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Effect of Microhabitat Variability on Body Size in *Drosophila subobscura**

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We assessed the variation in thorax size, wing size and wing loading in populations of *Drosophila subobscura* from two ecologically different habitats and within each habitat sampled during three periods of the day. The traits analyzed differed between laboratory reared samples and field collected samples. Differences were mainly caused by environmental factors and *genotype* x *environmental* interactions. While there were no significant differences between populations for particular periods of the day, within-population analysis for each sex showed specific differences. Results showed that wing loading was the least variable character in natural populations, also showing the lowest level of sexual dimorphism. The data are discussed from the aspect of the variability of gene arrangement frequencies over daytime periods obtained previously for the same samples. They are consistent with models of maintenance of genetic variability in multi-niche habitats, and are in favour of a type of reactive behaviour dependent on ecological niche qualities on a daily rhythm scale in *D. subobscura*.

Key words: Thorax size, wing size, wing loading, daily rhythm, genotype-environmental interactions, habitat variability.

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The ability to adapt to suboptimal environmental conditions enables the survival of species in ecologically variable habitats. Particular genotypes in a population interact with the environment and provide phenotypic variability at morphological, physiological, or behavioral levels. Variation in morphometric traits, such as body size parameters, can be studied as within-population variability between individuals and traits, or as between-population variability (SOULE 1982; COYNE & BEECHAM 1987), reflecting the source of variation.

Studies show that body size in natural populations of *Drosophila* varies with temperature, latitude, altitude, and other specific habitat variabilities (ANTIPIN *et al*. 2001; JENKINS & HOFFMANN 2000; MORIN *et al*. 1999; VAN T'LAND *et al*. 1999). Experimental results have shown that body size is associated with several fitness components such as mating success, longevity, fecundity (REEVE *et al*. 2000). Phenotypic and genetic variation of morphometric traits in *D. melanogaster* is influenced by temperature and nutrition as well (BUBLY *et al*. 2001; PETAVY *et al*. 1997; IMASHEVA *et al*. 1998).

Drosophila subobscura is a wild *Drosophila* species, ranging over almost all of Europe, populating habitats at altitudes from sea level to the upper forest boundary. This species exhibits uniquely rich inversion polymorphism along all five long chromosomes. This polymorphism shows a clear-cut geographical pattern but less clear annual variation, while there is little evidence of altitudinal, microgeographic and habitat-related variability (KARI & HUEY 2000; RODRIGUEZ-TRELLES *et al*. 1996; ORENGO & PREVOSTI 1996, 1999). The diurnal variability of gene arrangement frequencies was investigated in *D. subobscura* populations from ecologically different habitats (SAVKOVIĆ *et al*. 2004) and it largely depended on the variety and dynamics of ecological factors.

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Body size in *D. subobscura* also shows temporal variability (KARI & HUEY 2000) and a latitudinal cline of genetic origin, which suggests the action of natural selection on this trait (SANTOS *et al*. 2004). Studies on heritability of body size parameters in *D. subobscura* are scarce and show that genotypic variance is low in nature, but with strong differences between data obtained for different years (ORENGO & PREVOSTI 1999).

The *Drosophila subobscura* model system is appropriate for monitoring microevolutionary change as a way of studying the effects of global change (RODRIGUEZ-TRELLES *et al*. 1996, 1998). In the present paper microclimatic variability in morphological traits differently related to fitness in two *D. subobscura* populations is assessed. The degree of phenotypic variability is analysed among three body size parameters in populations of *D. subobscura* from two ecologically different habitats and within habitats sampled during three periods of the day, and in their laboratory reared progeny. The study also assesses the level of sexual dimorphism for the observed traits in the natural populations, as well as in their laboratory reared progeny. The goal is to assess the degree to which genetics, environment, and their interaction shape variation in these two populations of *D. subobscura.*

Material and Methods

D. subobscura flies were collected at Goč mountain, situated between 43° 33'- 43° 35' N and 18° 15'-18° 40' E in central Serbia. The local samples were taken from two forest communities (topographically about 4 km apart from each other), at about 700 m above sea level: the *Abieto-fagetum* and *Fraxineto-quercetum* habitats will be hereafter referred to as "beech" and " oak" woods, respectively.

Both populations were sampled simultaneously at the end of June, using fermented fruit traps. Ten traps were set 10 m apart in a square formation in oak forest, and in a rectangular formation in beech forest. The area covered by traps was thus 400 m^2 in each habitat. If 30 m are added in each direction, then according to BEGON (1976) and LOUKAS & KRIMBAS (1979) with regard to the dispersal of *D. subobscura*, the total area of the study site was 7000 m^2. Flies were sweeped with a net in three periods of the day: 06:00-08:00 – "morning sample", 12:00-14:00 – "noon sample" and 18:00-20:30 – "evening sample". Thus, six groups of isofemale lines were established according to habitat (beech-B and oak-O) and day period (morning-M, noon-N, evening-E). The F1 generation from these lines emerged under optimal laboratory conditions for *D. subobscura* (19°C, relative humidity, 60%, light intensity about 300 lux, light/dark daily intervals 12h/12h).

Males and females from each sample caught in the field were analyzed for wing and thorax length. Males were measured directly from the field, but females were first placed individually into vials to obtain isofemale F1 progeny. After F1 emerged, thorax and wing lengths were measured on 50 females and 50 males from each subgroup within habitats.

Thorax length was measured from the neck to the tip of the scutellum, on the left side view of etherized flies. The left wing from each fly was cut and prepared on a slide for measurement.

Wing length was taken as the distance from the intersection of the third longitudinal vein with the anterior crossvein to the wing tip where the third vein ends. Measurements were made under a binocular microscope, with a Leica/Cannon Image analysis system.

Wing loading (WL) was calculated as the thorax (T) to wing length (W) ratio (T/W) for each fly. The F/M ratio representing the degree of sexual dimorphism for all samples for each trait was obtained from the mean values in females (F) and males (M).

Prior to statistical analysis $\log_{10}$ transformation was done for all measurements. A post-hoc LSD test was done within three-way ANOVA (ZAR 1999) with generation, habitat and day period as sources of variability. The homogeneity of variances was tested by Leven's test using coefficients of variation for traits, habitats and sexes as effects.

Results

The means and coefficients of variation (CV) for thorax length, wing length and wing loading in *D. subobscura* from natural populations (NP) and F1 progeny are summarized for each population, sex and character (Table 1) Population and daytime variability analysis for the means of the thorax length, wing length, and wing loading of *D. subobscura* field samples and F1 progeny are given as through 3-way ANOVA results in Table 2 for both females and males. Table 3 shows the results of the homogeneity test of variances, using coefficients of variations for three traits, two habitats and two sexes.

In natural population samples, there is no significant difference in the means of all traits and both sexes between habitats (Table 2), as well as in their variability (Table 3). However, between generation variability and mean size differ significantly. Means of all traits were found to be significantly lower in the wild samples than in their offspring

Table 1

Means and coefficients of variation (in brackets) of three studied traits in *D. subobscura* females (f) and males (m), for morning (M), noon (N) and evening (E) samples, from beech (B) and oak (O) forest, natural populations (NP) and laboratory progeny (F1). Values were calculated for $\log_{10}$ transformed data. CV = (stand. deviation/mean) x 100%)

Sample	Wing		Thorax		Wing loading		Sample size	
	NP	F1	NP	F1	NP	F1	NP	F1
Females								
BM	2.09 (1.66)	2.13 (0.78)	1.87 (2.28)	1.93 (0.94)	0.90 (1.07)	0.91 (0.65)	23	50
BN	2.09 (1.95)	2.14 (0.90)	1.88 (2.69)	1.93 (0.84)	0.90 (1.09)	0.90 (0.75)	27	50
BE	2.11 (1.67)	2.13 (0.62)	1.90 (2.09)	1.93 (0.73)	0.90 (0.82)	0.91 (0.69)	29	50
OM	2.08 (1.49)	2.14 (1.20)	1.86 (1.98)	1.94 (0.91)	0.90 (0.84)	0.91 (0.74)	38	50
ON	2.09 (1.70)	2.14 (0.59)	1.88 (2.11)	1.94 (0.79)	0.90 (0.90)	0.91 (0.59)	28	50
OE	2.10 (1.88)	2.14 (0.77)	1.89 (2.43)	1.94 (0.73)	0.90 (0.83)	0.90 (0.62)	33	50
Males								
BM	2.06 (1.79)	2.09 (0.96)	1.83 (2.54)	1.87 (0.92)	0.89 (1.21)	0.90 (0.72)	31	50
BN	2.08 (1.79)	2.10 (0.68)	1.84 (2.46)	1.88 (0.78)	0.89 (1.01)	0.90 (0.74)	13	50
BE	2.06 (1.77)	2.10 (1.03)	1.83 (2.49)	1.88 (0.92)	0.89 (1.38)	0.90 (0.74)	48	50
OM	2.05 (1.59)	2.10 (0.76)	1.82 (2.61)	1.88 (0.71)	0.89 (1.43)	0.89 (0.62)	50	50
ON	2.07 (1.89)	2.10 (0.62)	1.83 (2.52)	1.88 (0.69)	0.89 (1.25)	0.90 (0.65)	12	50
OE	2.05 (1.92)	2.10 (0.70)	1.82 (2.52)	1.88 (0.76)	0.89 (0.99)	0.89 (0.80)	21	50
The whole population samples								
fB	2.10 (1.80)	2.13 (0.63)	1.88 (2.46)	1.93 (0.74)	0.90 (1.03)	0.91 (0.70)	79	150
fO	2.09 (1.75)	2.14 (0.89)	1.88 (2.25)	1.94 (0.82)	0.90 (0.85)	0.91 (0.66)	99	150
mB	2.07 (1.77)	2.10 (091)	1.83 (2.48)	1.88 (0.89)	0.89 (1.27)	0.90 (0.73)	92	150
mO	2.05 (1.75)	2.10 (0.70)	1.82 (2.57)	1.88 (0.72)	0.89 (1.30)	0.89 (0.70)	83	150

under laboratory conditions (Table 3, all $P<0.001$). Analysis of the interaction generation x habitat in both sexes (Table 2), shows that oak population samples have a higher increase in mean size in F1, which is also trait specific; a more significant increase for wing, less for thorax, while this interaction is absent for wing loading.

While there were no significant differences between populations for particular periods of the day, within-population variability analysis for each sex showed specific differences. Females were generally more variable in both wing and thorax lengths throughout the day than males. Significant differences for all three traits were found in natural samples from both populations. The highest values for traits were obtained for females sampled in the evening from both habitats, as shown by the daytime (DT) effect in ANOVA (Table 2). A significant generation x daytime (GENxDT) interaction shows that these differences between daytime samples do not occur in F1 reared in laboratory conditions.

Sexual dimorphism for wing length, thorax length, and wing loading were estimated as female/male ratios, calculated with means for each trait, each daytime sample, and field population, as well as for their F1. Generally, calculated for the populations on the whole, sexual dimorphism was the same in field samples and in F1. Females were always larger than males. In terms of each trait, and on average, the values were highest for thorax length (females were about 3% bigger) and lowest for wing loading (1%). Variability in sexual dimorphism within natural population samples is significant; the highest dimorphism was found in the evening field samples for thorax and wing length in both populations, but this changes in F1 progeny. Females in F1 from all daytime samples have on average a 3% larger thorax and 2% larger wings than males, while sexual dimorphism for wing loading is non-variable in natural populations but shows higher variability in F1, due to the smaller WL for males (Table 3, SEX effect)

Table 2

Three-Factor ANOVA for three traits in *D. subobscura* females (A) and males (B). Summary of all effects (generation GN, habitat HB and daytime DT). P<0.05*, P<0.01**, P<.001***.

(A)

Effect	df Effect	MS Effect (x10^{-3})			F			p		
		Wing	Thorax	Loading	Wing	Thorax	Loading	Wing	Thorax	Loading
GN	1	218.860	334.961	5.624	320.712	403.522	115.387	0.000***	0.000***	0.000***
HB	1	0.000	0.109	0.028	0.001	0.131	0.583	0.981	0.718	0.446
DT	2	4.424	6.358	0.196	6.482	7.659	4.021	0.002**	0.001***	0.019*
GNxHB	1	4.594	1.570	0.106	6.732	1.892	2.184	0.010**	0.170	0.140
GNxDT	2	3.777	8.511	0.313	5.535	10.253	6.418	0.004**	0.000***	0.002**
HBxDT	2	0.119	0.928	0.135	0.175	1.117	2.774	0.840	0.328	0.064
GNxHBxDT	2	1.703	1.030	0.005	2.496	1.241	0.097	0.084	0.290	0.907
	df Error	MS Error (x10^{-3})								
	466	0.682	0.830	0.049						

(B)

Effect	df Effect	MS Effect (x10^{-3})			F			p-values		
		Wing	Thorax	Loading	Wing	Thorax	Loading	Wing	Thorax	Loading
GN	1	119.285	234.668	7.359	179.590	252.588	99.346	0.000***	0.000***	0.000***
HB	1	1.281	1.741	0.021	1.928	1.874	0.272	0.166	0.172	0.602
DT	2	2.313	2.244	0.023	3.483	2.415	0.309	0.032*	0.091	0.735
GNxHB	1	7.610	3.805	0.052	11.458	4.096	0.706	0.001**	0.044*	0.401
GNxDT	2	2.293	1.208	0.031	3.453	1.300	0.417	0.033*	0.273	0.659
HBxDT	2	0.390	0.112	0.075	0.587	0.120	1.006	0.556	0.887	0.367
GNxHBxDT	2	0.306	0.369	0.038	0.461	0.397	0.512	0.631	0.672	0.600
	df Error	MS Error (x10^{-3})								
	463	0.664	0.929	0.074						

Table 3

Levene's test for homogeneity of variances for three traits, for separate effects (sex SX, habitat HB, generation GN). Degrees of freedom for all F's: 1.951.

Effect	MS Effect (x10^{-3})			MS Error (x10^{-3})			F			p-values		
	Wing	Thorax	Loading	Wing	Thorax	Loading	Wing	Thorax	Loading	Wing	Thorax	Loading
SX	0.630	0.014	0.293	0.489	0.717	0.035	1.286	0.020	8.431	0.257	0.888	0.004**
HB	2.281	0.822	0.047	0.584	0.963	0.045	3.904	0.854	1.033	0.049*	0.356	0.310
GN	23.335	39.151	1.560	0.374	0.483	0.034	62.400	81.022	46.349	0.000***	0.000***	0.000***

P<0.05*, P<0.01**, P<0.001***.

Discussion

Body size is a complex polygenic trait and its different parameters such as thorax size or wing size are easily affected by environmental conditions. It is shaped by natural selection in insects and *Drosophila* as well, with latitudinal and altitudinal clines and little data exist on seasonal changes and microhabitat variation (JENKINS & HOFFMAN 2000; HAERTY *et al*. 2003). Diurnal changes of combinations of environmental factors in a particular habitat represent the basis for microhabitat temporal variability. Similar factors that influence body size at a large scale, such as between seasons, may be attributable to variability within the day, if favorable genotypes are positively selected.

In the present paper, higher phenotypic variances were obtained for two natural populations of *D. subobscura* than for those in the laboratory. This was expected due to the extreme heterogeneity of the field conditions and confirms that phenotypic variation of morphometric traits declines in the laboratory, compared to field samples (BRYANT & MEFFERT 1998). The across generation differences between the two populations indicate genotype-environment interactions as the main source of variability. Several papers describe low heritability of body size obtained from natural *Drosophila* populations (COYNE & BEECHAM 1987) and heritability for wing length across environments was not obtained for several *Drosophila* species (JAENIKE 1991). Heritability of wing size in a natural population of *D. subobscura* was reported by ORENGO & PREVOSTI (1999), but a highly significant difference was obtained between the years sampled due to quite variable climate conditions.

Thorax and wing size are highly positively correlated within the samples, and variability studies across environments showed the correlations are greater in natural conditions, as expected. The traits analyzed in the present paper varied consistently under laboratory conditions, but not in the samples from the wild. Temperature sensitivity of wing size was consistently negative over temperature range (GILBERT & DE JONG 2001) in several *Drosophila* species investigated, and thorax size may be selected towards temperature compensation to achieve optimal physiological performance. The results of PETAVY *et al*. (1997) in the two sibling species, *D. melanogaster* and *D. simulans*, demonstrated clear-cut differences of thorax size, wing size, and wing loading between the two species, with varying temperature. In *D. subobscura,* MORETAU *et al*. (1997) surprisingly obtained different TMVs (temperatures of maximal value) for thorax and wing lengths, which are assumed to be positively correlated. It is possible that wing loading (thorax/wing ratio) is the target of natural selection, rather than the two traits themselves. Our results indicate that wing loading is a more stable character, which suggests that it may be more related to fitness.

While no significant between-population differences were presently found for a particular period of the day, within-population analysis showed some significant variabilities among the samples taken in the morning, noon, and evening. Although temperature has been implicated as a selective agent for clinal variability in body size, microhabitat specificities, the result of interactions among various factors, are considered optimal in a given ecological niche. Temperature and humidity are among the most important environmental factors affecting the adaptive strategies and evolution of insects. It has been shown that microclimatic contrasts can shape the direction of selection for body size in natural populations of *D. melanogaster* and *D. simulans* (NEVO *et al*. 1998).

Sexual dimorphism in body size exists in *Drosophila*, and studies confirm that it is a plastic trait (MORIN *et al*. 1999; REEVE *et al*. 1996, 2000). The low level of sexual dimorphism for wing loading suggests that the genetic correlation between sexes is high for this trait. The fact that the highest dimorphism was found for the thorax in the evening samples from wild populations, but not from laboratory progeny, indicates a strong sex x environment interaction. REEVE & FAIRBAIRN (1996) suggested that the trend of decreasing dimorphism is due to the complex nature of the growth process, influenced by temperature and food, occurring differently in both sexes. Little is known about the use of natural resources by *D. subobscura* (KRIMBAS 1993) which is not a generalist species. Feeding behaviour may differ between sexes throughout the day.

Although the primary signal that influences activity in *D. subobscura* is light, humidity appears to also be a limiting factor (ANDJELKOVIC *et al*. 1985; BEGON 1976). Local differences in topography and soil composition, as well as the distribution of dominant trees within " beech" and " oak" forests modify microclimates considerably. Generally, the temperature is the lowest in the morning, rises slowly towards noon, peaks at the time of maximum exposure and decreases during the rest of the day. The humidity values change oppositely to temperature. Due to the buffering effect of a dense, closed canopy of beech forest, both temperature and humidity changes are less intensive and rapid as opposed to open oak forest, in which rapid and more drastic temperature and humidity changes are due to more direct exposure. Also, the humidity changes in beech forest occur later than the exposure intensity and temperature changes; a humidity shift does not occur in the oak habitat.

The relationship between genetic variability, particularly chromosomal inversion polymorphism and body size, was studied in natural populations of *D. subobscura* (ORENGO & PREVOSTI 2002; SANTOS *et al*. 2004). SAVKOVIĆ *et al*. (2004) analysed samples of *D. subobscura*, used in the present paper, for diurnal and habitat variability in gene arrangements. Some selective pressure regarding habitat differences was observed, indicating the presence of different homokaryotypes or heterokaryotypes, depending on ecological factors, such as light, temperature and humidity, and their dynamics. Arrangement frequencies of the morning samples appeared particularly interesting, and were in accordance with similar studies

(GOSTELLI 1991). According to SANTOS *et al*. (2004) changes in O chromosome gene arrangement frequencies in *D. subobscura*, as a response to temperature, likely underlie the correlated changes in wing shape because of gene-inversion linkage disequilibria. The results of SAVKOVIĆ *et al*. (2004) point to an interaction between different coadapted parts of the genome as a mean of optimal habitat adaptation. Our data are consistent with models of the maintenance of genetic variability in multi-niche habitats, and are in favor of a type of reactive behavior depending on ecological niche qualities on a daily rhythm scale, rather than on a fixed circadian rhythm.

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References

ANDJELKOVIĆ M., STAMENKOVIĆ-RADAK M., SEKULIĆ M. 1985. The daily activity rhythm of *D. subobscura* at Ravniste (Jastrebac) locality, Yugoslavia. Acta Entomologica Yugoslavica **21**: 109-118.

ANTIPIN M. I., RAKITSKAYA T. A., IMASHEVA A. G. 2001. Stability of development and variability in morphological traits in a natural population of *Drosophila melanogaster*: seasonal dynamics in 1999. Russian J. Genet. **37**: 58-63.

BEGON M. 1976. Dispersal density and microdistribution in *Drosophila subobscura* Collin. J. Anim. Ecol. **45**: 441-456.

BRYANT E. H., MEFFERT L. M. 1998. Quantitative genetic estimates of morphometric variation in wild-caught and laboratory-reared houseflies. Evolution **52**: 626-630.

BUBLY O. A., LOESCHCKE V., IMASHEVA A. G. 2001. Genetic variation of morphological traits in *Drosophila melanogaster* under poor nutrition:isofemale lines and offspring-parent regression. Heredity **86**: 363-369.

COYNE J. A., BEECHAM E. 1987. Heritability of two morphological characters within and among natural populations of *Drosophila melanogaster*. Genetics **117**:727-737.

GIBERT P., DE JONG G. 2001. Temperature dependence of developmental rate and adult size in *Drosophila* species: biophysical parameters. J. Evol. Biol. **14**: 267-276.

GOSTELLI M. 1991. Differential flight activity among karyotypes:daily and weather-induced changes in chromosomal inversion polymorphism in natural populations of *Drosophila subobscura*. Genetica **84**: 129-136.

HAERTY W., GIBERT P., CAPY P., MORETAU B., DAVID J. R. 2003. Microspatial structure of *Drosophila melanogaster* populations in Brazzaville: evidence of natural selection acting on morphometrical traits. Heredity **91**: 440-447.

IMASHEVA A. G., LOESCHCKE V., ZHIVOTOVSKY L. A., LAZEBNY O. E. 1998. Stress temperatures and quantitative variation in *Drosophila melanogaster*. Heredity **81**: 246-253.

JAENIKE J. 1991. Across-environment heritability of wing length. Dros. Inf. Serv. **70**: 101-102.

JENKINS N. L., HOFFMANN A. A. 2000. Variation in morphological traits and trait asymetry in field *Drosophila serrata* from marginal populations. J. Evol. Biol. **13**: 113-130.

KARI J. S., HUEY R. B. 2000. Size and seasonal temperature in free-ranging *Drosophila subobscura*. J. Therm. Biol. **25**: 267-272.

KRIMBAS C. B. 1993. *Drosophila subobscura*. Biology, Genetics and Inversion Polymorphism. Verlag-Kovac, Hamburg, Germany.

LOUKAS M., KRIMBAS C. B. 1979. The genetics of *Drosophila subobscura* populations. X. A study of dispersal. Genetica **50**: 127-134.

MORIN J. P., MORETEAU B., PETAVY G., DAVID J. R. 1999. Divergence of reaction norms of size characters between tropical and temperate populations of *D. melanogaster* and *D. simulans*. J. Evol. Biol. **12**: 329-339.

MORETEAU B., MORIN J. P., GILBERT P., PETAVY G., PLA E., DAVID J. R. 1997. Evolutionary changes of nonlinear reaction norms according to thermal adaptation: a comparison of two *Drosophila* species. C. R. Acad. Sci. Paris **320**: 833-841.

NEVO E., RASKOVETSKY E., PAVLICEK T., KOROL A. 1998. A complex adaptive syndrome in *Drosophila* caused by microclimatic constrasts. Heredity **80**: 9-16.

ORENGO D. J., PREVOSTI A. 1996. Temporal changes in chromosomal polymorphism of *Drosophila subobscura* related to climatic changes. Evolution **50**: 1346-1350.

ORENGO D. J., PREVOSTI A. 1999. Wing size heritability in a natural population of *D.subobscura*. Heredity **82**: 10-196.

ORENGO D. J., PREVOSTI A. 2002. Relationship between inversion polymorphism and wing size in a natural population of *D. subobscura*. Genetica **115**: 311-318.

PETAVY G., MORIN J. P., MORETAU B., DAVID J. 1997. Growth temperature and phenotypic plasticity in two *Drosophila* sibling species: probable adaptive changes in flight capacities. J. Evol. Biol. **10**: 875-887.

REEVE J. P., FAIRBAIRN D. J. 1996. Sexual size dimorphism as a correlated response to selection on body size: an empirical test of the quantitative genetic model. Evolution **50**: 1927-1938.

REEVE M. W., FOWLER K., PARTRIDGE L. 2000. Increased body size confers grater fitness at lower experimental temperature in male *Drosophila melanogaster*. J. Evol. Biol. **13**: 836-844.

RODRIGUEZ-TRELLES F., ALVAREZ G., ZAPATA C. 1996. Time series analysis of seasonal changes of the O inversion polymorphism of *Drosophila subobscura*. Genetics **142**: 179-187.

RODRIGUEZ-TRELLES F., RODRIGUEZ M. A., SCHEINER S. M. 1998. Tracking the genetic effects of global warming: *Drosophila* and other model systems. Coservation Ecology 2 [online]. Available from the URL: http:/www.consecol.org/vol2/iss2/art2.

SANTOS M., IRIARTE P. F., CESPEDES W., BALANYA J., FONTDEVILA A., SERRA L. 2004. Swift laboratory thermal evolution of wing shape (but not size) in *Drosophila subobscura* and its relationship with chromosomal inversion polymorphism. J. Evol. Biol. **17**: 841-855.

SAVKOVIC V., STAMENKOVIĆ-RADAK M., ANDJELKOVIĆ M. 2004. Diurnal variability of gene arrangement frequencies in *Drosophila subobscura* populations from two habitats. J. Zool. Syst. Evol. Res. **42**: 208-214.

SOULE M. E. 1982. Allomeric variation. 1. The theory and some consequences. Am. Nat. **120**: 751-764.

VAN'T LAND J., VAN PUTTEN P., ZWAAN B., KAMPING A., VAN DELDEN W. 1999. Latitudinal variation in wild populations of *Drosophila melanogaster*: heritabilities and reaction norms. J. Evol. Biol. **12**: 222-232.

ZAR H. J. 1999. Biostatistical Analysis.Prentice-Hall, Upper Saddle River, New Jersey, USA.