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Sources of inter- and intra-individual variation in basal metabolic rate in the zebra finch, *Taeniopygia guttata*

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Contents

1. List of individual papers

I Bech, C., **Rønning, B.** and Moe, B. (2004) Individual variation in the basal metabolism of Zebra finches *Taeniopygia guttata*: no effect of food quality during early development. *International Congress Series* **1275:** 306-312.

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III **Rønning, B.**, Jensen, H., Moe, B. and Bech, C. (2007) Basal metabolic rate: heritability and genetic correlations with morphological traits in the zebra finch. *Journal of Evolutionary Biology* **20:** 1815-1822.

IV Moe, B., **Rønning, B.**, Verhulst, S. and Bech, C. (2008) Metabolic senescence in individual zebra finches. *Biology Letters* (submitted).

2. Introduction

2.1 Basal metabolic rate

The first measurements of energy expenditure in animals were conducted by the famous chemist Antoine Lavoisier and his collaborators late in the eighteenth century (Prentice 1995). In the twentieth century metabolic measurements, especially measurements of basal metabolic rate (BMR), became an important tool to answer questions regarding bioenergetics and growth (Hulbert and Else 2004). Consequently, BMR has become one of the most commonly measured physiological characters in animals. BMR is defined as "metabolic energy transformation calculated from the measurements of heat production or oxygen consumption in an organism in a rested, awake, fasting, and thermoneutral state" (IUPS Thermal Commission 2001). Hence, BMR represents an animal's maintenance cost, and because it is obtained under standardized conditions, it acts as a useful physiological standard for multiple comparisons of animal performance (McNab 1997; Hulbert and Else 2004; White and Seymour 2004). The whole body BMR is essentially determined by the metabolic intensity and mass of all different tissues and organs. Thus, it can be regarded as a rather complex trait that is prone to be influenced by a variety of factors. The most obvious determinant of BMR is body mass, and the relationship between these two variables, both within and between species, has been extensively studied (see White and Seymour 2005). However, the considerable variation in basal energy expenditure among species living in different environments is larger than what is expected by variation in body mass alone. Looking at the vertebrate class *Aves*, birds living in cold climates and at high latitudes are characterised by a high BMR (Ellis 1984; Gabrielsen *et al.* 1988; Bryant and Furness 1995), while low BMR is characteristics of birds living in the tropics (Hails 1983; Pettit 1985) and on islands (McNab 1994). Latitudinal differences in BMR are also found between populations at the intra-specific level (Wikelski *et al.* 2003; Broggi *et al.* 2005). These correlations between BMR and specific environments are believed to represent evolutionary adaptations reflecting different selection pressures which have worked upon metabolic traits in the past (Furness 2003).

2.2 Phenotypic plasticity

Avian BMR exhibits considerable phenotypic plasticity, which greatly complicates the identification of potential metabolic genetic adaptations (McKechnie 2008). One category of phenotypic plasticity is termed phenotypic flexibility and is, following Piersma and Lindstrøm (1997), defined as reversible within-individual phenotypic transformations in response to changes in environmental conditions. Phenotypic flexibility in metabolic traits is common, and may be attributed to changes in body composition, metabolic intensity (Selman and Evans 2005; Vézina and Williams 2005a; Rønning *et al.* 2008) or both. Thus, studies reporting differences between populations that are based on measurements of BMR in birds acclimatised to their specific habitat (e.g. Hudson and Kimzey 1966), should be interpreted with caution in the light of genetic adaptation. This is due to the problem that if birds are not acclimated to a common environment, the BMR differences can to some extent simply reflect phenotypic adjustments. Furthermore, the environment experienced during development can have an irreversible effect upon adult phenotype. This environmentally induced variability is known as developmental plasticity (Piersma and Lindstrøm 1997; West-Eberhard 2003). Hence, some of the intra-specific variation in BMR both between and within populations can potentially be attributed to environmentally induced variation in physiology during development. Food supply and food quality may vary greatly between breeding areas as well as between breeding seasons, and are thus potential environmental factors that during development could induce permanent physiological differences. Variability in the quality of food provided to growing chicks have been shown to induce permanent effects on body mass and body size (e.g. Boag 1987; Searcy *et al.* 2004; Arnold *et al.* 2007). Consequently, nutrition status during early development may also be a potential factor shaping the metabolic machinery setting the individual "level" of basal metabolism. Chicks have been shown to down-regulate their metabolism when facing a food shortage during development (e.g. Brzęk and Konarzewski 2001; Moe *et al.* 2005). However, whether early nutritional status, or other environmental factors experienced during early growth, contributes to individual variation in the adult metabolic phenotype is poorly investigated.

2.3 Heritable variation in basal metabolic rate

Differences in BMR between species and between populations are frequently reported (see references above). Although natural selection probably works upon several levels of organization (Gould and Lloyd 1999), it might be more interesting to look at variation across individuals than across species and populations when trying to interpret the micro-evolutionary potential of BMR. Individual variation is the most fundamental requirement for natural selection to occur (Garland and Carter 1994). Hence, if selection on a trait is to be effective, the trait must show some consistent inter-individual variation, i.e. it must be repeatable and have a genetic component. However, as metabolic traits exhibit phenotypic flexibility (e.g. due to short-term acclimatisation), it may be more difficult for selection to work upon such traits compared to more fixed morphological traits because the genetic component may be small (Hayes and O'Connor 1999). Repeatability is thought to represent the upper limit of heritability (Falconer and Mackay 1996), and a significant repeatability therefore indicates a genetic variation in the trait. In the last 20 years several studies have been investigating repeatability of metabolic traits, where the majority of studies have been focusing on maximal metabolic rate. This is probably because maximal metabolic rate sets the upper limit to sustained power output and is therefore thought to influence individual fitness (e.g. Chappell *et al.* 1995). Furthermore, selection for increased maximal metabolic rate is also suggested as an explanation for the evolution of endothermy (Bennett and Ruben 1979). In the recent years repeatability of BMR has been given increased attention. This is not surprising since BMR constitutes a significant component of the aerobic performance of endotherms, and is believed to have undergone adaptive changes (Bech *et al.* 1999). Basal metabolic rate is found to be a repeatable trait both in captive (Hõrak *et al.* 2002; Vézina and Williams 2005b) as well as in wild bird populations (Bech *et al.* 1999). Although non-significant repeatabilities of BMR are reported (e.g. Russell and Chappell 2006), the majority of studies have found this metabolic trait to be significantly repeatable (see Nespolo and Franco 2007 and references cited therein), indicating a between-individual genetic component of BMR. However, because the permanent differences between individuals could be attributed to environmental sources of variance, highly repeatable traits may not necessary be heritable (Merilä and Sheldon 2001). Hence, traits which are significantly repeatable need not to hold the possibility to change genetically between generations through selection.

Selection can induce within generation changes in the population trait mean regardless of the genetic basis of the trait. However, an evolutionary response to selection, where the mean value of the trait changes from one generation to another, depends on inheritance (Arnold and Wade 1984). Thus, for a trait to change across generation through selection the trait must exhibit some heritable (additive) genetic variation. Studies on quantitative genetic variation in physiological traits (e.g. metabolic traits) are scarce compared with more easily measured morphological traits, especially in birds (Merilä and Sheldon 2001). As far as I know, heritability studies on metabolic traits in birds are absent in the literature except from one study of domestic chicken (*Gallus gallus*) where Damme *et al.* (1986) found evidence of a genetic component in BMR. There are, however, some studies examining genetic variation in energetic traits in small mammals. Maximal metabolic rate in rodents is generally found to be significantly heritable (Dohm *et al.* 2001; Konarzewski *et al.* 2005; Nespolo *et al.* 2005; Sadowska *et al.* 2005), whereas studies on additive genetic variation in BMR are more inconclusive. While two recent studies report a moderately high heritability of BMR (Konarzewski *et al.* 2005 Sadowska *et al.* 2005), most previous studies on rodents have found the heritability to be low and not significantly different from zero (Lacy and Lynch 1979; Dohm *et al.* 2001; Nespolo *et al.* 2003; Bacigalupe *et al.* 2004; Nespolo *et al.* 2005). However, the fact that artificial selection on BMR has proven effective in mice (Książek et al 2004; Brzek *et al.* 2007), strongly suggest presence of an additive genetic variance in this metabolic trait. Thus, while morphological traits are generally found to be significantly heritable in both mammals and birds no general trend has yet been established for energy expenditure.

2.4 Energy metabolism and aging

The relationship between energy expenditure and different rates of aging between species has long been a subject of debate. In the last years, the effect of age on energy expenditure at the individual level (see below) has also been given some attention. Early in the previous century the "rate of living theory" emerged, suggesting that the total energy expenditure per lifetime is fixed (Rubner 1908; Pearl 1922). That is, animals with high metabolic rates "burn out" their energy faster compared to animals with lower metabolic rates, causing them to die earlier. The "free radical theory" proposed that aging was due to the cumulative damage caused by endogenous oxygen radicals generated in the cells (Harman 1956). By-products from oxidative phosphorylation in the mitochondria are the main source of free radicals and oxidants, collectively termed reactive oxygen species (ROS) (Cadenas and Davies 2000). ROS are generally small and highly unstable molecules that cause random damage to proteins, lipids and DNA (Cadenas and Davies 2000). Because this damage is believed to be cumulative, the "free radical theory" could be seen as a mechanistic explanation of the "rate of living theory". Today the "rate of living theory" is more or less disregarded, partly due to the observation that birds spend much more energy per lifetime compared with a similarly sized mammal (Holmes and Ottinger 2003). Differences in production of ROS per O_2 volume that is metabolized or in the ability to respond to the oxidative damage could weaken the correlation between lifespan and metabolic rate. The summed effect of oxidant generation, antioxidant protection and repair of oxidative damage is defined as oxidative stress (Beckman and Ames 1998). The "free radical theory" is today often referred to as the "oxidative stress hypothesis" in which senescence and eventually time of death is determined by the accumulated negative effects of oxidative stress on the cells.

Although substantial evidence exist that oxidative stress is at least partly responsible for the effects of aging, this does not explain why and how senescence evolved. Senescence (aging) is associated with a decreased fertility and increased mortality rate due to a decline in physiological functioning with age (Rose 1991; Holmes and Austad 1995; Jones et al. in press). Consequently, senescence is causing a decline in fitness with age, and it therefore seems sensible that natural selection should be working towards eliminating senescence from the life history. This makes the evolution of senescence somewhat paradoxical. The "disposable soma theory" looks at the evolution of senescence from a physiological point of view as a trade-off between cost of reproduction and antioxidant protection (somatic maintenance) (Kirkwood and Rose 1991; Kirkwood and Austad 2000). Investing more energy in somatic maintenance than needed to stay in good physiological condition during expected lifetime in the wild would be a wasteful adaptation, because this energy could potentially have been used for reproduction instead. The mean optimal use of energy for antioxidant defence and repair of oxidative damage of a species would therefore depend on the rate of extrinsic mortality (e.g. predation, contagious disease, starvation) experienced.

Energy metabolism is a potential determinant of lifespan and rate of aging, but energy metabolism may also be subjected to age-related changes itself (Navarro and Boveris 2007). Because aging is associated with a gradual decline in physiological performance, a decline in metabolic output as aging progresses may be expected. A decline in BMR with age is a well-known phenomenon in humans (e.g. Piers et al. 1998; Roberts and Rosenberg 2006). Studies on the relationship between BMR (or resting metabolic rate [RMR]) and age in various mammalian and avian species, partly belonging to the same animal classes, have however, given ambiguous results (Greenberg 1999; Even *et al.* 2001; O'Connor *et al.* 2002; Chappell *et al.* 2003; Miyasaka *et al.* 2003; Speakman *et al.* 2003; Broggi *et al.* 2007; Moe *et al.* 2007).

For the time being, the mechanistic explanations underlying age-related changes in BMR are not well understood, and neither are the selective forces behind the evolution of the species-specific differences in the relationship between age and BMR.

3. Aims of the thesis

This thesis investigates sources of variation in avian basal metabolic rate, both at the intra- and inter-individual level, using the zebra finch (*Taeniopygia guttata*) as a model species. Variation observed in avian basal energy expenditure can potentially be ascribed to both environmental and genetic factors. Differences in BMR have often been attributed to genetic differentiation; however the actual genetic basis of BMR in birds has hardly been studied. In the first three papers in this thesis I investigate environmental (**paper I, II and III**), and genetic (**paper II and III**) sources of inter-individual variation in BMR. In the last paper included in this thesis (**paper IV**) I examine the effect of age on BMR at the intra-individual level, and try to discuss this in the light of evolution.

The individual papers in this thesis aim to answer the following questions for the zebra finches;

1) Does diet quality during early development affect adult basal metabolic rate through developmental plasticity? **paper I**
2) Is there consistent inter-individual variation (repeatability) in basal metabolic rate which natural selection can work upon? **paper II**
3) Is there a heritable genetic component in basal metabolic rate? **paper III**
4) Does basal metabolic rate decline with age? **paper IV**

4. Methods

4.1 Study species and study population

The zebra finch (*Taeniopygia guttata*, Vieillot 1817) is a granivorous passerine bird species belonging to the estrildine group of finches. The domesticated finches used in our studies originate from the subspecies Australian zebra finch (*T. g. castanotis*, Gould 1837) native to Australia. Zebra finches are sexual dimorphic, and the male is easily distinguished from the more or less plain-coloured grey female by their rusty orange chest patches, brown coloured side flanking and zebra like pattern on their throat. This species will easily breed in captivity and has become a very common laboratory model system. Free-living zebra finches tend to maximise their breeding effort when food availability peaks (Zann 1996), while captive birds with unlimited access to food will produce new broods all year around. The maximum life span in the wild is around five years (Zann 1996), whereas captive birds are observed to live for more than seven years (own personal observation).

Our laboratory population of zebra finches are held in the animal unit at Norwegian University of Science and Technology in Trondheim. The temperature in the holding rooms is kept at 24 ºC, the humidity is 40 % RH and the light-dark regime is 12-12 h with light on at 07:00. When not breeding, the sexes are kept separated in large aviaries (10 m^3). The breeding birds are either mixed in large aviaries supplied with nest boxes, or paired in separate breeding cages. The individuals in the population are distributed over three different generations. The first generation birds (n = 63), from which all individuals in the population originated, came from three different breeders and were purchased through a local pet store in the end of 2000 and early in 2001. The first generation birds were breeding in two periods (2001 and 2002), while birds from the second generation were given the opportunity to breed several times from 2002 to 2005. Individuals from the second generation, fed either a low or a high quality diet (*n* = 86, hatched in 2001 and 2002), were used when investigating the effect of food quality during early development (**paper I**). Furthermore, individuals from the second generation provided a normal diet (*n* = 39, hatched in 2001) were used to estimate the repeatability of BMR (**paper II**). The same individuals were also used in the study of the effect of age on BMR (**paper IV**). The pedigree used in the heritability study (**paper III**) included individuals (*n* = 349) from all three generations.

4.2 Metabolic measurements

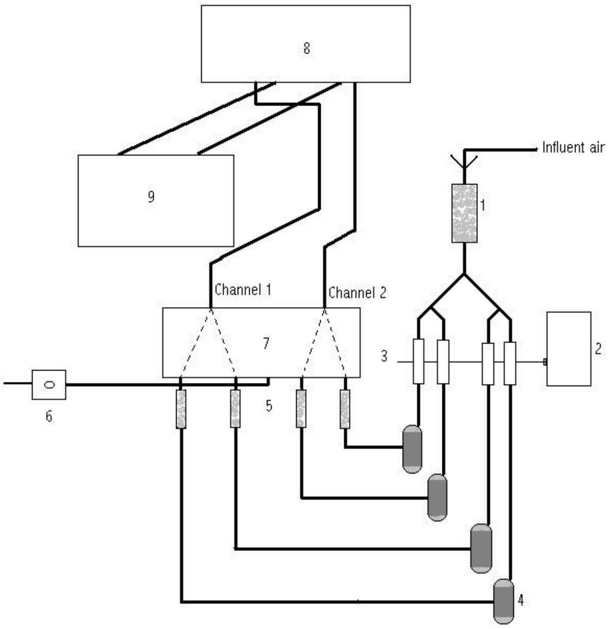

Figure 1. Dry outside air (set to 20.95% oxygen) was pumped through four metabolic chambers made from 1.5 L metal boxes (4). For each metabolic chamber a calibrated mass flowmeter (2, 3) was used to adjust the flow to 400 mL min^{-1}. Both influent and effluent air was dried (1, 5). After a reduction in flow (8), a two-channel oxygen analyser (9) measured the oxygen concentration in the effluent air. An automatic valve-system (7) switched between the chambers, so that two chambers were measured simultaneously for 26 min, with fresh air being pumped through the system (6) for four min between each switching. Numbers in parenthesis refers to number in the figure.

Basal metabolic rate was measured as O_2-consumption rates using an open flow system (Fig. 1). The respiration chambers containing the birds were located in a temperature controlled room at 35 ºC, which is within the thermoneutral zone for the zebra finch (Calder 1964). The voltage outputs from the oxygen analyser and mass flowmeters were stored on a Grant Squirrel, type 1200 data-logger (Cambridge, England) at 30-seconds intervals. The rate of oxygen consumption (VO_2) was calculated following Withers (1977), using a respiratory quotient of 0.71 (assuming a post absorptive state). The lowest 10 min running average VO_2 value was used to represent BMR. Body mass at the time of the lowest VO_2 (used when calculating mass-specific BMR) was found assuming a linear body mass reduction. The lowest VO_2 readings were usually obtained at the end of the measurement period, and the birds were consequently in a post-absorptive state.

5. Summary of the individual papers

5.1 Paper I

The aim of this study was to investigate whether food quality experienced during development influenced BMR later in life. Zebra finches were randomly assigned to two different breeding aviaries, and provided different diets. One group of birds were given a low quality diet consisting of only seeds, while the other group was given a high quality diet which in addition to seeds included a protein supplement and daily fresh hard-boiled eggs. All chicks produced in the two colonies were removed at an age of 6 weeks, and provided a normal diet. The BMR measurements of the birds raised on different diets were obtained when the birds were between 1.5 and 2 years old. The adult body mass in birds fed a low quality diet the first six weeks of life was lower than in birds fed a protein rich diet during early development. The effect of food quality on adult body mass was significant in both sexes. In addition to feeding regime, sex had a significant effect on adult body mass. Within both feeding regimes the adult body mass of the females was higher than in the males. In a multiple linear regression with BMR (ml O_2 h^{-1}) as dependent parameter and body mass, sex and experimental group as independent parameters, both sex and body mass were found to be significant determinants of the variation in BMR. Experimental group, however, did not contribute in explaining the BMR variation. Hence, despite inducing large variation in adult body mass, diet quality during development was not found to have an effect on BMR later in life (when controlling for the effect of body mass).

5.2 Paper II

Basal metabolic rate was measured six times in individual zebra finches over a 2.5 years period, to investigate if this metabolic trait was repeatable. The first four measurements were obtained over a 1.5 month period in 2002, and subsequent to these measurements the birds were allowed to breed. The two last BMR measurements were obtained in 2004. Body mass and sex were significant determinants of the variation in BMR (ml O_2 h^{-1}), and we consequently used residual values of BMR, controlled for the effect of body mass and sex, in our repeatability analyses. Variance components derived from a one-way analysis of variance were used to calculate the repeatability (R). BMR was found to be significantly repeatable in both males and females, and in the sexes combined (R ranging from 0.413 to 0.567). This was found both over the short (1.5 months) as well as over the long period (2.5 years) containing a breeding period. The short-term repeatabilities and long-term repeatabilities were not significantly different either in the sex-specific samples or in the pooled-sexes sample. Thus, there was consistent between individual variation in BMR and the repeatability of BMR was not affected by the length of the measurement period, suggesting that the performance ranking of BMR between the individuals was relatively constant during a substantially portion of their lifetime.

5.3 Paper III

To examine the genetic basis of basal metabolic rate in a captive population of zebra finches, we used metabolic and morphological measurements obtained in 349 individuals from three generations. To establish a pedigree, the birds were genotyped on six microsatellite loci to determine genetic parenthood. Heritabilities and genetic correlations were estimated using an animal model combined with a restricted maximum likelihood estimation procedure (VCE4 software; Neumaier and Groeneveld 1998). Basal metabolic rate and all morphological traits (tarsus length, head length, wing length and body mass) analysed in the zebra finch population were found to have heritabilities that were significantly different from zero. The heritability of BMR was not different between the sexes, and the genetic correlation between male- and female-BMR was not different from one. All traits analysed in this study were phenotypically correlated, and eight out of 10 additive genetic correlations were significantly larger than zero. All correlations, both phenotypic and genetic were positive. Because BMR was strongly genetically correlated to body mass (the trait showing the strongest genetic correlation to BMR), we calculated the heritability of BMR conditional on body mass by estimating the additive genetic variation in BMR that was independent of the additive genetic variance in body mass. When conditional on the genetic value of body mass the heritability of BMR was substantially reduced (from 0.250 to 0.041), suggesting that the potential for adaptation in BMR independent of body mass may be limited.

5.4 Paper IV

This paper aimed to study the effect of age on BMR in individual zebra finches. A longitudinal data-set with measurements of BMR in individual birds at one, three and five years of age was used to analyse the effect of age. Data were analysed using general linear mixed models, including individual bird as random effect. To separate the effect of between individual (i.e. selective disappearance/mortality) and within individual change in BMR with age the data were analysed following van de Pol and Verhulst (2006). There was no evidence for selective disappearance in either sex. Basal metabolic rate showed a significant age-related decline in females, but not in males. However, while body mass was independent of age in females, mass increased with age in males. The increase in body mass masked a decline in BMR in the males, because BMR declined with age in both sexes when mass was controlled for statistically.

6. Discussion

6.1 Phenotypic plasticity

6.1.1 Developmental plasticity

The large variation found in avian basal metabolic rate among populations and species has for a long time been attributed to genetic variance caused by adaptation to different environments (e.g. Weathers 1979). However, because both genetic and environmental mechanisms may contribute to the phenotype which is expressed, it is important to consider potential factors, other than genetic differentiation, which could explain some of the BMR variation in avian populations. The environment experienced during development early in life is known to have an effect on the phenotype expressed as adult (i.e. developmental plasticity). Both access to food and quality of food vary considerably in time and space in nature and can therefore be potential factors influencing adult phenotypes. Differences in nutritional status in growing chicks have been shown to induce a permanent effect on body mass in zebra finches (Boag 1987), but if this also could influence adult BMR has so far not been examined. In accordance with Boag (1987) we found that the protein content in the diet provided during the first weeks of development had a significant effect on adult body mass in zebra finches (**Paper I**). Despite this, the BMR of the adult birds raised on different diets did not differ beyond that expected based on variation in body mass alone. Thus, early nutritional status apparently did not influence adult body composition or organ/tissue metabolic intensity which would have changed the “level” of BMR for a given body mass. Although the birds raised on different diets did not show any differences in the relationship between BMR and overall mass, the birds fed low quality food did have a substantially lower body mass, giving them lower absolute energy requirements as adults. Thus, one can argue that BMR is actually affected by early nutrition, however only indirectly, as a correlated response to the decrease in body mass. Environmental quality experienced during development has been shown to influence heritability of body mass in birds (see Merilä 1997 and references cited therein). How nutrition quality experienced during early development might influence heritability of BMR or the genetic correlations with other traits is beyond the discussion in **paper I**. The fact that diet quality did not have any profound effect upon BMR do not exclude the possibility that other environmental factors experienced during development might induce permanent effects on BMR beyond that expected from variation in body mass.

6.1.2 Phenotypic flexibility

Inter-specific variations in BMR have been found to correlate with a variety of factors like e.g. aridity, latitude, temperature and primary production (e.g. Tieleman *et al.* 2003a). Consequently, these environmental factors are suggested to act as selective forces causing genetic differentiation in energy expenditure between species living in different habitats. Differences in BMR are also found within species between populations living in different environments (e.g. Wikelski *et al.* 2003; Broggi *et al.* 2005), indicating that natural selection has acted on this particular metabolic trait. However, we have to keep in mind that there are other potential "non-genetic" mechanisms that can induce the BMR differences observed between populations. This could theoretically also be the case even with the presence of genetic variance in BMR within the populations. In addition to developmental plasticity (see above), differences could reflect phenotypic flexibility often termed acclimatisation when referring to physiological traits. That is, a reversible change in the phenotype in response to different environmental conditions. Hence, observed differences between populations do not have to reflect genetic differences shaped by different selection pressures, but simply be a product of acclimatisation (McKechnie 2008). However, we should perhaps be careful terming phenotypic flexibility a non-genetic mechanism, as there could potentially exist genetic variance in acclimatisation capacity as well. One could hypothesize that individuals with the "best adapted" response to a given change in the environment would have a higher fitness than individuals with less well-adapted response patterns. In ectotherms there is some evidence suggesting the presence of genetic variation in thermal acclimatisation response (see Kingsolver and Huey 1998 and references therein). Although the ability to acclimatize through metabolic flexibility seems to be a basic trait in birds (e.g. Klaassen *et al*. 2004), little is known about differences in acclimatisation responses in avian BMR (but see Tieleman *et al.* 2003b). Thus, we can only speculate if the degree of flexibility in BMR in response to various environmental changes or life-cycle events exhibits some heritable genetic variation.

6.2 Genetic variation in basal metabolic rate

6.2.1 Repeatability

Most physiological traits are known to show some degree of phenotypic flexibility and may therefore vary over time. Consequently, comparative studies based on single individual measurements may be questionable. Repeatability is a measure of the portion of variance in a trait that occurs between, rather than within, individuals (Lessells and Boag 1986), and the repeatability between individual measurements of a flexible trait provides an indication of how reliable a current measurement is as a prediction of future measurements of the same trait. Moreover, repeatability is also interesting in quantitative genetics because a trait has to show some between-individual variation (i.e. repeatability) for natural selection to be able to operate upon it (Falconer and Mackay 1996). Thus, a trait showing consistent between-individual variation could potentially change through natural selection provided that the trait is heritable. The repeatability of a trait is given by the sum of genetic and general environmental variance divided by the phenotypic variance, and is therefore thought to set the upper limit to heritability (Falconer and Mackay 1996; but see Dohm 2002). Unfortunately, the repeatability value does not tell anything about the actual heritability of the trait. This is because the analysis of repeatability does not separate between genes and environment as the source of the inter-individual variation. However, a significant repeatability indicates genetic differences in a trait among individuals and a quantitative genetic approach may therefore be worthwhile. Studies of quantitative genetics require large sample sizes, and a test for repeatability may thus be a good screening tool to see if the trait is convenient for studies of natural selection.

Because of phenotypic flexibility, repeatability of physiological traits could be affected by the length of the measurement period in such a way that repeatability decreases with the length of the measurement period (Chappell *et al.* 1995). However, the basal metabolic rate in our captive zebra finches was found to be equally repeatable over a short and a long period constituting a considerable portion of this species lifetime (**Paper II**). These findings contrast to other studies measuring repeatability of BMR in birds (Bech *et al.* 1999; Hõrak *et al.* 2002; Vézina and Williams 2005b), reporting a significant decrease in BMR repeatability with time. However, when analysing available data on repeatability of various metabolic traits, the repeatability values were more or less independent of time between measurements

(Konarzewski *et al.* 2005, but see Nespolo and Franco 2007). Zebra finches are known to increase their resting metabolism during breeding (Vézina and Williams 2005b; Vézina *et al.* 2006). In spite of these phenotypic adjustments we found BMR to be significantly repeatable when comparing measurements obtained before and after a breeding period. In another study of zebra finches Vézina and Williams (2005b) found RMR (equivalent to BMR) to be repeatable between two consecutive breeding periods. Thus, at least in the zebra finch, the between-individual variation in BMR seems to persist regardless of these phenotypic adjustments. In other words, phenotypic adjustments during breeding that changes the population mean value of BMR do not seem to alter the ranking much between the individuals within the population. The fact that the ranking of BMR between individuals remains more or less constant over time, regardless of phenotypic adjustments (at least adjustments during breeding), will facilitate any effects of selection on BMR provided that this trait is heritable. If metabolic traits like for example BMR actually do exhibit heritable genetic variation necessary for a genetic change between generations to occur is still poorly investigated. However, metabolic traits, including BMR, are generally found to be significantly repeatable in both mammals and birds (Nespolo and Franco 2007). Hence, it seems safe to conclude that individual differences in basal energy expenditure normally exist in homoeothermic animals upon which natural selection could potentially work.

6.2.2 Heritability

Data on quantitative genetics of physiological traits are highly underrepresented in the literature compared with morphological and behavioural traits (Merilä and Sheldon 2001; Artacho *et al.* 2005). One obvious problem associated with quantitative genetic studies of physiological traits (e.g. metabolic traits), is that measuring such traits are often laborious and time-consuming. Thus, it may be hard to obtain enough individual measurements as required in any pedigree-based design (Artacho *et al.* 2005). This problem is probably partly responsible for the scarcity of studies on quantitative genetics of physiological traits, despite the long history of comparative physiology research. Another potential problem with quantitative genetic analyses of at least some physiological traits is connected to the difficulty of obtaining accurate measurements. For example, metabolic measurements using indirect calorimetry may induce large measurement errors (Konarzewski *et al.* 2005; Walsberg and Hoffman 2005). A large measurement error will induce a low repeatability and hence a large phenotypic variance causing the heritability estimate to be deflated (Merilä and Sheldon

2001). Thus, comparing heritability values obtained in accurately measured morphological traits with metabolic measurements, which are prone to be less accurate, may be somewhat problematic.

Despite the problems associated with heritability studies on metabolic traits, there are some studies that have examined quantitative genetics underlying metabolic traits, with the majority of studies focusing on rodent species. There is evidence for a significant heritability of maximal oxygen consumption in rodents (Dohm *et al.* 2001; Konarzewski *et al.* 2005; Nespolo *et al.* 2005; Sadowska *et al.* 2005). However, studies examining heritable genetic variation in BMR, have given more ambiguous results. The majority of studies report the heritability of BMR to be low and not significantly different from zero (Lacy and Lynch 1979; Dohm *et al.* 2001; Nespolo *et al.* 2003; Bacigalupe *et al.* 2004; Nespolo *et al.* 2005). In contrast, two recent studies on rodents have found BMR to have a moderately high heritability ($h^2 \sim 0.4$, Konarzewski *et al.* 2005; Sadowska *et al.* 2005). Heritability estimates can be expected to vary both between populations and between environments (Lynch and Walsh 1998). Hence, these contrasting results could in theory be attributed to a true difference in additive genetic variance in BMR. However, the power of quantitative genetic analyses where e.g. heritability is estimated are highly dependent on the number of individuals measured (Konarsewski *et al.* 2005), and the findings of insignificant heritabilities of BMR in some studies are therefore likely to be attributed to an inadequate pedigree size (Sadowska *et al.* 2005). Compared to mammals, even less is known about the genetics underlying metabolic traits in avian populations. In a population of captive zebra finches we found evidence of a heritable genetic component in BMR (**paper III**), and this is in agreement with a study on domestic fowl by Damme *et al.* (1986). Furthermore, in a wild great tit (*Parus major*) population BMR is also found to exhibit some heritable genetic variation (Nilsson, J.Å., personal communication). Thus, although the knowledge about genetics underlying metabolic traits is still scarce, an increasing number of studies do in fact indicate that individual metabolic intensity is transferred across generations both in mammals and birds. Further evidence for additive genetic variance in BMR comes from selection studies in small mammals, where BMR has been shown to respond to artificial selection (Książek *et al.* 2004; Brzek *et al.* 2007). Finding significant additive genetic variance in BMR is however not evidence that natural selection is operating on this trait.

6.2.3 Natural selection

For natural selection to act directly upon a trait, variation in the trait has to be associated with differences in fitness (Falconer and Mackay 1996). It has been suggested that differences in reproductive rates and growth rates observed between mammalian species can be attributed to differences in biosynthesis reflecting their energy expenditure, e.g. BMR (McNab 1980). This view is supported by findings of correlations between several life history traits and variation in BMR at the inter-specific level in mammals (White and Seymour 2004). Contrasting results were found by Trevelyan *et al.* (1990) who failed to find any correlation between RMR and offspring production and neonatal growth in a comparative study using data from 325 bird species. However, to understand the micro-evolutionary potential in metabolic traits it might be more interesting to look at parameters causing fitness differences between individuals at the intra-specific level. Is there a relationship between BMR and intra-specific variation in reproductive performance, survival or other fitness components? There is some evidence in birds suggesting this. Basal metabolic rate is found to correlate positively with social rank (i.e. dominance) in several passerine species (Røskaft *et al.* 1986; Hogstad 1987; Bryant and Newton 1994; Kerimov and Ivankina 1999). Because dominance in monogamous bird species has been shown to influence characters favoured by natural selection like survival and reproductive success (Arcese and Smith 1985; Schubert *et al.* 2007), these findings indicate that selection may favour increased basal energy expenditure needed to defend social rank in the flock. Furthermore, in marsh tits (*Parus palustris*) an increased brood size is associated with an increase in BMR (Nilsson 2002). In house sparrows (*Passer domesticus*), high BMR is associated with early breeding, and thus production of more clutches, both at the individual and population level (Chastel *et al.* 2003). In contrast, BMR has been found to have the opposite effect in leach's storm petrels (*Oceanodroma leucorhoa*) where high BMR in males was associated with late breeding (Blackmer *et al.* 2005), indicating that the correlation between BMR and reproduction (and possibly fitness) differs between species.

In addition to direct selection on BMR, a genetic differentiation in BMR could also be attributed to responses to selection on correlated traits. If there is a positive genetic correlation between traits, direct selection on one trait would result in an indirect selection on other traits (Lande and Arnold 1983; Price and Langen 1992). Basal metabolic rate in zebra finches was positively genetically correlated to body mass and body size (**paper III**). Selection on body mass would thus result in a correlated response in BMR. However, some of the additive genetic variation was independent of body mass, indicating that BMR, at least to some extent, may evolve independently of body mass in the zebra finch. Mass of various highly metabolic active internal organs is often found to be significant determinates of variation in BMR (e.g. Piersma 2002). Because internal organs are important in "fuelling" the metabolic machinery used for sustained aerobic activity, they are potential targets for natural selection. In laboratory mice, selection for high and low mass corrected BMR induced a correlated response in the mass of several internal organs, strongly suggesting a positive genetic correlation between BMR and organ masses (Książek *et al.* 2004). Furthermore, the aerobic capacity model for the evolution of endothermy suggests that the high BMR characterizing endotherms evolved as a correlated response through selection on maximal aerobic capacity (Bennet and Ruben 1979). In fact, inter-specific variation in BMR controlled for the effect of body mass does indeed correlate with maximal metabolism (cold induced) in birds (Rezende *et al.* 2002). Exercise induced maximal aerobic capacity (VO_{2max}) was not found to be phenotypically correlated with BMR in our zebra finches (Bech *et al.* 2007). However, in bank voles (*Clethrionomys glareolus*), a positive genetic correlation between BMR and VO_{2max} was present, despite a lack of correlation between these metabolic traits at the phenotypic level (Sadowska *et al.* 2005). Thus, the absence of a phenotypic correlation between BMR and VO_{2max} in the zebra finches does not exclude the possibility of a genetic correlation. Finally, given that BMR is likely to be a complex polygenetic trait, probably genetically correlated with several morphological as well as physiological traits, answering the questions regarding if and how natural selection operates upon BMR will be a challenge.

6.3 Basal metabolic rate and aging

For the time being, the ultimate and proximate factors explaining the relationship between BMR and age are not well understood. To increase our knowledge of age-related physiological changes, studies on birds may be especially interesting, given the paradox that they usually have longer life-spans compared to similar sized mammal, in spite of having higher metabolic rates, higher body temperatures and higher blood glucose levels (Holmes and Ottinger 2003). Using measurements obtained in a captive zebra finch population, BMR was found to decrease substantially with age (**paper IV**). Although extrinsic mortality is prone to be higher in wild populations compared to populations kept in captivity the intrinsic mortality (due to senescence) is probably not very different. Thus, results from studies on captive populations are relevant for understanding senescence in natural avian populations (Ricklefs 2000).

The mechanistic factors explaining an age-related decrease in BMR may either be a reduction of lean body tissue and organ masses, a reduction in metabolic intensity in tissues and organs, or a combination of these factors. A reduction in metabolic intensity can possibly be ascribed to a variety of different factors influencing any levels in the metabolic pathway. Thus, age-related changes in BMR may be attributed to a numerous set of mechanisms which for the time being is not well understood. Studies on humans and rats, which are known to show a decrease in BMR with age, suggest that both the mass of metabolically active tissues and their specific metabolic activity decline with age (Fukagawa *et al.* 1990; Piers *et al.* 1998; Even *et al.* 2001). In rats, this reduction in tissue metabolic activity seems not to be due to reduced mitochondrial density, but rather an increased mitochondrial dysfunction with age (Navarro and Boveris 2007). Thus, a gradual accumulation of oxidative damage on mitochondrial structures causing mitochondrial dysfunction (reducing ATP-production) is one potential explanation for a decline in BMR with age. However, the fact that the functional decline in the “metabolic machinery” with age can be counteracted by antioxidant defence and repair of oxidative damage (Beckman and Ames, 1998) makes another explanation for a reduction in BMR with age plausible. If we assume that the antioxidant protection has an energetic cost which is reflected in BMR, a decline in BMR could also be expected if progressively less energy is devoted to counteract oxidative damage with age. These two potential explanations are not mutually exclusive, and it may be difficult to distinguish them given the lack of knowledge about how antioxidant defence and repair contribute to the rate of basal metabolism.

Several species including both mammals (Greenberg 1999; Even *et al.* 2001; Miaysaka *et al.* 2003; Speakman *et al.* 2003) and birds (**Paper IV**; Broggi *et al.* 2007) show a decline in BMR with age, whereas others species of mammals (O’Connor *et al.* 2002; Chappell *et al.* 2003) and birds (Blackmer *et al.* 2005; Moe *et al.* 2007) do not. The “disposable soma theory” suggests an evolutionary explanation for the observed inter-specific differences in the relationship between BMR and age. This theory states that the investment in antioxidant defence and repair of oxidative damage (somatic maintenance) is related to the rate of extrinsic mortality and hence expected lifespan (Kirkwood and Rose 1991; Kirkwood and Austad 2000). Short-lived animals experiencing high extrinsic mortality should benefit from investing energy in early reproduction at the sacrifice of somatic maintenance. Long-lived animals on the other hand would benefit from investing energy in somatic maintenance to prevent/reduce a decline in physiological performance with age. Thus, if evolution of optimal somatic maintenance is dependent on lifespan the relationship between age and BMR are also expected to be dependent on lifespan. There is some evidence both in birds and mammals suggesting that this may be true. Zebra finches and great tits, which both are short-lived species, show a decline in BMR with age (**paper IV**; Broggi *et al.* 2007), while in two long-lived species, leach's storm petrels and snow petrels (*Pagodroma nivea*), BMR does not decline with age (Blackemer *et al.* 2005; Moe *et al.* 2007). In mammals, short-lived rat species show a decline in BMR with age (Greenberg 1999; Even *et al.* 2001; Miyasaka *et al.* 2001), while BMR in the long-lived naked mole rat (*Heterocephalus glaber*) seems unaffected by age (O'Connor *et al.* 2002). Humans do however diverge from this pattern by showing a substantial decline in BMR with age in spite of being very long-lived (Piers *et al.* 1998). Hence, there does not always seem to be a strict relationship between age-effects on BMR and lifespan, pointing to possible complex evolutionary and mechanistic causalities.

7. Conclusions and future perspectives

- Diet quality during development in zebra finches had an effect on adult body mass, and thus on total basal energy expenditure. However, the difference in BMR between the diet groups was not greater than expected from the variation in body mass. Thus, early nutrition quality (different protein content) apparently does not have any direct effect on BMR in zebra finches. That is, developmental plasticity elicited by variable early nutrition quality does not seem to contribute to variation in basal metabolic intensity.
- Basal metabolic rate showed a significant repeatability in captive zebra finches, thus, one of the prerequisites for natural selection to operate upon this trait is fulfilled. Furthermore, the variation among individuals persisted over a considerable portion of the expected lifespan in this species and individual BMR differences could thus potentially be subject to selection at any time throughout life.
- Basal metabolic rate was found to be a significant heritable trait in zebra finches. The genetic correlations between BMR and morphological traits tended to be stronger than the phenotypic correlations, suggesting that the environment disrupts some of the underlying genetic links between these traits. Only one sixth of the additive genetic variance of BMR was independent of the genetic variance in body mass. Thus, a potential for adaptation in BMR independent of body mass (metabolic intensity) is probably present, but may be limited.
- BMR declined with age in the zebra finches. This effect was not confounded by selective disappearance, and was therefore attributed to within-individual change in BMR as the finches got older. Assuming that the age-related decline in BMR was related to a true physiological decline with age, the study gives support to the disposable soma theory for the evolution of senescence.

Evolutionary physiology is a new discipline in biological science, and our understanding of the genetics underlying physiological traits is consequently scarce compared to e.g. morphological and behavioural traits. Much work remains to fill this gap of knowledge regarding the micro-evolutionary potential in physiological traits (e.g. metabolic traits). Selection experiments under laboratory conditions could be a powerful tool to analyse the strength of selection and genetic architecture of metabolic traits. On the other hand, there exists some scepticism regarding the relevance of laboratory studies when trying to interpret genetic architecture of natural populations. Thus, it could be argued that quantitative genetic studies in wild populations should be preferred. However, quantitative genetic studies require large samples of individuals with known genetic relationships, which may be an obstacle when sampling data from wild populations. One solution to this problem could be to incorporate studies of physiological traits in broad research programmes of evolution and ecology which are already established.

Physiological and evolutionary approaches are needed to increase our knowledge of the selective forces which have caused evolution of species-specific relationships between energy metabolism and age. Comparative studies of animals with different extrinsic mortality and lifespan could serve as potentially fruitful models. Furthermore, the various mechanisms causing a decrease in BMR with age (as found in the zebra finch) are still poorly understood and deserve further attention.

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Errata

Paper I

The first sentence in the second paragraph in the results should be: “In order to test for influence of experimental group (either low-quality or high-quality food during early life) in addition to body mass and sex, which could be presumed to influence BMR, we performed a multiple linear regression with BMR (expressed as mass dependent oxygen consumption; ml O_2 h^{-1}) as the **dependent*** parameter, and group, sex and body mass as **independent** parameters.”

***** Changes from the original manuscript are indicated with bold text.

Paper I

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Individual variation in the basal metabolism of Zebra finches *Taeniopygia guttata*: no effect of food quality during early development

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Abstract. We investigated the physiological background to individual variation in basal metabolic rate (BMR) in a laboratory population of a small passerine bird, the Zebra finch, *Taeniopygia guttata*. We especially explored whether food-quality during early development influenced the BMR later in life. Zebra finches were raised on two different food regimes; one ('low quality') involving only mixed seed diet, while the other ('high quality') in addition including a protein supplement and daily fresh hard-boiled eggs. The different diets were administrated to the birds only during the first 6 weeks of life. At an age of 1.5 to 2 years, and after a breeding period of their own, BMR was measured in all individuals. Food quality had a significant effect on body mass. Adult finches raised on high-quality protein-rich food were significantly heavier (2.2 g for males and 1.9 g for females) than their counterparts raised on low-quality food. Despite this, the BMR of the adult birds did not differ beyond that expected based on variation in body mass alone. Hence, the early feeding regimes, which induced a non-genetic phenotypic variability in body mass, did apparently not induce any long-term changes in body composition or in organ metabolic intensity. © 2004 Elsevier B.V. All rights reserved.

Keywords: Allometry; Basal metabolic rate; Diet quality; *Taeniopygia guttata*; Zebra finch

1. Introduction

The basal metabolic rate (BMR) represents the lowest sustainable aerobic metabolism of a resting, postabsorptive, endothermic organism at thermoneutral conditions [1]. The

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BMR differs greatly between endothermic organisms, both within and between species. The interspecific variation have been attributed to adaptations to either specific environmental conditions, or to certain behavioural traits of the species. In birds, for instance, a high BMR is characteristic of species living in colder climates and at higher latitudes [2–4], in species living in an aquatic environment [5] and in species with a high level of aerobic activity [6]. In contrast, low BMR is characteristic of birds living in the tropics [7,8], of birds living on islands [9], and of night-active birds [5].

Intraspecific variations in BMR may be of two types. Firstly, variation in BMR may occur between populations. Such variations usually correlate with different environments. In House finches (*Carpodacus mexicanus*), for example, those individuals living in Colorado and Michigan have a significantly higher winter-BMR compared to those individuals living in southern California, which experience much milder winters [10]. Secondly, BMR may also vary within populations. For instance, a significant repeatability of BMR, measured over two consecutive years, have been described within an arctic population of Black-legged kittiwakes (*Rissa tridactyla* [11]). Other studies have also shown BMR to be a repeatable parameter [12,13]. Hence, within populations, some individuals may be ‘high-metabolic’ while others may be ‘low-metabolic’. The reasons to such differences in metabolic rate have hardly been studied. Some of the within-species BMR-variation may have a genetic component [14,15]. However, intraspecific variation in BMR within endothermic populations, may potentially also be attributed to variations in the physiology of the individuals caused by non-genetic factors. Such phenotypic flexibility is commonly found in living organisms [16], but it is not known whether this also would pertain to BMR. One factor which potentially could be involved in ‘setting’ the level of the basal metabolic rate, is the nutritional status during the early development. Both the amount and quality of food provided to avian nestlings have been shown to influence later adult body mass [17,18]. The amount of food may also influence body composition of nestlings [19,20]. Since both body mass and body composition are predictors of BMR, one could hypothesise that the level of BMR also would be influenced by early nutritional status.

In the present study, we test this possibility, by studying whether the BMR of adult Zebra finches will vary according to different nutritional status during their early growth.

2. Material and methods

The Zebra finch (*Taeniopygia guttata*) is a small finch native to the Australasia. The species will easily breed in captivity and is consequently often used in laboratory studies. We randomly assigned 15 pairs of finches to each of two large (10 m^3) walk-in aviaries. Twenty nest-boxes were placed in each aviary. These were accessible from the outside and were checked every day at around noon. All breeding birds were provided with a mixed seed diet (‘Life Care’; protein-content: 10.8% of wet mass; water content: 11.7%). In one colony (low quality, LQ) this was the only food provided. In the other colony (high quality, HQ), the finches were in addition provided a commercial protein-supplement (‘Eggfood Witte Molen’; protein-content: 11.3% of wet mass; water content: 9.8%) and daily fresh hard-boiled eggs (protein-content: 13.2% of wet mass; water-content: 76.4%). All types of food were provided ad libitum, as was drinking water. Ambient temperatures in the rooms with both breeding aviaries and ‘holding aviaries’ were kept at 24 °C and the relative humidity was kept at 40% RH. There was a light–dark regime of 12:12 h with light on at 07:00.

Breeding was recorded in both colonies from August 2001 to June 2002. All chicks produced in the two colonies were removed at an age of 6 weeks, at which time they were fully independent of their parents. After removal from the breeding colonies, they were kept in sex-specific larger holding aviaries until September 2002, when they themselves were assigned to breeding aviaries. During the ‘holding period’ and the subsequent breeding period, which lasted until June 2003, they all received a diet of normal ‘medium’ quality (NQ), consisting of the mixed seed diet and the protein-supplement. After the breeding period (in June 2003), all birds were again assigned to separate sex-specific holding aviaries and received the NQ food until September 2003, when measurements of BMR were obtained. Hence, at the time of BMR-measurements the birds were 1.5–2 years of age and the only experimental difference were in the food-quality they received during the first 6 weeks of age.

The basal metabolic rate was measured using an open-flow system. Dry air was pumped through metabolic boxes made from 1.5-l metal boxes painted flat black on the inside. Each metabolic chamber received an air flow of approximately 400 ml/min; measured accurately by a calibrated mass flowmeter (Bronkhorts). The effluent air was dried using drierite before the oxygen concentration was measured by an oxygen analyser (Servomex, two-channel analyser, type 4200). The oxygen analyser was calibrated using dry outside air (set to 20.95% oxygen) and pure stock nitrogen. Rates of oxygen consumption (VO_2) were calculated using formula 3A given by Withers [21], assuming an RQ of 0.71, and corrected for wash-out delay in the system by the method described by Niimi [22]. In this way, we obtained the instantaneous oxygen consumption rates. Birds were placed in the metabolic chamber in the evening (at about 19:00) and stayed overnight in the metabolic chamber. They were taken back to their holding aviaries at about 08:00 the next morning. An experimental set-up was constructed so that we could measure up to four birds per night. An automated valve-system switched between the four metabolic chambers, so that two chambers simultaneously were measured at a time for 26 min, interspersed between shorter periods of 4 min, during which fresh air entered the oxygen analyser. Hence, each individual was measured 26 min every hour during the night. All voltage outputs from the oxygen analyser and the mass flowmeter were stored at 30-s intervals on a datalogger (Grant Squirrel, Type 1203) and later transferred to a computer for analysis.

The lowest night-time level of VO_2, which was taken as representative of BMR, was calculated as the lowest 10-min running average of instantaneous oxygen consumption. This was usually experienced during the latter half of the night. The body mass (M_b) was measured (with an accuracy of 0.01 g) immediately before and after the experiment and a linear decrease in body mass was assumed when assessing the body mass value at the time of BMR-measurements. All measurements of BMR were conducted at an ambient temperature of 35 °C, which is within the thermoneutral zone of the Zebra finch (own unpublished results).

Before performing linear analysis on the relationship between M_b and BMR all data were log10-transformed. Linear regressions between M_b and BMR are presented as both ordinary least squares regression (OLS) as well as reduced major axes (RMA). The latter method is used because OLS might underestimate the true allometric exponent (see Pagel and Harvey [23]). The RMA-values were calculated according to Sokal and Rohlf [24]. All statistics were performed using SigmaStat ver. 3.0 (SPSS). Results are shown as mean±1 S.D.

3. Results

Food quality during the first 6 weeks of life had a distinct effect on the resultant adult body mass of Zebra finches. Hence, those individuals which were fed only seeds during early growth had an adult body mass significantly smaller that of the individuals which received a protein-rich food during early development. This was the case for both females (13.6±2.1 g for LQ-fed individuals vs. 15.5±1.1 g for HQ-fed; $t_{2,42}$=3.967, P<0.001) and for males (12.3±0.9 g for LQ-fed individuals vs. 14.5±1.5 g for HQ-fed; $t_{2,40}$=4.898, P<0.001). Within both feeding regimes, the adult body mass of the females was significantly higher than that of the males (P<0.001 for both).

In order to test for the influence of experimental group (either low-quality or high-quality food during early life) in addition to body mass and sex, which could be presumed to influence BMR, we performed a multiple linear regression with BMR (expressed as mass dependent oxygen consumption; ml O_2 h^{-1}) as the independent parameter, and group, sex and body mass as dependent parameters. Both sex and body mass were significant determinants of the variation in BMR (both P<0.001). Experimental group, however, did not contribute in explaining the BMR-variation (P=0.738). Hence, whether birds experience low- or high-quality diet during early life, will not affect their BMR later in life, despite large variation in body mass. The combined regression lines (OLS) for adults males is BMR (ml O_2 h^{-1})=$10.12*M_b^{0.504}$ (n=42, r^2=0.452, P<0.001) and for females BMR (ml O_2 h^{-1})=$9.51*M_b^{0.548}$ (n=44, r^2= 0.452, P<0.001). By using the

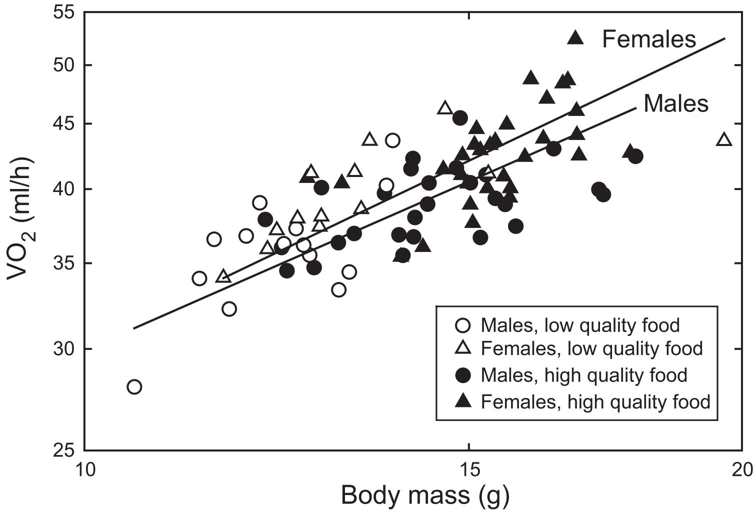

Fig. 1. Basal metabolic rate, expressed as oxygen consumption, in adult Zebra finches fed either low quality food (open symbols) or high-quality protein-rich food (filled symbols) during the first 6 weeks after hatching (circles=males, triangles=females). Regression lines (reduced major axes) are shown for males and females separately. See text for statistics.

reduced major axes method, we arrive at slightly larger exponents, the regression line being for males being BMR (ml O_2 h^{-1})= 5.34*$M_b^{0.749}$ and that for females BMR (ml O_2 h^{-1})=4.63*$M_b^{0.815}$ (Fig. 1).

4. Discussion

It is important to notice that the present study has not involved a food-deprivation experiment, since the birds in both experimental groups had free access to food at all time. Only the quality, through the protein content, were manipulated. Hence, our data are not directly comparable with the many studies showing that reduced food intake, will alter the adult morphology including body mass [18,25,26].

Our finding that food quality during early development significantly influences the adult body mass concur with the results of Boag [17] and Birkhead et al. [27] also on Zebra finches. Boag [17] likewise manipulated the protein-content of the diet given to Zebra finch nestlings and found that both the growth rate as well as the adult body mass was higher when chicks were fed high-protein food than when fed a low-protein diet. The body mass difference was found to be evident for up to one year of age. Hence, the present study supplements that of Boag [17] in that we found the diet-induced body mass difference to be present up to an age of 2 years. Other studies, in which manipulation of food quality have been performed have produced mixed results. Hochachka and Smith [28] supplemented the food of free-living Song Sparrows (*Melospiza melodia*), and in this way manipulated the food-quality of the nestlings. They also found an immediate positive effect on the growth rate in the food-supplemented nestlings. However, when measured at an age of between 56 to 108 days, there was no difference in body mass between the experimental groups. A similar lack of long-term effect on adult body mass was also reported by Ohlsson and Smith [29] after manipulation of the protein-content of the diet fed to chicks of the Ring-necked pheasants (*Phasianus colchicus*) (although in this species the tarsus length was affected). We can offer no explanation as to why food quality during early life has a strong effect on body mass in Zebra finches and apparently not in other species. It is, however, difficult to compare the results directly because they involve quite different food-manipulation techniques. Our results together with that of Boag [17] and Birkhead et al. [27] clearly show that drastic phenotypic variation can be induced by environmental effects, even in a physiological character such as body mass which generally is known to be highly heritable [30].

Since BMR is the sum of the metabolic rates of all organs and components of an organism, any change in body mass will in turn affect the BMR. Consequently, correlative relationships between body composition and BMR have been repeatably reported [19,20,31–34]. The relationship between body mass and BMR is generally described by exponential relationships, and if all organisms were built in proportionally the same way and the organs had similar metabolic intensity, the mass exponent of such relationships would be 1.0. Normally, however, mass exponents will be lower or higher than 1.0. Our finding of intraspecific allometric exponents for the Zebra finches (0.75–0.82 for RMA estimates and 0.50–0.55 for OLS estimates) is strikingly similar to what is normally found for interspecific relationships in birds [5]. In contrast, our exponents are below that normally reported for intraspecific BMR–body mass relationships in birds, where exponents well above 1.0 generally are reported (see Kvist and Lindström [35]). It is difficult to ascertain exactly why this is so, except that in contrast to most other bird species, high body mass in Zebra finches is apparently not paralleled by disproportionally larger organs having a high metabolic intensity, which have been the usual explanation for allometric exponents above 1.0. On the contrary, high body mass in Zebra finches must be paralleled by either disproportional smaller high-metabolic organs or their organs must have lower metabolic intensity. One explanation could be that heavier individuals are carrying larger amounts of fat, which has little metabolic activity compared to most other internal organs. Lindström and Rosén [36] recently showed that the accumulation of fat carried a high metabolic cost in Greenfinches (*Carduelis chloris*). However, their experiments were carried out over a very short time span (days) where such an immediate 'cost' would be eminent. Presumably, there are fewer metabolic costs involved when fat is permanently stored as in heavier vs. lighter individuals.

Irrespective of the reason for the low intraspecific allometric exponent in the Zebra finches, our study has demonstrated that despite the significant effect on body mass incurred by manipulation of the food-quality during the nestling stage, the BMR of the resultant adult birds did not differ beyond that expected based on variation in body mass alone. Hence, the early feeding regimes have apparently not induced any long-term changes in body composition or in organ metabolic intensity. Our results imply that the non-genetic phenotypic variability in body mass, which we have induced during nestling development by different feeding regimes, will not result in disproportional organ masses inducing different metabolic rates.

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Paper II

Paper III

Paper IV