

Testing the Randomness of the Samples of Body Size in a Replicated Design in *Drosophila melanogaster* Populations from the Eastern Mediterranean*

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Abstract: Strong body size correlates, wing and thorax lengths, of *Drosophila melanogaster* populations from the Eastern Mediterranean were measured and tested for the presence of autocorrelation, i.e. nonrandom alternation of individual measurements within a sample. The experimental design was a laboratory setup aiming at overall homogeneity in fly culture conditions, which was achieved. A *t-test*, first proposed by Von Neumann, was performed for the autocorrelation analysis in each population. No significant autocorrelation was detectable, indicating very low bias in the experimental design for nonrandom picking of individuals for measurements. The test is suggested for routine use in studies before any inference is made about the state of a character chosen.

Key Words: *Drosophila*, body size, randomness

Doğu Akdeniz'den Toplanan *Drosophila melanogaster* Populasyonlarında Vücut Büyüklüğü Örneklerindeki Rastgeleliğin Tekrarlı Bir Dizayn Kullanılarak Ölçülmesi

Özet: Doğu Akdeniz'den örneklenen *Drosophila melanogaster* populasyonlarında, vücut büyüklüğü ile yakın ilişkili olan kanat ve toraks uzunlukları ölçüldü ve bir örneklemdaki bireysel ölçümlerin rastgele-olmayan sıralanması demek olan otokorelasyonun varlığı test edildi. Deneysel tasarım, sineğin yetiştirme koşullarındaki homojenliği amaçlayan bir laboratuvar düzeneği idi. Sonuçta bu koşul sağlandı. İlk kez Von Neumann tarafından geliştirilen bir rasgelelik testi olan bir *t-testi* otokorelasyon analizi için her populasyon açısından kullanıldı. İstatistiksel anlamlı herhangi bir otokorelasyon düzeyinin olmadığı görüldü ve bu durumun deneysel tasarımdaki rasgele-olmayış açısından oldukça düşük bir yönlülük taşıdığı sonucuna varıldı. Sonuç olarak, burada kullanılan test, seçilen bir karakterin durumu açısından bir çıkarsama yapılmadan önce düzenli biçimde kullanılması için önerilmektedir.

Anahtar Sözcükler: *Drosophila*, vücut büyüklüğü, rasgelelik

Introduction

The reliability of statistics from a set of measurements depends highly on the randomness of the samples taken (e.g., Sokal and Rohlf, 1995). This randomness rule becomes apparently relevant when the respective measurements taken are from an experimental design aiming at a broader conclusion, e.g., inferences for natural populations from laboratory results in a population biologist's case. Most of the methodologies used in population biology harbor a variance analysis structure, one of the basic tenets of which is the

randomness of the sample(s) taken. This randomness helps underline the degree of correspondence between the field and laboratory inferences. Especially in studies concerning natural populations, the traits chosen can have sharp differentiation profiles even in very small geographical ranges (Özsoy, 2004) due to almost universally narrow dispersal distances of various species of a wide range of taxa (Endler, 1977). In this respect, the detection of randomness in any trait sample is one of the enforcing elements to infer the correct differentiation structure of a geographical variation pattern.

*This study comprises a part of the work for the PhD thesis of E.D.Özsoy

In the present study we show the efficiency of several statistical tests in detecting the randomness of some traits varying within and among natural *Drosophila melanogaster* populations. Strong body size correlates, wing and thorax lengths, were chosen for the main analysis. To show the overall homogeneity of the design, sample sex ratios and viabilities were also used as sub-experimental variables to test given biological assumptions, i.e. equality of sex numbers and population specific viabilities.

Materials and Methods

Populations

Flies were sampled at 5 locations in Turkey, and at 1 location, Tel Aviv, in Israel. The Turkish populations consisted of 2 local samples from Ankara (coded 1 and 2, respectively), and 2 local samples from Kirsehir (coded 1 and 2, respectively). The Turkish locations are not Mediterranean in the sense that they do not have typical Mediterranean characteristics, i.e. hot dry summers accompanied by mild rainy winters. However, they are well within the regions relevant to Mediterranean biogeography (see Blondel and Aronson (1999) for the description and delimitation of the Mediterranean region). The Israeli sampling site is genuinely Mediterranean in the above gross climatic sense. All samples were collected between August and November 1999.

Wing and thorax lengths

After 6 generations of breeding under standard conditions (i.e. at 25 °C and 60% R.H.), populations from Ankara (1 and 2), Kirsehir (1 and 2) and Tel Aviv were allowed to lay eggs for 3 h at 25 °C. For each population, eggs were distributed into 10 replicate vials, with each vial having 50 eggs. When all flies had emerged, 40 males per population were selected at random to establish the experimental populations. Randomization was done as follows. When all the flies in all the populations had emerged, there was variation in the number of adults across populations (Table 1). Emerging flies per population were first collected into separate glass bottles with standard *Drosophila* medium. After the full collection (i.e. when all the pupae in replicate vials per population had hatched), the flies were

tipped into 2 separate empty glass bottles and moderately etherized. Then from each of those bottles 20 males were picked up haphazardly and put into a vial with standard medium. Each experimental sample was thus constructed with 30 to 41 males in total per population.

Thorax lengths of slightly etherized males were measured in lateral view from the neck to the tip of the scutellum in micrometers. Wing length was measured as the distance from the anterior crossvein to the wing tip in micrometers. The Figure shows the distances measured. Individual measurements with low resolution were

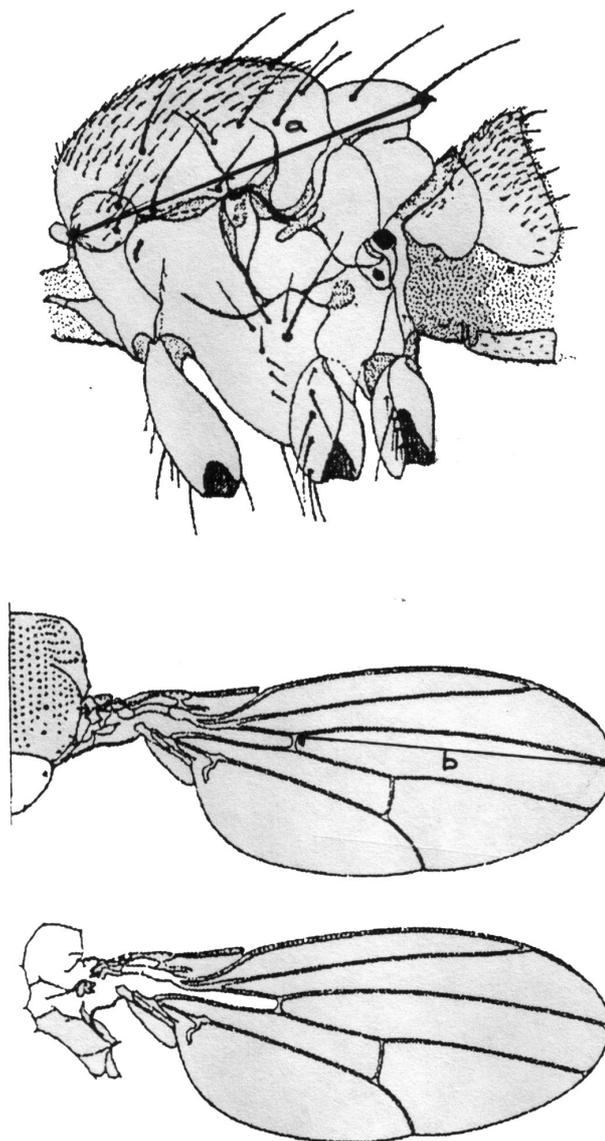


Figure. The thorax (a) and wing (b) lengths measured for the study (pictures from www.flybase.bio.indiana.edu).

Table 1. Viabilities and sex-ratios of flies in the wing-thorax lengths randomness tests.

Population	Number of eggs planted	Number of adults	% Viability	Number of adult males	Adult male ratio
Tel Aviv	500	417	83.4	233	0.56
Ankara-1	500	430	86	188	0.44
Ankara-2	500	428	85.6	202	0.47
Kırşehir-1	500	452	90.4	228	0.50
Kırşehir-2	500	454	90.8	209	0.46

ignored. Both thorax and wing lengths were determined in 2 replicates per individual by a light microscope connected to a computer. The average of those 2 replicates was treated as a single measurement from each individual fly.

Statistical tests for randomness

Wing and thorax lengths were tested for randomness using a test proposed by Von Neumann (Von Neumann et al., 1941; Sokal and Rohlf, 1995 p. 393). This test is a kind of *t-test* that quantifies the degree of positive autocorrelation, and the self-similarity of measurements of a trait chosen, and is based on successive differences between the normally distributed measurements in a sample. It specifically tests the degree of nonrandom alternation of the magnitude of the measurements using a threshold value (Sokal and Rohlf, 1995 p. 394).

Results and Discussion

In Table 1, the number of adults that developed and the proportion of adult males used in the randomization are shown. The number of adults that developed per population was high, indicated by a very high respective viability. The male numbers were also high and a moderate variation occurs among the populations with respect to male sex proportion (coefficient of variation (CV): 9.6%). Population-wise viability as the number of flies developed from the eggs varies little (CV: 3.7%). However, when the viabilities are expressed as deviations from 100% expected viability fixed among populations (i.e. the expected total number of adults developed, 500, per population), viability deviations give a highly significant figure ($\chi^2 = 42.79$, d.f. = 4, $P < 0.001$). This may reflect population-specific viability differences still

emergent under homogenized laboratory culture conditions; viabilities can be grouped in 3 different regions, i.e. Tel Aviv (Israel), Ankara, and Kırşehir (Table 1). One would think that viability could be correlated with sex ratio as if there were a sex-dependent component of viability. This possibility does not seem to hold in our case as there is a low, insignificant correlation between the percent viability and adult male proportion (Pearson correlation coefficient, $r = -0.391$, $P = 0.516$). In brief, the trivial variation seen in viability suggests that our design provided a homogenized environment for the populations for the egg-to-adult component, notwithstanding effectors of population specific viabilities still being in operation, and the viability variation did not have a correlated effect on male sex ratio.

After all the flies had developed from each population, we discarded the females and used the males for the analyses. Randomization was performed such that up to 41 fully developed males (adults) were picked haphazardly from the total male collection of each population. The data set of the wing and thorax lengths thus consisted of small subsamples of the total collections, randomizations of which were the subject of this study. The results are shown in Table 2. We obtained very small values of test statistics, Von Neumann's *t*, in our case. The results clearly indicate that no significant autocorrelation, i.e. nonrandom alternation of individual measurements due to experimental bias, occurred in our randomization design for strong body size correlates, wing and thorax lengths. Thus it would be highly reliable to construct working hypotheses on any test statistics obtained with wing or thorax length data within any population biological context, together with other assumptions that should hold in a variance analysis (see Sokal and Rohlf, 1995 chap. 13 for those assumptions). The test performed here is a simple and elegant *t-test* and we propose its use for any study concerning samples

Table 2. Von Neumann statistics (t_s) for randomness of the wing and thorax lengths measurements.

Population	N	t_s (wing)	t_s (thorax)
Tel Aviv	41	0.0001	0.0005
Ankara-1	39	0.0001	0.001
Ankara-2	30	0.001	0.001
Kırşehir-1	40	0.0001	0.0002
Kırşehir-2	39	0.0002	0.001

N: number of the male flies from which the measures were taken

$$t_{.05(29)} = 2.45$$

$$t_{.05(40)} = 2.021$$

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from a larger data set constructed in the laboratory or from a field collection trip. It would be very worthwhile to determine the randomness of the measurements before any inference is drawn for a character under study.

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