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THE VII UKRAINIAN ANTARTIC EXPEDITION TRANSATLANTIC JOURNEY IMPACT ON THE *DROSOPHILA MELANOGASTER* CROSSING OVER

The processes of adaptation and, fimaley survival of species in a changeable environment largely depend on its fitness and plasticity. Crossing over, or homologous recombination, is one of the mechanisms providing biological species with the vital variability. The mechanisms of recombination are practically universal and characteristic of diverse taxa. However, recombination frequency is known to be substantially depends' on the various factors. The rapid changes of latitudinal (geomagnetic field inclination and intensity) and climatic factors during the transequatorial passage make such journey unique for the genetic experiment.

In this study we have made an attempt to investigate the effect of latitude and climatic factors on recombination in the sex chromosomes of *Drosophila melanogaster* based on the data obtained during the transequatorial passage as a part of the VII Ukrainian Antarctic expedition. Effectively, such a systems was assumed to elicit the processes that might take place in the biological systems during sudden changes in the environment (abrupt climate changes, ecological catastrophes, extreme living conditions, etc). Fly crosses were obtained during two transequatorial marine journeys: in winter (outbound from Sevastopol for Ushuaia) and in spring (Ushuaia–Sevastopol) in 2002. We have measured recombination frequency between the genes *white* (*w:* 1-1.5) and *cut* (*ct:* 1-20.0). Concurrent control experiments were performed at Taras Shevchenko National University of Kyiv. In spite of the putative sensitivity of homologous recombination of the external factors, we have found out no significant differences in recombination frequencies between the control and expedition experiments. In spring, we have even observed a slight decrease in recombination frequency between the genes *white* and *cut*, both in the control and expedition groups. These results do not resolve well with the reputed high sensitivity of the applied test to external influences. Further research is necessary to clarify the observed stability of recombination frequency.

Key words: *Drosophila melanogaster*, recombination chromosomes, latitude, climatic factor

Variability is an important evolutionary factor and an ultimate property of all biota. This phenomenon is ascribed a chief responsibility for adaptation of organisms to changing environments. One of the mechanisms which provide biological species with a range of variability and result in biodiversity and uniqueness of any given genotype is crossing over, or homologous recombination [1-3]. The mechanisms behind recombination are practically universal and are found to be much the same in nearly any investigated animal or plant [1, 2].

The frequency of recombination between two loci of the same chromosome has been shown to be constant. However, it can vary within a certain range if affected by various biotic and abiotic factors, such as temperature, humidity, population density, age, genotype features, the distance to centromere, ionizing radiation etc. [1, 3, 4, 5]. Research on the mechanisms of adaptation to changing environmental conditions, including its genetic basis, is important for prediction of possible genetic processes driven by adaptation. From the viewpoint of applied ecology and fundamental grounds of adaptation theory, this is an important question.

Drosophila is a convenient research object. The effect of various factors on genetic processes in whole and crossing over in particular have traditionally been studied on fruit flies [6, 7]. The conditions of a transequatorial passage are unique for a genetic experiment because of the rapid changes of latitudinal and climatic factors accompanying such a journey. Studies of the effect of these conditions on the mechanisms of variability are necessary for understanding the processes taking place in biological systems during sudden changes of environment in whole (rapid climate changes, ecological catastrophes, extreme conditions of existence) and a transequatorial passage in particular. The recombination test is known to be reliable and sensitive [1, 3, 5]. Many papers report the effects of abiotic factors on crossing over in drosophila, however publications on the impact of latitude and longitude are lacking.

Actually, the list of latitudinal and/or longitudinal factors that could potentially influence recombination rates in a given genome region is subject to speculation, as their influence on organismal development is as yet poorly understood. Among such potential factors, we can put forth the geomagnetic field or variation in the solar radiation intensity and composition to number a few. Ambient magnetic field has been reported to influence some cellular processes, such as, for instance, cell division symmetry, in fish embryos [8]. The Earth's magnetic field is known to vary and form latitudinal gradients of its intensity and inclination. Therefore, geographic location-specific parameters of the geomagnetic field might potentially constitute a factor affecting recombination during cell division in *Drosophila*.

In this study we attempted to investigate the effect of latitude factors on recombination in the sex chromosomes of *Drosophila melanogaster* based on data obtained during two transequatorial passages as part of the VII Ukrainian Antarctic expedition.

Materials and methods

We used two strains of *Drosophila melanogaster* from our laboratory: *Canton S* (wild type) and a strain carrying a recessive mutation in the genes *white* (*w:* 1-1.5) and *cut* (*c:* 1-20.0) located on the first (sex) chromosome.

Recombination frequency was calculated as the frequency of crossover flies in the second generation. The crossing scheme is shown in Fig. 1. Flies (three females in one tube) were maintained on standard medium at 25 ^{o}C [9]. Standard error was measured according Fleis [10].

Crossings were performed on the research ship "Horyzont" during transequatorial marine journeys of the VII Ukrainian Antarctic expedition in the winter (outbound from Sevastopol for Ushuaia) and in the spring (Ushuaia – Sevastopol) in 2002. Concurrent control tests were performed at the Department of General and Molecular Genetics of Taras Shevchenko National University of Kyiv. A total of six crossings were made (two on the way from Sevastopol to Ushuaia and four during the reverse journey).

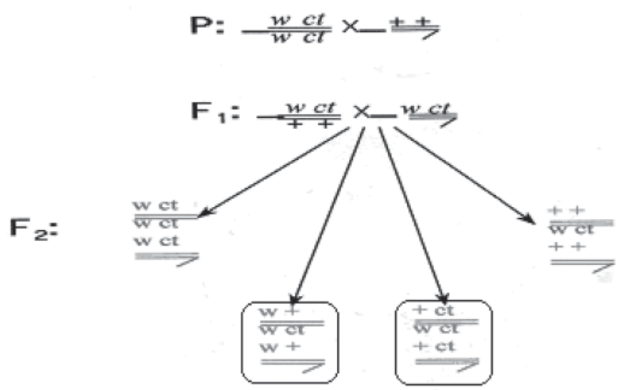

Figure 1. The crossing scheme used in the test for recombination between the genes *white* and *cut*.

Results

The experiment was performed during two seasons (the Antarctic summer and Antarctic autumn) from early January (8.01) through mid-April (15.04) in 2002. Six crossings were made: two during the way from Sevastopol to Ushuaia (1, 2 points), four on the way back (3-6 points). The first, second and sixth series of crossings were made in the Northern Hemisphere with the extreme point of 11°49'N; 25°18'W; the third, fourth and fifth were made in the Southern Hemisphere with the extreme point of 54°48'S; 68°18'W (Table 1).

The winter in the Northern Hemisphere concurs with the summer in the Southern Hemisphere. Therefore, the crossings of the first journey which were done in the Northern Hemisphere in January concurred with the Antarctic summer, while the crossings during the reverse journey which were done in the Southern Hemisphere in March and April (except for the last point) concurred with the Antarctic autumn. As a result, we obtained the following data: crossing over frequency for experiments conducted during the Sevastopol – Ushuaia way (1) was significantly higher than for

Table 1

Recombination frequency between the genes *white* and *cut*.

Point number	Date	Geographic coordinates	Recombination frequency, %	
			Experiment	Control (experiment date in Kyiv, 50°39'N; 30°47'W),
1	08.01.2002	11°49'N; 25°18'W	20,62±2,06	18,21±1,82 (4.01)
2	13.01.2002	10°50'N; 31°50'W	21,71±2,17	19,41±2,09 (11.01)
3	27.03.2002	54°48'S; 68°18'W	13,94±1,4	19,4±1,94 (29.03)
4	03.04.2002	33°44'S; 47°56'W	13,21±1,32	15,51±1,51 (5.04)
5	09.04.2002	7°41'S; 33°24'W	14,47±1,45	16,34±1,63 (12.04)
6	15.04.2002	10°54'N; 24°44'W	14,57±1,46	14,52±1,45 (19.04)

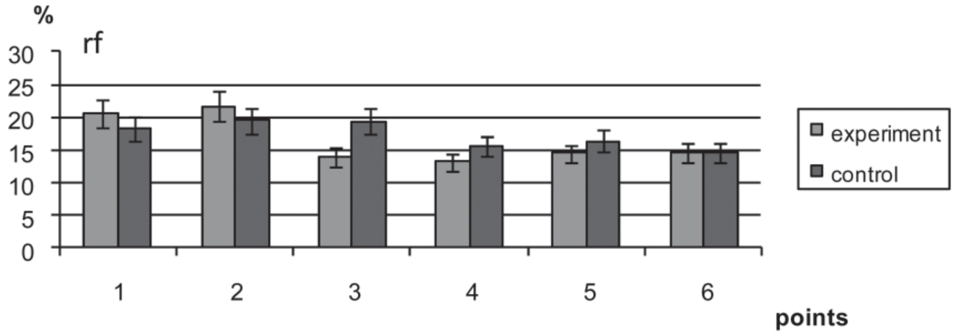

Figure 2. Crossing over frequency between the genes *white* and *cut* in a sex chromosome of *D. melanogaster*.

experiments conducted during Ushuaia – Sevastopol way (2). Meanwhile, the results of two crossings conducted with periodicity in 7 days in the Antarctic summer (08.01.2002 and 13.01.2002) did not differ significantly, just as it did not in the four experiments conducted with periodicity in around 7 days during the Antarctic autumn. Still, seasonal differences were registered (Table 1, Figure 2), but were also found to occur in control.

Thus recombination in the tested flies closely followed patterns observed in control. In spite of the reputed sensitivity of recombination processes to environmental factors, latitudinal effects were not detected.

Discussion

Recombination frequency is known to be sensitive to many environmental factors [1]. Therefore the seasonal dependence of crossing over frequencies obtained in our experiments was expected. However, the absence of geographic position effects was a little surprising, as was the similar recombination pattern in tested and control flies.

A difference with control was only evident for one point (27.03.2002). A steep decline between point 2 (13.01.2002) and point 3 (27.03.2002) was revealed in experimental flies (Figure 1). Crossing at point 2 was done during the journey from Sevastopol to Ushuaia in the Northern Hemisphere, while mating at point 3 was done on the return way to Sevastopol in the Southern Hemisphere. At the same time, a decline in control was observed between points 3 and 4 (29.03.2002 and 05.04.2002, correspondingly). This might be related to seasonal changes as well: point 2 mating in the experiment was done in mid-January, while that of point 3 was done in late March. March in the coordinates (54°48'S; 68°18'W) coincides with the autumn to early winter. The analysis of the obtained results is limited by the relatively small sample sizes and few mating points. However, the results are interesting. Finding out the reasons of such unusual stability of homological recombination frequency in different latitudinal and longitudinal zones requires further research.

Conclusions

Crossing over frequencies were significantly higher in experiments during the Sevastopol–Ushuaia journey compared to the return trip.

Contrary to the reputed sensitivity of the recombination test to external factors, we found no reliable difference in the recombination frequency between control (Kyiv) and expedition experiments.

References

1. *Ghuchenko A. A., Korol A. B.* Recombination in evolution and selection. – M.: Nauka, 1985. - 400 p.
2. *Kushev V. V.* Mechanisms of genetic recombination. - Leningrad, 1971. – 364 p.
3. *Smirnov V. G.* Cytogenetics. – M.: Visshaya shkola, 1991. - 247 p.
4. *Gong W. J., Golic K. G.* Genomic Deletions of the *Drosophila melanogaster Hsp70* Genes // Genetics – 2004. - V.168 (3) – P. 1467–1476.
5. *Totskiy V. M.* Genetics. - Odessa: Astroprint, 1998. - V. 1, 2: 476p., 276 p.
6. *Kozeretskaya I. A., Topchij I. A.* Using Fruit Fly as the Test-system to Detect Soil Pollution by Heavy Metals // Sixty International Symposium & Exhibition on Environmental Contamination in Central and Eastern Europe and the Commonwealth of Independent States. – Prague, 2003. – P. 84.
7. *Al-shibli* Suitability and crossing over frequency for mutants *Drosophila melanogaster* in relation to age and artificial genotype reconstruction: Aauthor's abstract of Ph.D. thesis, Odessa - 2003.- 17 p.
8. *Tanski A., Formicki K., Korzeletska-Orkisz A., Winnicki A.* Spatial orientation of fish embryos in magnetic field // Electronic Journal of Ichthyology –2005. - V. 1. – P. 21-34.
9. *Roberts D. B.* Drosophila a practical approach. – Oxford, 1986. - 295 p.
10. *Fleis G.* Statistical methods for proportions and percents tables study. - translation from English (Russian version)/ Under the editorship of Yu.N. Blagoveshchenskiy. – M.: Finances and statistics, 1989.- 319 p.

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ВЛИЯНИЕ ТРАНСАТЛАНТИЧЕСКОГО ПЕРЕХОДА НА КРОССИНГОВЕР У *DROSOPHILA MELANOGASTER*

Процессы адаптации и, в конечном счете, выживания видов в изменчивых условиях окружающей среды во многом зависят от размаха изменчивости. Кроссинговер, или гомологическая рекомбинация, один из механизмов создания генетичекого разнообразия. Механизмы рекомбинации универсальны как в животном так и в растительном мире. В то же время, на частоту рекомбинации влияют различные факторы, как внешней, так и внутренней природы. Быстрая смена широтных и климатических факторов, а также магнитного поля Земли уникальные условия для генетичекого эксперимента, которые создаются в процессе трансатлантического перехода.

Изучали зависимость частоты рекомбинации от широтно-долготных характеристик месторасположения экспедиции. Установление последствий таких влияний на механизмы изменчивости необходимы для понимания процессов, которые имеют место в биологических системах в условиях быстрого изменения факторов окружающей среды (климатические изменения, экологические катастрофы, экстермальные условия существования, и т. д.). Постановка скрещиваний проводилась во время трансатлантического перехода VII Украинской антарктической экспедиции зимой (Севастополь-Ушуая) и весной (Ушуая-Севастополь) в 2002 г. Изучали частоту рекомбинации на участке между генами *white* (*w:* 1-1.5) и *cut* (*ct:* 1-20.0). Одновременно, в качестве синхронных условно контрольных экспериментов аналогичные скрещивания были проведены в Киевском национальном университете имени Тараса Шевченко. Результаты експериментов, полученных во время экспедиции и в Киеве, статистически достоверно не отличались, не смотря на чувствительность частоты рекомбинации к воздествию факторов окружающей среды. В то же время, показано достоверное снижение частоты рекомбинации между генами *white* and *cut* в весенний период, как в экспедиционных условиях, так и в контроле. Установление причин описанных феноменов требует проведения дальнейших исследований.

Ключевые слова: *Drosophila melanogaster*, рекомбинация, хромосомы, широта, климатический фактор

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ВПЛИВ ТРАНСАТЛАНТИЧНОГО ПЕРЕХОДУ НА КРОСИНГОВЕР У *DROSOPHILA MELANOGASTER*

Процесси адаптації та виживання видів в мінливих умовах оточуючого середовища значно залежать від розмаху їх мінливості. Кросинговер, або гомологічна рекомбінація, один з механізмів формування генетичного різноманіття. З однієї сторони механізми рекомбінації універсальні у тваринннних та рослинних організмів, з іншої їх частота значно залежить від коливання факторів оточуючого середовища. Швидка зміна широтних факторів, а також магнітного поля Землі унікальні умови для генетичного експерименту, які формуються в умовах трансатлантичного переходу.

Вивчали залежність частоти рекомбінації від координатних та кліматичних характеристик місцярозташування експедиції. Ідентифікація наслідків таких впливів на механізми мінливості необхідна для розуміння процесів, які мають місце в умовах швидкої зміни факторів оточуючого середовища (кліматичні зміни, екологічні катастрофи, екстремальні умови існування, і т. ін.). Постановка схрещувань проводилася під час трансатлантичного переходу VII Української антарктичної експедиції зимою (Севастополь-Ушуая) та весною (Ушуая-Севастополь) 2002 р. Досліджували частоту рекомбінації на ділянці між генами *white* (*w:* 1-1.5) та *cut* (*ct:* 1-20.0). Одночасно, в якості синхронних, умовно контрольных експериментів, аналогічні схрещування проводилися в Київскому національному університеті імені Тараса Шевченка. Результати експериментів отриманих протягом експедиції та в Києві статистично достовірно не відрізняються, не дивлячись на чутливість частоти рекомбінації до впливу факторів зовнішнього середовища. В той же час, продемонстровано достовірне зниження частоти рекомбінації між генами *white* та *cut* в весняний період, як в експедиційних умовах, так і в контролі. Встановлення причин описаних феноменів потребує подальших досліджень.

Ключові слова: *Drosophila melanogaster*, рекомбінація, хромомоми, широта, кліматичний фактор.