Available online at www.sciencedirect.com
ScienceDirect
Mechanisms of Ageing and Development 127 (2006) 802–807
mechanisms of ageing and development
www.elsevier.com/locate/mechagedev

Consequences of artificial selection on pre-adult development for adult lifespan under benign conditions in the butterfly *Bicyclus anynana*

Jeroen Pijpe [a,*], Klaus Fischer [b], Paul M. Brakefield [a], Bas J. Zwaan [a]

[a] *Institute of Biology, Leiden University, NL-2311 GP Leiden, The Netherlands*
[b] *Department of Animal Ecology I, Bayreuth University, D-95440 Bayreuth, Germany*

Received 11 May 2006; received in revised form 28 June 2006; accepted 12 July 2006
Available online 30 August 2006

Abstract

The genetic architecture underlying the regulation of lifespan is shaped by evolutionary history, thus, including selection in past environments. In particular, the developmental environment is important, because selection pressure for survival is highest during development. From this life-history point of view, the ageing phenotype is the outcome of these factors, and links between the developmental and adult life stage are expected. In this study, we specifically address whether genetic variation in pre-adult traits affects adult lifespan. We use lines artificially selected for divergence in development time, pupal mass or egg size, thus, exploiting the standing genetic variation in pre-adult traits present in natural populations of *Bicyclus anynana*. We then reared individuals from each line and the unselected base population in a common environment, and recorded each selected trait and adult longevity. In general, differences in adult lifespan across selection lines were small. This is not surprising given the benign conditions used here. The minor differences in adult survival were only partially the result of environmental influences, as indicated by low phenotypic correlations. However, significant genetic correlations point to possible intrinsic mechanisms involved in lifespan regulation. Genetic variation in egg mass or pupal mass did not contribute to variation in lifespan. However, we found a negative genetic correlation between developmental time and lifespan, suggesting a genetic coupling of faster development with a longer adult lifespan in this species. A follow-up study with an identical set-up that introduces stress during development should give a more detailed insight into the role of development in the regulation of lifespan.

Keywords: Ageing; Longevity; Genetic correlation; Phenotypic correlation; Egg size; Pupal mass; Development time

1. Introduction

Arguably, two main life stages can be differentiated in successfully reproducing organisms: development and adulthood. Classically, in sexual multicellular organisms development initiates with conception, although pre-zygote factors are recognised to be influential (Mousseau and Fox, 1998). Development ends with sexual maturity and the start of the reproductive period, while adulthood ends with death.

The transition from development to adulthood can be fairly gradual in mammals whilst in holometabolous insects these two stages are highly distinctive. Development essentially ends in metamorphosis and the eclosion of an imago or adult. The major transition of metamorphosis occurs in the pupa, when imaginal discs developed from epidermal cells in the embryo divide and grow to form the adult (Wolpert, 1998). The role of development in shaping the adult phenotype is well established. In insects, many fitness-related traits, such as body size, fat content, colour pattern and the timing of eclosion are determined during development (Brakefield et al., 2003). Thus, the environment during development may leave its footprint in adulthood, influencing adult life history, including ageing (Zwaan, 2003; Gluckman and Hanson, 2004; Brakefield and Frankino, 2006).

When investigating the role of development on lifespan, it is crucial to discriminate between variation in development that results from environmental or genetic sources, or by their interaction (Zwaan, 2003). Much research on environmentally induced variation in adult lifespan has been performed in the past using *Drosophila* (Miller and Thomas, 1958; Lints and Soliman, 1977; Zwaan et al., 1991), mostly in relation to the developmental theory of ageing (Lints and Lints, 1971). It has

* Corresponding author. Tel.: +31 71 5275191.
E-mail address: j.pijpe@biology.leidenuniv.nl (J. Pijpe).

0047-6374/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.mad.2006.07.006

proven to be difficult to show a clear relation of developmental life history traits with lifespan. This has two main reasons. First, the environmental manipulation that was used in such experiments introduces additional variation. Secondly, the context of the natural environment was missing. As a consequence, the attention of ageing science has diverted away from studying development. An exception is the influence of stress during development on adult lifespan (Zwaan et al., 1991; Sorensen and Loeschcke, 2001; Brakefield and Frankino, 2006). This has led to the stress theory of ageing, which states that fitness traits in development and adulthood are linked through stress resistance, which is omnipresent in all life stages (Parsons, 2003). Investigating developmental variation caused by genetic factors typically involves mutants of large-effect (Clancy et al., 2001) or transgenic manipulation (Aigaki et al., 2002) that allow successful screening for genes involved in longevity.

It is unclear to what extent standing genetic variation in developmental traits contributes to variation in adult lifespan. This information is important because variation in lifespan can only be fully understood when taking into account the whole life-history, including development. Natural selection should result in pre-adult growth traits that maximise fitness in the adult stage. All developmental traits are fitness related and have been under strong selection. Lifespan may evolve, but never independently of development and the genetic architecture underpinning development. This is recognised by a recent initiative that highlights the role of development in ageing (see Westendorp and Wimmer, 2005). By focusing on the standing genetic variation present in natural populations it is possible to identify the genes that matter in the evolution of lifespan, which may eventually help to the implementation of treatments for age-related diseases in humans (Kirkwood and Austad, 2000; Zwaan, 2003).

An excellent organism to study natural variation in life-history traits in relation to development is the tropical butterfly *Bicyclus anynana*. This African species occurs in an environment with wet and dry seasons and has evolved adaptive phenotypic plasticity in the form of seasonal polyphenism (Brakefield and Frankino, 2006). Many traits of the adult phenotype are determined late in development through endocrinological responses to temperature (Brakefield et al., 1996; Koch et al., 1996; Zijlstra et al., 2004). We have used artificial selection to establish separate pairs of upward and downward lines with divergent phenotypes for development time, pupal mass and egg size, thus exploiting the standing genetic variation in life history traits present in natural populations of *B. anynana*. These are all key developmental traits and closely related to fitness (Brakefield et al., 2003). We use these to tease apart the environmental and genetic effects on variation in adult lifespan.

The present study aims at uncovering the role of genetic variation in several pre-adult life-history traits on adult lifespan. Our experiment is unique in that we have used the same base population representing an outcrossed stock to set up independent pairs of selected lines for each of the key life history traits in pre-adult development and then used a common garden experiment to examine the consequences for adult life span. Since the experiment was performed in a single benign environment, we can investigate the influence of genetic variation in pre-adult traits on variation in adult lifespan directly, minimising the introduction of additional variation (e.g. caused by different environments).

2. Materials and methods

2.1. Experimental populations

All butterflies were derived from a single stock population that has been reared in the laboratory for over 100 generations since the parental generation was collected in Malawi in 1988. High levels of heterozygosity have been maintained (van't Hof et al., 2005). Butterflies from seven selection lines established from this stock were used in the present experiment. Six lines were artificially selected for increases or decreases in the values of the three major life-history traits: egg size, pupal mass and development time (egg to adult). In addition, the stock population was used as unselected control. Please refer to Table 1 for an overview and their names.

The unique designs of the present experiment posed practical limitations and forced us to use only a single replicate of each selection line. However, all lines were replicated with significant realised heritabilities and replicates generally showing comparable results (egg size selection: Fischer et al., 2006; pupal mass selection: Frankino et al., 2005; multiple selection lines, including development time: Brakefield and Kesbeke, 1997; Zijlstra et al., 2003). In addition, all past selection was performed at 27 °C in environments similar to the one used in the present experiment. Consequently, the extensive phenotypic divergence of the lines in the targeted traits has a clear genetic basis, and on the basis of our previous work (Fischer et al., 2006), are typical representatives of the full set of selection lines.

2.2. Experimental design

A total of 700 individuals, 100 per line, were followed individually from egg to death. Temperature was 27 °C (±1 °C), humidity 70% (±5%) and photoperiod 12:12 h light:dark throughout the experiment. The parental generation of all lines was also reared under these conditions to eliminate potential cross-generational effects.

Eggs were laid on maize cuttings over a 12 h light period, then weighed to the nearest 1×10^{-4} mg on a micro-balance and transferred to 1.5 ml Eppendorf tubes. Hatchlings were individually transferred to *Oplismenus africanus* plants, a natural food plant of *B. anynana*. The plants were propagated vegetatively, to standardize environmental conditions as far as possible. Larvae were reared in a common garden environment (one level of a single climate room).

Table 1
Overview of the lines used in this experiment and their abbreviation (code) used throughout this paper

Life-history trait	Egg size	Pupal size	Development time	Unselected
Line name (code)				
Selection *increased* trait value (↑)	Large egg size (LES)	Large pupae (LP)	Long time (LT)	Stock (stock)
Selection *decreased* trait value (↓)	Small egg size (SES)	Small pupae (SP)	Short time (ST)	