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Effects of temperature and maternal and grandmaternal age on wing shape in parthenogenetic *Drosophila mercatorum*

Anders Kjærsgaard[a,*,1], Søren Faurby[a,**,1], Ditte Holm Andersen[a,b], Cino Pertoldi[a,c], Jean R. David[d], Volker Loeschcke[a]

[a]*Department of Ecology and Genetics, Institute of Biological Sciences, University of Aarhus, Ny Munkegade, Building 1540, DK-8000 Aarhus C, Denmark*
[b]*Istituto Nazionale per la Fauna Selvatica, Via Cà Fornacetta 9, 40064 Ozzano dell'Emilia (BO), Italy*
[c]*Department of Wildlife Ecology and Biodiversity, National Environmental Research Institute, Kalø Grenåvej 14, DK-8410 Rønde, Denmark*
[d]*Laboratoire Populations, Génétique et Evolution, CNRS, 91198 Gif-sur-Yvette cedex, France*

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Abstract

The effects a stressful developmental temperature and maternal and grandmaternal age was investigated on plasticity of wing shape, using the Procrustes method, in two parthenogenetic strains of *Drosophila mercatorum*.

Shape was significantly modified by both factors. Maternal and grandmaternal age were more important than temperature when compared in terms of larger Mahalanobis distances.

Our results suggest that, in populations harbouring low genetic variability, the direct shape changes caused by different developmental temperatures may be smaller than indirect changes mediated through changes in age composition of the maternal and grandmaternal generation.

Keywords: Global change; Maternal effects; Procrustes analysis; Phenotypic plasticity; Ageing; Growth temperature

1. Introduction

Climate is changing globally at a speed that is faster than presumed changes in the past 10,000 years. On average, global temperature has increased by 0.7 °C over the past century and climate models indicate that the global temperature will increase approximately 2.8 °C within the next century (Wang et al., 1995). Several studies have already demonstrated ecological changes in response to climate changes (Walther et al., 2002) and are usually considered as physiological responses (Crick et al., 1997; Bradshaw and Holzapfel, 2001), but see Rodriguez-Trelles and Rodriguez (1998) and Umina et al. (2005). Although the effects seen are mainly plastic, it is extremely difficult to determine how large the influence of genetic variation and selection is. Natural selection will shape the response to climatic change in large and genetically variable populations, but in small populations or populations with little genetic variation the only possibility to respond to climatic change is by phenotypic plasticity and maternal effects (Rossiter, 1996; Réale et al., 2003).

Maternal effects, which can be defined as transfers of environmental and parental effects through non-genetic pathways (Rossiter, 1996), can in some cases be very important (McAdam et al., 2002; Pakkasmaa et al., 2003, but see Huey et al., 1995; Crill et al., 1996). Furthermore, some maternal effects have been shown to be subjected to natural selection and not being merely random fluctuations due to environmental stochasticity (Mousseau and Fox, 1998).

Temperature-induced developmental plasticity is known to influence thermal tolerance, the rate and timing of development, body size at maturity and to generate persistent effects on the adult phenotype (Johnston and

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*Corresponding author. Tel.: +45 89427242; fax: +45 86127191.
**Also for correspondence.
E-mail addresses: anders.kjaersgaard@biology.au.dk (A. Kjærsgaard), soren.faurby@biology.au.dk (S. Faurby).

[1] Both authors contributed equally to this work.

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Temple, 2002; David et al., 2004). The effect of temperature on wing size is well understood and has been commonly studied in laboratory experiments on thermal evolution, with lower temperature often resulting in increased wing size (a surrogate for body size) (McCabe and Partridge, 1997; Reeve et al., 2000; Nijhout, 2003; Pertoldi et al., 2005, David et al., 2006).

Compared to size, shape is thought to be controlled to a higher degree by genetic factors, and is subject to higher developmental constraints (Workman et al., 2002; Plejdrup et al., 2006). Here we want to evaluate if temperature changes have a significant impact on shape even though plasticity is believed to be low.

Most experiments have focused only on a single environmental factor but since organisms are subjected to many different environmental conditions this does not provide a realistic evaluation of the relative importance of the given factor. Likewise, only few studies have investigated the impact of both maternal effects and temperature on the progenies' phenotype (Hercus and Hoffmann, 2000; Faurby et al., 2005). It has been found that, like temperature, the age of both the mother and the grandmother may influence size and shape of offspring phenotypic characters (Røgilds et al., 2005; Faurby et al., 2005).

In this paper further analyses have been conducted on data obtained from a previous experiment by Faurby et al. (2005) on the impact of maternal and grandmaternal age, and temperature on different unidimensional uncorrelated wing traits. The data has been analysed by the Procrustes method, based on landmark coordinates which preserves the geometry of the wing but removes size variation and allows the different structural traits to be analysed separately (Rohlf and Slice, 1990; Klingenberg and McIntyre, 1998). The main emphasis of the original study was the effect of maternal and grandmaternal age, whereas this study is more focussed on the effects of temperature. Two parthenogenetic strains of *Drosophila mercatorum* were chosen as model organisms (Templeton et al., 1976). Due to the lack of genetic variance within strains we are able to evaluate how size and shape of wings are affected by rearing temperature as well as by maternal and grandmaternal age. Caution should be taken in the interpretation of results obtained from laboratory organisms regarding the ecological relevance. This problem may be greater in our case since parthenogenetic *Drosophila* are not found in the wild and they appear to have developmental problems (Templeton et al., 1976). We do believe, though, that the advantages of parthenogenetic organisms outweigh these disadvantages, especially since the common alternative of using iso-female lines is not ecologically relevant either.

2. Material and methods

2.1. Experimental design

We used two different parthenogenetic strains of *Drosophila mercatorum*: Iv-23-o-Im isolated from a sexually reproducing population in 1990 (Kramer and Templeton, 2001) hereafter referred to as strain 1, and strain 2 which was founded in a Brazilian laboratory (Ezio Mattioli, pers. comm.). Crowding and chemical stress was avoided as described in Faurby et al. (2005).

We used old and young mothers as defined by Røgilds et al. (2005). Young mothers (referred to as "Y") were 3–6 days and old mothers (referred to as "O") were 15-18 days (post-emergent age). We used the four age-classes from the different combinations of young and old mothers and grandmothers. They are referred to as YY, YO, OY and OO, where the first letter states the age of the grandmother and the second letter the age of the mother. A previous pilot study found that there was no significant difference between the numbers of offspring from the different age-classes (Røgilds et al., unpublished). Both the maternal and grandmaternal generation was kept at 25 °C. The offspring were then either reared at 25 °C or transferred to 28 °C after eggs were laid over 3 days and kept at the respective temperature for the remainder of their development. For details of the design see Faurby et al. (2005).

2.2. Measurements

Wings of flies were removed and placed in a droplet of lactic acid, on a microscope slide and covered with a cover slip. Wings were named referring to their strain, temperature and grandmaternal and maternal age group. They were photographed by using a camera attached to a dissecting microscope and a computer with the software IM1000 version 1.2 and measured by the use of the software package ImageJ version 1.33u (Rasband, 2001). Nine landmarks were measured (A–I) (Fig. 1). For each strain at each temperature at each age-class, 150 wings were measured.

In order to quantify possible measurement errors, we chose 20 flies at random. For each of these flies all landmarks were measured three times (by the same person) and the within individual coefficient of variation for each mean was taken as an estimate of the measurement error, adding Haldane's (1955) correction for small sample size.

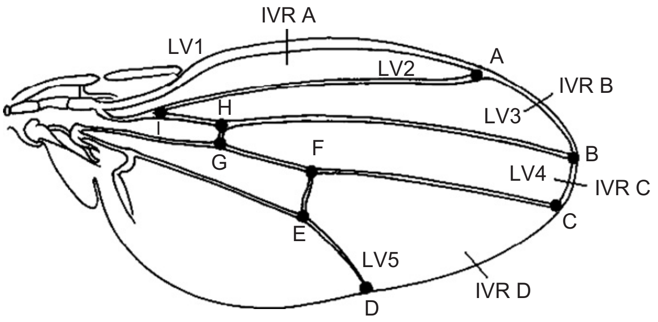

Fig. 1. Positions of the nine landmarks for measurements (A–I). LV, longitudinal veins; ACV and PCV anterior and posterior cross veins; IVR intervein regions as defined by Birdsall et al. (2000) (modified from Loeschcke et al., 1999).