

Adult body size was estimated as right wing length (Table 3), as length of the third longitudinal vein, from the anterior cross vein to the distal edge (Partridge *et al.*, 1987).

Flies reared at 18°C were about 10% larger than flies of the corresponding sex reared at 25°C, and this difference, estimated by t-test, was highly statistically significant.

Effects of growth temperature on traits that we have observed are consistent with literature data, while some deviations, always present when comparing different populations and/or different samples are consequences of the genetic differences between those populations (see *e.g.*, Trotta *et al.*, 2006), as well as specific interactions among environmental conditions, genetic basis and experimental design. Using the *BGSK* strain, we intend to observe effect of growth temperature also on some other, less investigated phenotypic characteristics, especially on some behavioral traits.

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### **Growth temperature, mating latency, and duration of copulation in *Drosophila melanogaster*.**

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For ectotherm species, such as *Drosophila*, temperature is one of the crucial environmental factors (David *et al.*, 1983; Precht *et al.*, 1955). Many papers considering influence of temperature on *Drosophila* geographic distribution, their adaptation on local thermal regimes, on phenotypic plasticity, as well as on different processes in all developmental stages were published (see *e.g.*, Anderson, 1973; James and Partridge, 1995; Hoffmann *et al.*, 2003; David *et al.*, 2004; Ayrinhac *et al.*, 2004; Trotta *et al.*, 2006). Delayed effects of growth temperature (temperature at which development occurs, from egg laying up to eclosion) on different phenotypic traits of adults are especially interesting (David *et al.*, 1983; Atkinson, 1994; French *et al.*, 1998). However, very little is known about delayed effect of growth temperature on behavior of *Drosophila* adults.

The present study examined the effect of two different growth temperatures (18°C and 25°C) on two components of mating behavior: *mating latency* (time between introduction of females and males into mating vial until inception of copulation) and *duration of copulation* (time from inception to the termination of copulation). Mating latency is an important component of fitness in *Drosophila* (Prakash, 1967) and is correlated with different fitness components, like fecundity, fertility and longevity (Hegde and Krishna, 1999; see also Rose *et al.*, 2004). Duration of copulation is primarily under genetic control, but may be affected by different factors, like previous mating experience (Singh and Singh, 2004; Pavković-Lučić and Kekić, 2006).

*D. melanogaster* flies used in this experiment belonged to the *BGSK* laboratory strain (see Obradović *et al.*, 2007). Flies were maintained on yeast's substrate (dry baker's yeast - sucrose - water - agar - nipagin), in mass culture, in 250 ccm glass bottles, without competition, at 25°C, relative humidity of 60%, and light conditions 12h L: 12h D, light from 8 a.m. to 8 p.m. From F<sub>67</sub> generation, 12 males and 12 females were taken and 12 families were formed. Part of the eggs collected from each pair was put into a climate room at 18°C, while the other part was maintained at 25°C. After eclosion, males that had developed on both temperatures were aspirated and kept into separate flacons during acclimatization at room temperature (about 23°C) until experiment started. Of course, as duration of development differed at different temperatures (see Obradović *et al.*, 2007), it was necessary to synchronize eclosion, as it was important to use males of similar age (4 to 8 days). In experiments, virgin females of the same age from basic laboratory population, reared at 25°C were used.

Mating behavior was tested during morning hours, at room temperature, in *female choice* experimental design: in the first place, males (10 males that developed at 25°C + 10 males developed at 18°C) were introduced into mating vials (250 ccm glass bottles), followed by 10 virgin females. All males introduced into one mating vial were full-sibs. Two replicates *per* family were made, or, in total, 24 replicates. Males that had developed at different temperatures were marked 24 hours before experiment started with fluorescent UV dust, as it does not influence their activity (Crumpacker, 1974; Terzić *et al.*, 1994). Observation lasted one hour *per* replicate; mating pairs were gently aspirated from mating vial and put into separate flacons.

Mean values of observed components of mating behavior were pooled for all 12 families and presented in Table 1. No significant differences in mating latency, as well as in duration of copulation between full-sib males which had developed on different temperatures were observed.

Table 1. Mean mating latency (*log* transformed) and duration of copulation (in *minutes*) in mating pairs in which males differed in growth temperatures.

Males growth temperature	Mating latency					Duration of copulation				
	N	$\bar{X} \pm SE$	t	df	P	N	$\bar{X} \pm SE$	t	df	P
18°C	112	2.761 ± 0.044	1.03	216	>0.05	103	18.37 ± 0.43	1.65	194	>0.05
25°C	106	2.695 ± 0.046				93	19.34 ± 0.38			

Males that had developed at 18°C were significantly larger than those reared at 25°C. Adult body size was approximated as right wing length, as length of the third longitudinal vein, from the anterior cross vein to the distal edge (Partridge *et al.*, 1987). Mean wing length of males developed at 18°C was:  $\bar{X} \pm SE = 114.14 \pm 0.26$ ; N = 107; at 25°C it was  $\bar{X} \pm SE = 102.63 \pm 0.28$ ; N = 94; this difference, estimated by t-test, was highly statistically significant (t = 29.51; P < 0.01; df = 199).

On the other side, when body size variation was created by varying the degree of crowding among larvae from an inbred strain of *D. melanogaster*, copulation duration was shown to depend on female body size, but either not or much less on male body size (Lefranc and Bundgaard, 2000). Unfortunately, in our experiment, we did not use females that had developed at 18°C; it is possible that variation in female body size created by growth temperature has more influence on copulation duration than variation in male body size. Our previous results also suggested much more active and more important role of females in control of duration of copulation (Pavković-Lučić and Kekić, 2006).

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### **Amplifications of orthologous DNA fragments in three *Drosophila* species endemic to India.**

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The footprint of evolution is very often reflected at the molecular organisation in the closely related taxa (Takano, 1998). Such footprints can easily be traced by the identification of conserved DNA sequences across different taxa (otherwise known as orthologous DNA fragments). These conserved DNA sequences can then be utilized to reconstruct phylogenetic positions of these taxa (Goodstadt and Ponting, 2006) and to infer detailed evolutionary history of a group of closely related taxa. Thus, much attention has been paid in recent years to use of single-locus homologous DNA markers for these kinds of study. However, it has been shown that evolutionary history reconstruction through the use of multilocus DNA fragments provides robust inference to this aspect (Kopp and Barmina, 2005).

To this respect, the model organism *Drosophila* has been used to test this hypothesis in great detail. Since wide varieties of *Drosophila* species are sympatric to many tropical habitats of the globe, it is interesting to understand evolutionary history of these species and thus to correlate with ecological adaptations. Evolutionary history inferred from multilocus DNA fragments, that too using putatively neutral markers are especially important, as demographic events related to species differentiation could be inferred barring the effect of natural selection. *Drosophila* species, due to manifold advantages for use in both classical and molecular genetic work, are organisms of choice to test the differential evolutionary hypotheses and also the role of different evolutionary forces in speciation.