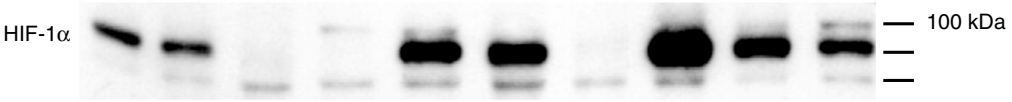

Fig. 5. Variation in the normoxic level of hypoxia-inducible factor 1α (HIF) in crucian carp *Carassius carassius*. Data from Rissanen et al. (Rissanen et al., 2006). It is likely that the three bands in the gel are HIFs with different levels of phosphorylation. While some of the individual variation can be explained by variations in fish mass, most variation remains unexplained.

remains unexplained. Since hypoxia-inducible factor 1α is a transcription factor, coded for by a single gene, and regulates the expression of many (up to more than a hundred) genes, individual variation observed in the level of this transcription factor and its function in normoxic conditions will lead to differences in the responses of animals to environmental changes.

Third, the retina of many fishes is avascular. In several species, the Root effect of haemoglobin (decrease of oxygen capacity at atmospheric oxygen tension with decreasing pH) is considered to be a mechanism ensuring oxygen delivery at a high oxygen tension in the eye (Ingermann, 1982; Ingermann and Terwilliger, 1982). There is, however, large individual variation in the oxygen tension profile of rainbow trout eye, as measured by an electrode (Desrochers et al., 1985; Waser and Heisler, 2005). While some of the individual differences are likely to be caused by the method, the whole variability of maximal oxygen tension from ca. 150–700 mmHg (Fig. 6) is probably not. Again, since each individual globin chain is coded for by a single gene, it is possible to associate physiological and genetic responses.

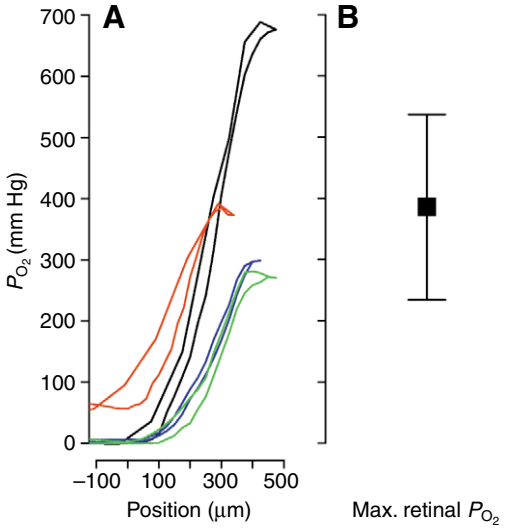

Fig. 6. (A) Selected oxygen profiles measured in the retina of rainbow trout. Positions of electrode tip (*x*-axis) range from vitreous humor (negative values) to inner surface of retina (0 μm) to the outer limit of the retina (approx. 450 μm). Lowest P_{O_2} values were generally measured at the inner surface of the retina, while highest P_{O_2} were associated with the outer limit of the retina. Duplicate lines in each profile result from insertion and withdrawal of the measuring electrode (cf. Waser and Heisler, 2005). (B) Mean and standard deviation of maximum intraretinal P_{O_2} (N=20). Since almost identical values (mean, s.d., N) are given by Desrochers et al. (Desrochers et al., 1985), it is unlikely that the applied methods are the sole cause of the variation.

All of the above examples are functions that can probably be pinpointed to a single or few genes, and will influence the success of individuals. However, the studies were performed to characterize basic physiological mechanisms, so the variation observed was not discussed from an evolutionary perspective. Bridging the gap between evolutionary ecology, genetics and physiology requires that the role of this individual variability for individual fitness is evaluated in conditions as natural as possible.

Conclusions

Integrative functions such as metabolic rate or lifetime reproductive success are properties that most interest evolutionary biologists. These functions are normally the products of several genes functioning in concert (molecular responses) to produce an integrative function. It is important to note that ‘integrative’ and ‘molecular’ are not opposites. Rather, as stated above, an integrative function is the result of several molecular responses (i.e. integration of several gene functions). In systems biology (with associated mathematical modelling) one tries, as one goal, to figure out the pathways that associate the molecular responses to an integrative function.

To study evolutionary changes at the functional level of individuals, one must first show that the integrative function is selected for. This point already brings the ecological observations and physiological measurements together. Recently, the physiological basis of life-history trade-offs has been intensively studied, and in addition to the ‘traditional’ energetic focus, the studies included focus on other physiological phenomena (for a review, see Zera and Harshman, 2001). In this context, it is very important to relate the function, in physiological studies often measured in the laboratory, to field conditions; ‘field physiology’ is needed to integrate ecology, genetics, evolution biology and comparative physiology (e.g. Irschick, 2003; Costa and Sinervo, 2004). Second, the integrative functions must be dissected into components. In such investigations, use of QTL mapping (unless all or most of the components of the response are known – in such cases the role of the components in the total response can be evaluated directly) is one way of progressing. Studies on natural populations of vertebrates, which would analyze cellular and molecular functions from evolutionary and ecological perspectives, are scarce. Designing experiments

from the evolutionary perspective requires that, as already pointed out (Bennett, 1987), individual variability is increasingly taken into account in cellular and molecular studies. With regard to functional genomics studies with data sets that are already large, this makes the data sets required even larger and further complicates the analysis. Further development of evolutionary physiology in vertebrates also requires that relevant physiological measurements are found that can both be associated with specific genes and measured an adequate number of times. Whenever one tries to associate a physiological function to genetic, evolutionary adaptation (for natural populations) it is important that the statistical treatment of the data is more detailed and complex than has traditionally been the case for physiological studies, that the comparative method has been properly utilized, including appropriate use of phylogenetic information, and that the studies take into account the individual variability inherent in natural populations (Arnold, 1983; Bennett, 1987; Arnold, 1988; Arnold, 2003; Irschick, 2003; Costa and Sinervo, 2004).

Thanks are due to Jose Cano Arias, Chikako Matsuba, Juha Merilä, Robert O'Hara and Craig Primmer for valuable comments on the manuscript. The authors' work was supported by Centre of Excellence grants from the Academy of Finland and the University of Turku. Because of space limitations, not all relevant literature could be included in the reference list. The authors apologize for any omissions.

References

Arnold, S. J. (1983). Morphology, performance and fitness. *Am. Zool.* **23**, 347-361.

Arnold, S. J. (1988). Behavior, energy and fitness. *Am. Zool.* **28**, 815-827.

Arnold, S. J. (2003). Performance surfaces and adaptive landscapes. *Integr. Comp. Biol.* **43**, 367-375.

Bennett, A. F. (1980). The thermal dependence of lizard behavior. *Anim. Behav.* **28**, 752-762.

Bennett, A. F. (1987). Interindividual variability: an underutilized resource. In *New Directions in Ecological Physiology* (ed. M. E. Feder, A. F. Bennett, W. W. Burggren and R. B. Huey), pp. 147-169. Cambridge: Cambridge University Press.

Bennett, A. F. and Lenski, R. E. (1999). Experimental evolution and its role in evolutionary physiology. *Am. Zool.* **39**, 346-362.

Bennett, A. T. D. and Cuthill, I. C. (1994). Ultraviolet vision in birds: what is its function? *Vision Res.* **34**, 1471-1478.

Bost, B., Dillmann, C. and de Vienne, D. (1999). Fluxes and metabolic pools as model traits for quantitative genetics. I. The L-shaped distribution of gene effects. *Genetics* **153**, 2001-2012.

Bronikowski, A. M., Morgan, T. J., Garland, T. and Carter, P. A. (2006). The evolution of aging and age-related physical decline in mice selectively bred for high voluntary exercise. *Evolution* **60**, 1494-1508.

Clark, R. M., Wagler, T. N., Quijada, P. and Doebley, J. (2006). A distant upstream enhancer at the maize domestication gene tb1 has pleiotropic effects on plant and inflorescent architecture. *Nat. Genet.* **38**, 594-597.

Cossins, A. R. and Crawford, D. L. (2005). Opinion – Fish as models for environmental genomics. *Nat. Rev. Genet.* **6**, 324-333.

Cossins, A., Fraser, J., Hughes, M. and Gracey, A. (2006). Post-genomic approaches to understanding the mechanisms of environmentally induced phenotypic plasticity. *J. Exp. Biol.* **209**, 2328-2336.

Costa, D. P. and Sinervo, B. (2004). Field physiology: physiological insights from animals in nature. *Annu. Rev. Physiol.* **66**, 209-238.

Crawford, D. L. and Powers, D. A. (1992). Evolutionary adaptation to different thermal environments via transcriptional regulation. *Mol. Biol. Evol.* **9**, 806-813.

Derome, N., Duchneshe, P. and Bernatchez, L. (2006). Parallelism in gene transcription among sympatric lake whitefish (*Coregonus clupeaformis* Mitchill) ecotypes. *Mol. Ecol.* **15**, 1239-1249.

Desrochers, P. E., Pratt, K. A., Fromm, P. O. and Hoffert, J. R. (1985). Oxygen diffusion in the trout retina. *Exp. Eye Res.* **41**, 607-618.

Dumke, C. L., Rhodes, J. S., Garland, T., Maslowski, E., Swallow, J. G., Wetter, A. C. and Cartee, G. D. (2001). Genetic selection of mice for high voluntary wheel running: effect on skeletal muscle glucose uptake. *J. Appl. Physiol.* **91**, 1289-1297.

Erickson, D. L., Fenster, C. B., Stenoien, H. K. and Price, D. (2004). Quantitative trait locus analyses and the study of evolutionary process. *Mol. Ecol.* **13**, 2505-2522.

Feder, M. E. (2002). Plant and animal physiological ecology, comparative physiology/biochemistry, and evolutionary physiology: opportunities for synergy: an introduction to the symposium. *Integr. Comp. Biol.* **42**, 409-414.

Feder, M. E. and Walser, J.-C. (2005). The biological limitations of transcriptomics in elucidating stress and stress responses. *J. Evol. Biol.* **18**, 901-910.

Feder, M. E., Bennett, A. F. and Huey, R. B. (2000). Evolutionary physiology. *Annu. Rev. Ecol. Syst.* **31**, 315-341.

Feder, M. E., Bedford, T. B. C., Albright, D. R. and Michalak, P. (2002). Evolvability of Hsp70 expression under artificial selection for inducible thermotolerance in independent populations of *Drosophila melanogaster*. *Physiol. Biochem. Zool.* **75**, 325-334.

Fordyce, J. A. (2006). The evolutionary consequences of ecological interactions mediated through phenotypic plasticity. *J. Exp. Biol.* **209**, 2377-2383.

Foster, S. A. and Baker, J. A. (2004). Evolution in parallel: new insights from a classic system. *Trends Ecol. Evol.* **19**, 456-459.

Gao, Y., Hu, X. X., Du, Z. Q., Deng, X. M., Huang, Y. H., Fei, J., Feng, J. D., Liu, Z. L., Da, Y. and Li, N. (2006). A genome scan for quantitative trait loci associated with body weight at different developmental stages in chickens. *Anim. Gen.* **37**, 276-278.

Garland, T., Jr and Carter, P. A. (1994). Evolutionary physiology. *Annu. Rev. Physiol.* **56**, 579-621.

Garland, T., Jr and Kelly, S. A. (2006). Phenotypic plasticity and experimental evolution. *J. Exp. Biol.* **209**, 2344-2361.

Garland, T., Jr, Bennett, A. F. and Rezende, E. L. (2005). Phylogenetic approaches in comparative physiology. *J. Exp. Biol.* **208**, 3015-3035.

Giallourakis, C., Henson, C., Reich, M., Xie, X. H. and Mootha, V. K. (2005). Disease gene discovery through integrative genomics. *Annu. Rev. Genomics Hum. Genet.* **6**, 381-406.

Goldsmith, T. H. (1994). Ultraviolet receptors and color vision: evolutionary implications and a dissonance of paradigms. *Vision Res.* **34**, 1479-1487.

Gomes, F. R., Rezende, E. L., Bunkers, J. L., Rivas, D. A., Yaspelkis, B. B. and Garland, T. (2004). Muscle glucose transporters (GLUT-4) and glycogen storage of mice selectively bred for high activity levels. *Integr. Comp. Biol.* **44**, 560.

Gracey, A. Y. and Cossins, A. R. (2003). Application of microarray technology in environmental and comparative physiology. *Annu. Rev. Physiol.* **65**, 231-259.

Gracey, A. Y., Troll, J. V. and Somero, G. N. (2001). Hypoxia-induced gene expression profiling in the euryoxic fish *Gillichthys mirabilis*. *Proc. Natl. Acad. Sci. USA* **98**, 1993-1998.

Guryev, V., Koudijs, M. J., Berezikov, E., Johnson, S. L., Plasterk, R. H. A., van Eeden, F. J. M. and Cuppen, E. (2006). Genetic variation in the zebrafish. *Genome Res.* **16**, 491-497.

Hoffmann, A. A. and Merila, J. (1999). Heritable variation and evolution under favourable and unfavourable conditions. *Trends Ecol. Evol.* **14**, 96-101.

Hoffmann, A. A., Sgro, C. M. and Lawler, S. H. (1995). Ecological population genetics: the interface between genes and the environment. *Annu. Rev. Genet.* **29**, 349-370.

Huey, R. B., Gilchrist, G. W., Ward, K., Maves, L., Pepin, D. and Houle, D. (2003). Mutation accumulation, performance, fitness. *Integr. Comp. Biol.* **43**, 387-395.

Huynen, M. A. and Bork, P. (1998). Measuring genome evolution. *Proc. Natl. Acad. Sci. USA* **95**, 5849-5856.

Ingermann, R. L. (1982). Physiological significance of Root effect hemoglobins in trout. *Respir. Physiol.* **49**, 1-10.

Ingermann, R. L. and Terwilliger, R. C. (1982). Presence and possible function of Root effect hemoglobins in fishes lacking functional swim bladders. *J. Exp. Zool.* **220**, 171-177.

Irschick, D. J. (2003). Measuring performance in nature: implications for studies of fitness within populations. *Integr. Comp. Biol.* **43**, 396-407.

Irschick, D. J. and Garland, T., Jr (2001). Integrating function and ecology in studies of adaptation: investigations of locomotor capacity as a model system. *Annu. Rev. Ecol. Syst.* **32**, 367-396.

Ivan, M., Kondo, K., Yang, H., Kim, W., Valiando, J., Ohh, M., Salic, A., Asara, J. M., Lane, W. S. and Kaelin, W. G., Jr (2001). HIFα targeted for VHL-mediated destruction by proline hydroxylation: implications for O_2 sensing. *Science* **292**, 464-468.

Jaakkola, P. Mole, D. R., Tian, Y.-M., Wilson, M. I., Gielbert, J., Gaskell, S. J., von Kriegsheim, A., Hebestreit, H. F., Mukherji, M., Schofield, C. J. et al. (2001). Targeting of HIFα to the von Hippel-Lindau ubiquitylation complex by O_2-regulated prolyl hydroxylation. *Science* **292**, 468-472.

Jackson, T. R., Ferguson, M. M., Danzmann, R. G., Fishback, A. G., Ihssen, P. E., O'Connell, M. and Crease, T. J. (1998). Identification of two QTL influencing upper temperature tolerance in three rainbow trout (*Oncorhynchus mykiss*) half-sib families. *Heredity* **80**, 143-151.

Kingsolver, J. G. and Huey, R. B. (2003). Introduction: the evolution of morphology, performance, and fitness. *Integr. Comp. Biol.* **43**, 361-366.

Kaelin, W. G. (2002). How oxygen makes its presence felt. *Genes Dev.* **16**, 1441-1445.

Koskinen, H., Pehkonen, P., Vehniainen, E., Krasnov, A., Rexroad, C., Afanasyev, S., Molsa, H. and Oikari, A. (2004). Response of rainbow trout transcriptome to model chemical contaminants. *Biochem. Biophys. Res. Commun.* **320**, 745-753.

Kruuk, L. E. (2004). Estimating genetic parameters in natural populations using the 'animal model'. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **359**, 873-890.

Kucerova, J., Lund, M. S., Sorensen, P., Sahana, G., Guldbrandtsen, B., Nielsen, V. H., Thomsen, B. and Bendixen, C. (2006). Multitrait quantitative trait loci mapping for milk production traits in Danish Holstein cattle. *J. Dairy Sci.* **89**, 2245-2256.

Loeschcke, V., Sorensen, J. G. and Kristensen, T. N. (2004). Ecologically relevant stress resistance: from microarrays and quantitative trait loci to candidate genes – A research plan and preliminary results using Drosophila as a model organism and climatic and genetic stress as model stresses. *J. Biosci.* **29**, 503-511.

Mackay, T. F. C. (2001). Quantitative trait loci in Drosophila. *Nat. Rev. Genet.* **2**, 11-20.

Martyniuk, C. J., Perry, G. M. L., Mogahadam, H. K., Ferguson, M. M. and Danzmann, R. G. (2003). The genetic architecture of correlations among growth-related traits and male age at maturation in rainbow trout. *J. Fish Biol.* **63**, 746-764.

McDonald, J. F., Chambers, G. K., David, J. and Ayala, F. J. (1977). Adaptive response due to changes in gene regulation: a study with Drosophila. *Proc. Natl. Acad. Sci. USA* **74**, 4562-4566.

Melvin, R. G. and Ballard, J. W. O. (2006). Intraspecific variation in survival and mitochondrial oxidative phosphorylation in wild-caught *Drosophila simulans*. *Aging Cell* **5**, 225-233.

Moen, T., Fjalestad, K. T., Munck, H. and Gomez-Raya, L. (2004). A multistage testing strategy for detection of quantitative trait loci affecting disease resistance in Atlantic salmon. *Genetics* **167**, 851-858.

Moller, A. P. and Merila, J. (2004). Analysis and interpretation of long-term studies investigating responses to climate change. *Adv. Ecol. Res.* **35**, 111-130.

Montooth, K. L., Marden, J. H. and Clark, A. G. (2003). Mapping determinants of variation in energy metabolism, respiration and flight in Drosophila. *Genetics* **165**, 623-635.

Nespolo, R. F., Lardies, M. A. and Bozinovic, F. (2003). Intrapopulational variation in the standard metabolic rate of insects: repeatability, thermal dependence and sensitivity (Q10) of oxygen consumption in a cricket. *J. Exp. Biol.* **206**, 4309-4315.

Nikinmaa, M. (2002). Oxygen-dependent cellular functions – why fishes and their aquatic environment are a prime choice of study. *Comp. Biochem. Physiol.* **133A**, 1-16.

Nikinmaa, M. and Rees, B. B. (2005). Oxygen-dependent gene expression in fishes. *Am. J. Physiol.* **288**, R1079-R1090.

Nikinmaa, M. and Tiihonen, K. (1994). Substrate transport and utilization in fish erythrocytes. *Acta Physiol. Scand.* **152**, 183-189.

O'Hara, R. B. and Merila, J. (2005). Bias and precision in QST estimates: problems and some solutions. *Genetics* **171**, 1331-1339.

O'Malley, K. G., Sakamoto, T., Danzmann, R. G. and Ferguson, M. M. (2003). Quantitative trait loci for spawning date and body weight in rainbow trout: Testing for conserved effects across ancestrally duplicated chromosomes. *J. Hered.* **94**, 273-284.

Oleksiak, M. F., Kolell, K. J. and Crawford, D. L. (2001). Utility of natural populations for microarray analyses: isolation of genes necessary for functional genomic studies. *Mar. Biotechnol.* **3**, S203-S211.

Oleksiak, M. F., Churchill, G. A. and Crawford, D. L. (2002). Variation in gene expression within and among natural populations. *Nat. Genet.* **32**, 261-266.

Oleksiak, M. F., Roach, J. L. and Crawford, D. L. (2005). Natural variation in cardiac metabolism and gene expression in *Fundulus heteroclitus*. *Nat. Genet.* **37**, 67-72.

Orr, H. A. (1998). Testing natural selection vs. genetic drift in phenotypic evolution using quantitative trait locus data. *Genetics* **149**, 2099-2104.

Peichel, C. L., Nereng, K. S., Ohgi, K. A., Cole, B. L. E., Colosimo, P. F., Buerkle, C. A., Schluter, D. and Kingsley, D. M. (2001). The genetic architecture of divergence between threespine stickleback species. *Nature* **414**, 901-905.

Perry, G. M. L., Ferguson, M. M., Sakamoto, T. and Danzmann, R. G. (2005). Sex-linked quantitative trait loci for thermotolerance and length in the rainbow trout. *J. Hered.* **96**, 97-107.

Pigliucci, M. (2003). Phenotypic integration: studying the ecology and evolution of complex phenotypes. *Ecol. Lett.* **6**, 265-272.

Pohjanvirta, R. and Tuomisto, J. (1994). Short-term toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in laboratory animals: effects, mechanisms, and animal models. *Pharmacol. Rev.* **46**, 483-549.

Pough, F. H. and Andrews, R. M. (1984). Individual and sibling-group variation in metabolism of lizards–the aerobic capacity model for the origin of endothermy. *Comp. Biochem. Physiol.* **79A**, 415-419.

Powers, D. A., Greaney, G. S. and Place, A. R. (1979). Physiological correlation between lactate dehydrogenase genotype and haemoglobin function in killifish. *Nature* **277**, 240-241.

Powers, D. A., Lauerman, T., Crawford, D. and DiMichele, L. (1991). Genetic mechanisms for adapting to a changing environment. *Annu. Rev. Genet.* **25**, 629-659.

Reid, D. P., Szanto, A., Glebe, B., Danzmann, R. G. and Ferguson, M. M. (2005). QTL for body weight and condition factor in Atlantic salmon (*Salmo salar*): comparative analysis with rainbow trout (*Oncorhynchus mykiss*) and Arctic charr (*Salvelinus alpinus*). *Heredity* **94**, 166-172.

Rissanen, E., Tranberg, H. K., Sollid, J., Nilsson, G. E. and Nikinmaa, M. (2006). Temperature regulates hypoxia-inducible factor-1 (HIF-1) in a poikilothermic vertebrate, crucian carp (*Carassius carassius*). *J. Exp. Biol.* **209**, 994-1003.

Roesner, A., Hankeln, T. and Burmester, T. (2006). Hypoxia induces a complex response of globin expression in zebrafish (*Danio rerio*). *J. Exp. Biol.* **209**, 2129-2137.

Rolfe, D. F. and Brown, G. C. (1997). Cellular energy utilization and molecular origin of standard metabolic rate in mammals. *Physiol. Rev.* **77**, 731-758.

Semenza, G. L. (2000). HIF-1: mediator of physiological and pathophysiological responses to hypoxia. *J. Appl. Physiol.* **88**, 1474-1480.

Shine, R. (2005). Life-history evolution in reptiles. *Annu. Rev. Ecol. Evol. Syst.* **36**, 23-46.

Siitari, H., Honkavaara, J. and Viitala, J. (1999). Ultraviolet reflection of berries attracts foraging birds. A laboratory study with redwings (*Turdus iliacus*) and bilberries (*Vaccinium myrtillus*). *Proc. R. Soc. Lond. B Biol. Sci.* **266**, 2125-2129.

Siitari, H., Honkavaara, J., Huhta, E. and Viitala, J. (2002). Ultraviolet reflection and female mate choice in the pied flycatcher, *Ficedula hypoleuca*. *Anim. Behav.* **63**, 97-102.

Slate, J. O. N. (2005). Quantitative trait locus mapping in natural populations: progress, caveats and future directions. *Mol. Ecol.* **14**, 363-379.

Sollid, J., Rissanen, E., Tranberg, H. K., Thorstensen, T., Vuori, K. A. M., Nikinmaa, M. and Nilsson, G. E. (2006). HIF-1 alpha and iNOS levels in crucian carp gills during hypoxia-induced transformation. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **176**, 359-369.

Steyermark, A. C. (2002). A high standard metabolic rate constrains juvenile growth. *Zoology Jena* **105**, 147-151.

Steyermark, A. C., Miamen, A. G., Feghahati, H. S. and Lewno, A. W. (2005). Physiological and morphological correlates of among-individual variation in standard metabolic rate in the leopard frog *Rana pipiens*. *J. Exp. Biol.* **208**, 1201-1208.

Thompson, J. D. (1991). Phenotypic plasticity as a component of evolutionary change. *Trends Ecol. Evol.* **6**, 246-249.

Tiihonen, K., Nikinmaa, M. and Lappivaara, J. (1995). Glucose transport

in carp erythrocytes: individual variation and effects of osmotic swelling, extracellular pH and catecholamines. *J. Exp. Biol.* **198**, 577-583.

Ton, C., Stamatiou, D., Dzau, V. J. and Liew, C. C. (2002). Construction of a zebrafish cDNA microarray: gene expression profiling of the zebrafish during development. *Biochem. Biophys. Res. Commun.* **296**, 1134-1142.

Tse, C.-M. and Young, J. D. (1990). Glucose transport in fish erythrocytes: variable cytochalasin-B- sensitive hexose transport activity in the common eel (*Anguilla japonica*) and transport deficiency in the paddyfield eel (*Monopterus albus*) and rainbow trout (*Salmo gairdneri*). *J. Exp. Biol.* **148**, 367-383.

Tuomisto, J. T., Viluksela, M., Pohjanvirta, R. and Tuomisto, J. (1999). The AH receptor and a novel gene determine acute toxic responses to TCDD: segregation of the resistant alleles to different rat lines. *Toxicol. Appl. Pharmacol.* **155**, 71-81.

Vasemagi, A. and Primmer, C. R. (2005). Challenges for identifying functionally important genetic variation: the promise of combining complementary research strategies. *Mol. Ecol.* **14**, 3623-3642.

Viitala, J., Korpimaki, E., Palokangas, P. and Koivula, M. (1995). Attraction of kestrels to vole scent marks visible in ultraviolet light. *Nature* **373**, 425-427.

von Schalburg, K. R., Rise, M. L., Cooper, G. A., Brown, G. D., Gibbs, A. R., Nelson, C. C., Davidson, W. S. and Koop, B. F. (2005). Fish and chips: Various methodologies demonstrate utility of a 16,006-gene salmonid microarray. BMC *Genomics* **6**, 126.

Walker, C. H., Hopkin, S. P., Sibly, R. M. and Peakall, D. B. (2006). *Principles of Ecotoxicology* (3rd edn). London: Taylor & Francis.

Waser, W. and Heisler, N. (2005). Oxygen delivery to the fish eye: root effect as crucial factor for elevated retinal P_{O_2}. *J. Exp. Biol.* **208**, 4035-4047.

Wenger, R. H. (2002). Cellular adaptation to hypoxia: O_2-sensing protein hydroxylases, hypoxia-inducible transcription factors, and O_2- regulated gene expression. *FASEB J.* **16**, 1151-1162.

Whitehead, A. and Crawford, D. L. (2006a). Neutral and adaptive variation in gene expression. *Proc. Natl. Acad. Sci. USA* **103**, 5425-5430.

Whitehead, A. and Crawford, D. L. (2006b). Variation within and among species in gene expression: raw material for evolution. *Mol. Ecol.* **15**, 1197-1211.

Wray, G. A., Hahn, M. W., Abouheif, E., Balhoff, J. P., Pizer, M., Rockman, M. V. and Romano, L. A. (2003). The evolution of transcriptional regulation in eukaryotes. *Mol. Biol. Evol.* **20**, 1377-1419.

Zeng, Z. B. (2005). QTL mapping and the genetic basis of adaptation: recent developments. *Genetica* **123**, 25-37.

Zera, A. J. and Harshman, L. G. (2001). The physiology of life history trade-offs in animals. *Annu. Rev. Ecol. Syst.* **32**, 95-126.