



## Temperature-induced phenotypic plasticity of flight

### performance in *Sepsis punctum*

#### Aim of the visit

Learn new techniques that build on my previous work with wing morphometrics in *Drosophila* (Faurby *et al.* 2005; Kjærsgaard *et al.* 2007; Krag *et al.* 2009; Kjærsgaard *et al.* 2010) and to study in detail how morphometric traits may affect flight performance. My previous work did not directly test the link between observed body size, wing morphological patterns and flight performance, although this would have been highly fruitful.

#### Project introduction

Insect flight is usually associated with considerable metabolic demands e.g. (Rankin & Burchsted 1992; Feder *et al.* 2010). Therefore optimization of flight in different environments can be expected to occur *via* plastic or evolutionary responses in traits that are related to flight performance. Morphological traits such as wing size and shape, thorax size, overall body size and their interrelationships are considered important in this respect (e.g. Loeschcke *et al.* 1999). It has been shown repeatedly that the relative changes with developmental temperature in these traits are often not isometric, suggesting that adaptive adjustments or developmental constraints may indeed affect flight performance (e.g. Hoffmann *et al.* 2007; Frazier *et al.* 2008). Most notably many studies have observed an increase in the wing size/body size ratio with decreasing temperature. This has been hypothesized to be an adaptation to decreased wing beat frequency and muscle power output by decreasing the wing load (body weight per wing area) (Starmer & Wolf 1989; Azevedo *et al.* 1998). The majority of studies that have discussed morphometric traits and flight capacity have not measured flight performance directly, so the link between these remains to some degree obscure and mostly based on correlational data.

The aim of this experiment was to test the adaptive significance of developmental plasticity associated with flight in the black scavenger fly *Sepsis punctum* by:



1. Testing the effect of high or low developmental temperature on flight performance using a flight mill setup.
2. Dissecting differences in flight performance by comparing morphological traits within and between developmental temperatures.

## **Experimental design**

### *Study organism*

*Sepsis punctum* is well-studied in the laboratory of Prof. Blanckenhorn serving as the primary model species here. It is widely distributed over temperate zones in the northern hemisphere where it is closely associated with faeces of several vertebrates wherein eggs are oviposited and the larvae develop. European populations are sexually dimorphic with males usually being larger than females.

### *Experimental treatments*

Flies collected in the field near Zurich, Switzerland and Stockholm Sweden, were propagated under standard laboratory conditions at 24°C, which is considered close to optimal for this species, and a 16/8 light/dark cycle for two generations to avoid cross-generational effects. The F3 generation was split into two groups and allowed to develop at constant temperatures at the high (30°C) and low (18°C) ends of the temperature range of development. At the same time these temperatures lie within the non-stressful realm so as to avoid erroneous conclusions caused by pathological developmental effects. These treatments result in morphologically measurable differences in the progeny due to plastic responses to developmental temperature in e.g. body and wing size. To test if and how these phenotypic changes translate into differential flight performance, flies from each developmental temperature were tested in flight mills at the optimal temperature (24°C) from which they were initially derived.

### *Flight mill setup*

When attached on the flight mill, the flies can fly only in circles in the horizontal plane. The mill is composed of a top piece with four arms which balances on a thin vertical pin. Flies can be attached to two



of the arms so that one experimental fly is placed on one arm and a dead fly is attached to the opposite arm a counter balance. The arms are L-shaped so that the fly flies in a circle.

In this experiment, anaesthesia was avoided when attaching flies since this can have a large negative impact on sepsid flies. The flies were instead individually caught in the population cage with a piece of cotton and the legs fixed while the fly was mounted to a piece of thick human hair extending from the mill arm. It was mounted with a small amount of fluid (hot) candle wax to the thorax.

The ten flight mills were placed in a climate cabinet at the standard conditions described above and connected to a computer placed adjacent to the cabinet. The flies were mounted between 10 and 11 a.m. and data was then collected for ten hours thus approximately reflecting dispersal capacity or propensity in one day in the absence of food and water. The first 15 minutes were removed in order to remove the most intensive initial reactivity caused by handling. The numbers of revolutions performed by individual flies were automatically counted by the computer as a light beam was broken twice for each revolution, and summed in bins of 5 s intervals. These were then subsequently transformed into metres flown over the whole 10 h period.

#### *Morphometric measurements*

After the flies were removed from the flight mill they were frozen for morphometric measurements. Head width was used as a surrogate for body size and wing length was computed so that wing loading could be estimated from the wing length/head width ratio. This design facilitated an analysis of functional aspects of morphometric traits on flight performance.

#### *Statistical analysis*

All analyses were conducted in R. Since there was large variation in flight distance, the data were log transformed to increase homogeneity. A lower cut-off of 50 metres flight was used, as shorter distances may not be biologically meaningful considering that the flies are deprived of mates, egg-laying substrate, food and water. This unfortunately lowered the sample size of the 30°C developmental temperature drastically (2-4 observations per treatment), so I restricted analyses involving flight to the 18°C treatment. A linear mixed effects model was fitted with population and sex as fixed factors and flight mill as a random



factor. Post hoc permutation tests were conducted for meaningful combinations of pairs of populations or sexes.

Linear mixed effects models were also fitted with head width, wing length and wing load as covariates to evaluate the effects of these metrics on flight distance (similar to analysis of covariance (ANCOVA)).

## Results

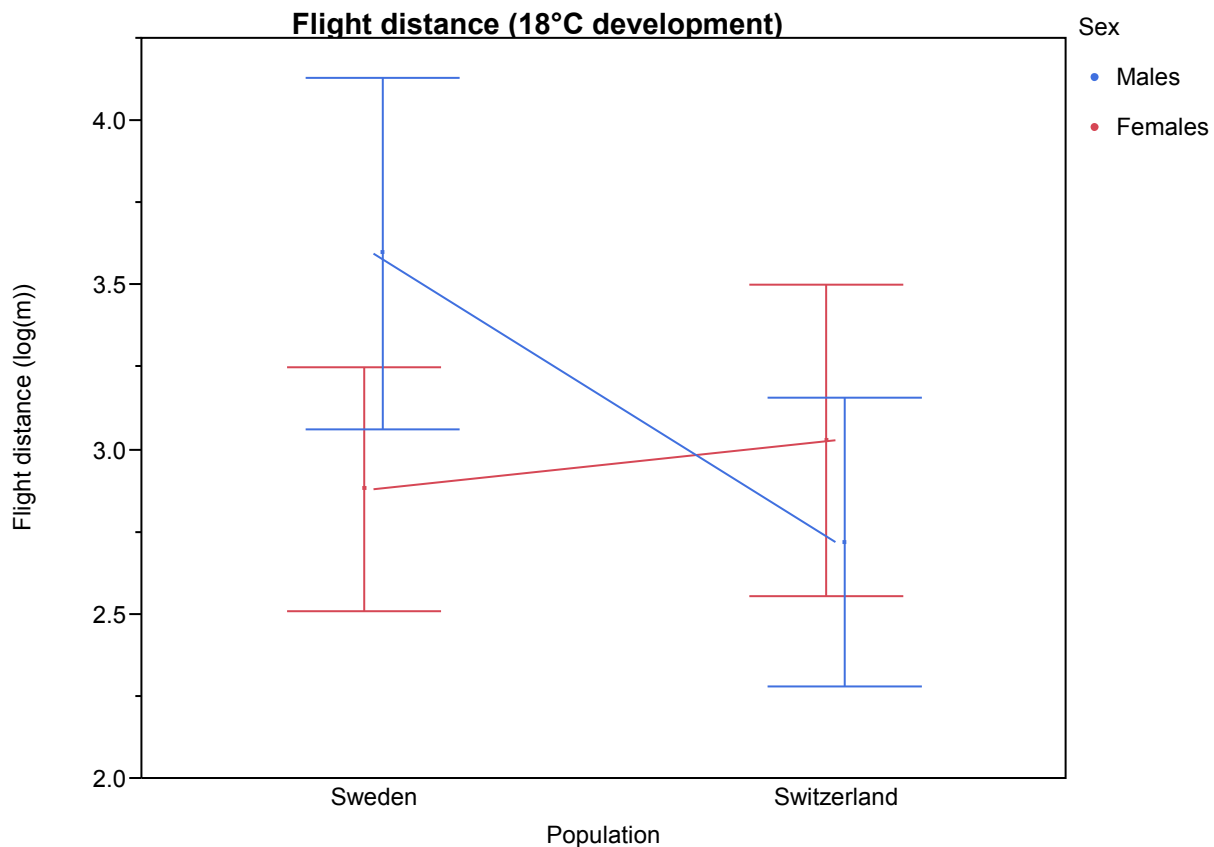
### *Flight*

Both population and sex were significant in the full model (pop:  $P = 0.017$ ; sex:  $P = 0.039$ ). The population by sex interaction was, however, only marginally significant ( $P = 0.081$ ) so model simplification was attempted by removal of the interaction term. This was justified albeit only barely ( $P = 0.067$ ). The full model was therefore retained. Fig. 1 shows that the effects were mainly due to longer flight distances of the Swedish males compared to the other three groups. This was confirmed by permutation t-tests where the Swedish males vs. females and vs. Swiss males were nearly significant or significant before correcting for multiple tests ( $P = 0.054$  and  $P = 0.040$ ).

Incorporation of the covariate head width (see fig. 2) revealed that this had a significant effect on flight distance ( $P = 0.004$ ), and that this effect was different for males and females ( $P = 0.012$ ) whereas this was not the case for the population by head width interaction ( $P = 0.089$ ).

Wing length did not affect flight distance as interpreted from the linear mixed effects model. The fit of the model was however also worse than for the other covariates judging from visual inspection and the multiple R-squared (0.21) obtained from standard ANCOVA (i.e. leaving out the random effect of flight mill). In comparison, the R-squared for head width and wing loading were 0.45, and 0.44.

Wing loading, i.e. the composite measure of the previous covariates, did have a significant effect on flight distance ( $P = 0.004$ ) and this effect was similar for both populations ( $P = 0.28$ ). As with head width, the effect was sex specific though ( $P = 0.009$ ). Three of the four groups showed a negative relationship of flight distance with wing loading whereas Swedish females showed a reversed pattern (sample size was however quite low).



**Fig. 1.** Flight distance (log10) of the two populations and sexes. Errorbars are 95% confidence limits.

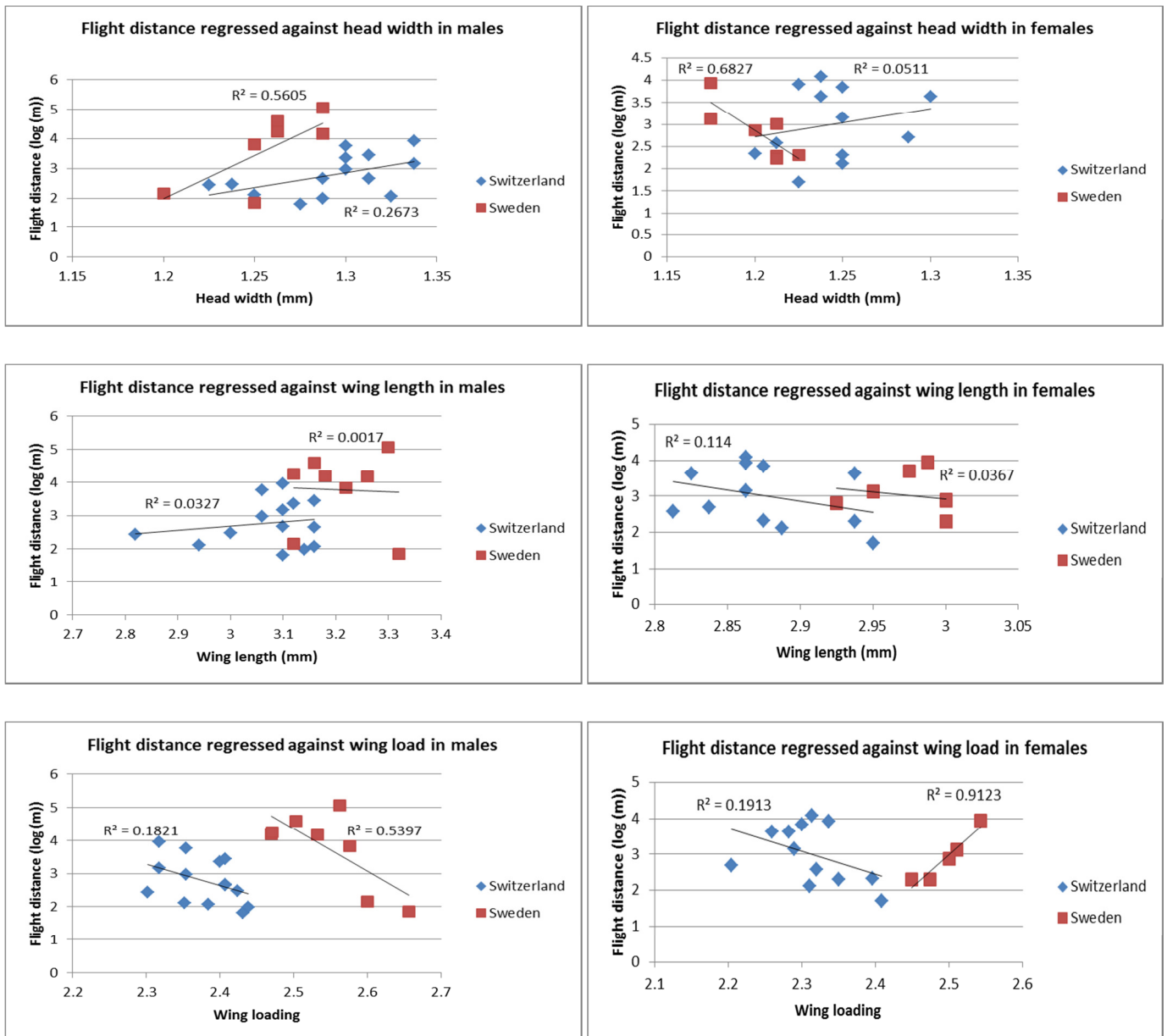
### *Morphometric traits*

The effect of the factors population, developmental temperature and sex on the body size surrogate “head width” were analysed with a linear model. None of the interaction terms were significant so the minimum adequate model contained only the main effects which were all highly significant after simplification.

The equivalent analysis for wing length showed that there were no interactions involving population so the model reduced to all highly significant effects of the factors development temperature and sex and their interaction + the main effect of population. The same was the case for wing loading.

### **Project outcomes**

The aim of the visit was three-fold: 1) to become familiarized with a new technique, 2) to investigate the effect of developmental temperature and the resulting adult morphology on flight capacity, and 3) to further increase collaboration with the host institution.



**Fig. 2.** Regressions of flight distance (log<sub>10</sub>) against the different morphological measures. Notice that axis scales are not fixed.

Adapting the technique to sepsids was not entirely without problems since these are sensitive to anaesthetization with CO<sub>2</sub> and chilling would make little sense when investigating temperature related effects. This problem was overcome by physically restraining the individuals, which took some practice. Sepsid flies also have strong legs so in some instances they managed to detach themselves from the mill.

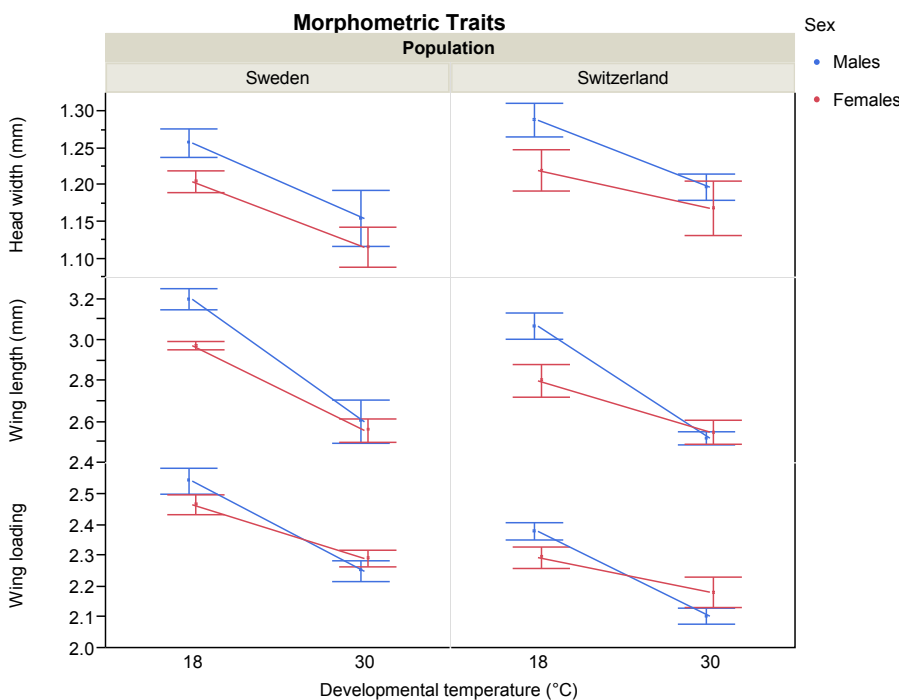
A noticeable result was that quite a lot of observations had to be removed due to very limited or no flying (i.e. < 50 m). This was particularly pronounced at the high developmental temperature, suggesting that flight capacity or propensity was affected by developmental temperature. High  $F_{st}$  values for European



populations have recently been found (unpublished data), supporting the notion that these flies are not strong fliers and/or are reluctant to fly long distances. Sample sizes therefore also did not quite suffice to make any strong inferences as of this moment, but some patterns emerged.

It was somewhat peculiar that wing length did not have any effect on flight distance, so the effect of wing loading was therefore mediated mainly by the body size (head width) effect on flight. A negative correlation between size and flight distance as seen in the Swedish females could be due to early reproductive effort. Looking at wing loading in isolation revealed an effect of population with the Swedish flies having higher wing loading ( $P \ll 0.001$ ). This may be an adaptation to flying at lower temperatures because the larger wing area may compensate for a lower wing beat frequency.

Meanwhile, the work with the flight mills has been expanded to a larger experiment and collaboration on house flies (*Musca domestica*), which is reported elsewhere and further studies are planned. This has all been possible because the visits to the host institution gave rise to a 2 year post doc in the Blanckenhorn group.



**Fig. 3.** The relationship of the three morphologic traits among populations and sexes shown graphically with 95% confidence limits.



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