A HIGH FREQUENCY OF HERITABLE CHANGES IN NATURAL POPULATIONS OF *DROSOPHILA MELANOGASTER* IN UKRAINE

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Spontaneous mutations are thought to have a stable rate for a given species. If non-adaptive, they appear at low frequencies and are governed by drift. However, environmental factors have been reported to cause spread of non-adaptive mutations in populations, governed by mechanisms, such as genetic assimilation. In the present study, we report a simultaneous appearance of a mutant and apparently non-adaptive C2 vein in Drosophila melanogaster at higher than expected frequencies in several distant populations, which excludes the role of drift or selection as the cause of the reported mutation frequencies. We discuss explanations of the phenomenon, including the role of external factors, such as temperature, in the possible genetic assimilation of the trait.

***Key words**: mutation rate, natural populations, genetic assimilation, Drosophila melanogaster.*

Introduction. For *D. melanogaster* spontaneous mutation rate has been estimated at $8.5 \cdot 10^{-9}$ per bp per generation [1]. Meanwhile, both temporal and spatial variations in mutation frequencies are well-known from various fruit fly populations [2–6]. Under a stable mutation rate such changes can result from selection, drift, migration or other known population phenomena [7, 8]. However, other reasons have been proposed that are not universally recognized, such as genetic assimilation of acquired traits, described by Waddington in 1953, when he showed that defects in the C2 cross vein in drosophila caused by temperature exposure at 40 °C for 4 h at the pupa stage was inherited by part of the progeny of the flies that had undergone the treatment [9].

We identified elevated frequencies of heritable changes in the C2 vein in natural populations of *D. melanogaster* from Ukraine which might be caused by genetic assimilation of acquired traits occurring in the wild.

Materials and Methods. *Natural populations sampled.* Flies were collected during late summer through early fall (August through September) in 2011–2014 from natural populations of *D. melanogaster* from Ukraine. Three of the sampled populations inhabit the Chornobyl Zone of Exclusion (Table 1). Flies were collected by insect nets at both random and near fruit-baited sites.

Phenotypic and hybridological analysis. Collected flies were transported to the lab in vials with standard nutritional media and inspected under the binocular for phenotypic deviations relative to wild type. The detected phenotypically deviation flies were isolated into separate vials to obtain progeny. Wild type females were also isolated to obtain isofemale strains [10], which were then inspected for five generations of inbreeding for visible mutations. Females from the inbred progeny that had deviation phenotypic traits were then further isolated to identify which of the detected phenotypic deviations were heritable.

All flies were kept on the standard nutritional media [11] at room temperature. Ethoxyethane was used for anaesthesia.

Analysis of type of inheritance and penetrance of deviant C2 veins. Mutant flies were crossed with laboratory wild type flies of the *Canton-S* strain to determine the type of inheritance.

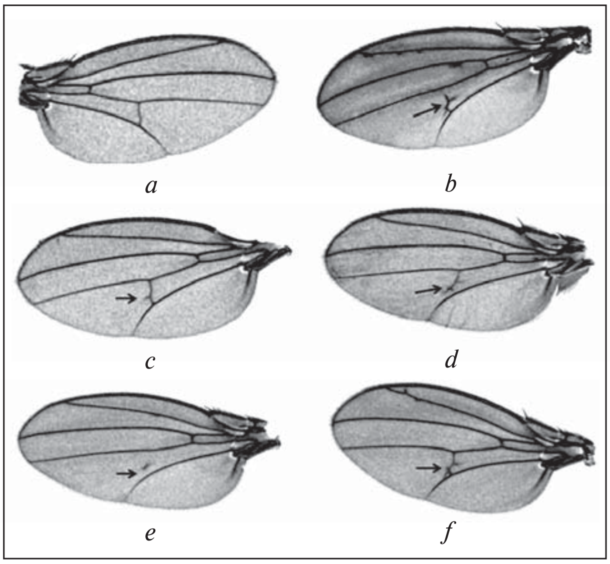
To determine penetrance, virgin females with mutant C2 were crossed with males having the same mutant phenotype. Progeny then were analysed visually.

Statistical analysis. The frequency of the C2 mutation was calculated as the ration of the number of flies bearing the trait relative to the number of all the analysed flies of both sexes. If mutant flies were not detected in a population, their frequency was thought to less than the ratio of 1 to all the analysed flies from the given population. In isofemales strains, the mutation frequency was estimated as the ratio of the number of isofemale strains in which the mutation was detected at least once through all the 5 inbred generations to the number of all the analysed isofemale strains. Error in the analysis of penetrance was calculated the standard error of a proportion.

Results. The frequency of imago flies with the aberrant C2 vein ranged within 0 % in 2010 in all sampled populations to 5 % in populations from the cooling pond of the Chornobyl Nuclear Power Station collected in 2012 (Table 1). In total, the aberrant C2 cross-vein was detected in the most sampled populations starting from 2011. In 2014, mutant flies were no longer detected in 4 of the sampled populations. In the vast majority of detected cases, the aberration was heritable. Therefore, we detected an event of elevated frequencies of mutant flies in *D. melanogaster* natural populations, which has been reported previously from other populations [12–14].

Phenotypically the mutations manifested in a punctuated C2 vein, branching of the vein of various magnitudes, with infrequent occurrence of additional vein-like structure in the inter-vein spans of the wing and callous formations on longitudinal veins (Figure). Noteworthy, the expressivity of the trait differed in progeny from different populations. Overall, the strains that demonstrated higher penetrance also showed higher expressivity.

When transferred to culture, a significant portion of the isofemale strains founded from the 2012 collections (48–75 % depending on the population) demonstrated cross vein aberrations in at least one of the 5 inbred generations. Hereby, the frequency of isofemale strains with the aberrant progeny within one generation ranged within 0–63 % in different populations (Table 2).



Wild type wing (*a*) and different kind of mutation wings (*b–f*)

Mutant alleles found in natural populations seem to be dominant, as their phenotype appeared in the first generation in crosses *Canton-S* flies with aberrant vein (in flies originating from Odesa the frequency of the phenotype was 3.7 ± 1.63 and 12.94 ± 2.57 %, and in males 1.07 ± 1.07 and

Table 1. **The frequency of flies with aberrant C2 vein in natural populations of *D. melanogaster* from Ukraine**

Collection site	Habitat	Frequency				
		2010	2011	2012	2013	2014
Magarach	Fruit canning vine laboratory	<0.013	0.0029	0.0071	0.0023	0.0041
Odesa	Orchard	<0.0052	<0.0063	0.0069	0.0065	0.0043
Uman’	Fruit canning factory	<0.0035	0.0132	0.0015	0.0121	<0.002
Varva	Fruit canning factory	<0.0078	0.0011	0.0027	0.0005	<0.0007
Kyiv	Fruit canning factory	<0.01	0.0142	0.0071	0.0079	0.0063
Kharkiv	Orchard	–	–	0.0111	0.0046	0.0029
Motovylivka	Orchard	<0.0032	<0.0023	0.0052	–	–
Drogobych	Orchard	<0.0062	<0.0196	0.0042	0.0027	<0.0041
Polis’ke	Abandoned orchard	<0.01	0.0117	<0.0238	0.0108	<0.0116
Chornobyl’ City	Abandoned orchard	<0.0065–	0.0022	<0.0016	0.0028	0.0014
Cooling pond	Two wild pear trees	<0.004	0.002	0.0222	–	0.0022

Note: «–» flies were not collected.

7.29 ± 2.12 %). Penetrance in the populations sampled range from 10 % up to almost 100 % (Table 3). The revealed alleles manifest in a sex-linked manner, whereby the frequency of the aberrant phenotype is higher among females (except for populations from Odesa in which it was equal) (Table 3).

Discussion. We detected flies with the aberrant second cross vein in natural populations of *D. melanogaster* from Ukraine. The trait was heritable and demonstrated incomplete penetrance and certain variability in different populations. As long as species-specific rates of spontaneous mutation are considered to be a stationary parameter [15], the elevated frequencies of the mutations we observed must have been caused by some external or internal factors that have been active in the majority of the studied populations concurrently, being global and not a population-specific. One of such factors could have been the ambient temperature. Temperatures above 40 °C have been common in Ukraine in the last years during July through August which lasted for over 4 h per day. Such temperatures have been shown to induce morphological aberrations in the drosophila C2 vein. Waddington has demonstrated that these changes can be induced by changes in various genes that control C2 vein development [9]. It is already known that the function of such genes can be disturbed not only by the changes in the genes themselves, but in the function of miR which regulate gene expression in certain signalling pathways [16]. It should be pointed out that genes responsible for wing development in drosophila are controlled by multiple micro RNAs, such as miR-2b-1, miR-8, miR-92b, miR-275, miR-303, miR-310, miR-312, and miR-313, and disruption in the work of these RNAs can lead to phenotypes described by Waddington in wild flies [16]. An ambient temterature has been shown to affect the expression levels of miR in the rockcress, *Arabidopsis thaliana* [17]. Therefore, high temperature-induced changes in miR expression could also be possible in drosophila, which might be one reason why C2 mutation can demonstrate cross-population frequency outbursts. Moreover, miR have been shown to be transported from somatic cells into germline cells, which may lead to temporary inheritance of the changes they induce [18]. This temporally restricted inheritance might have been one possible cause of the phenomenon we observed, whereby similar phenotypic aberrations are seen concurrently in distant populations. The molecular mechanisms that could ensure such a phenomenon call for further research.

Table 2. **The frequency of isofemale strains with the aberrant C2 progeny**

Collection site	Frequency					
	F1	F2	F3	F4	F5	Total in isofemale lines
Magarach	0.32	0.00	0.30	0.10	0.30	0.48
Odesa	0.29	0.38	0.33	0.29	0.29	0.67
Uman’	0.25	0.35	0.37	0.23	0.40	0.75
Varva	0.21	0.52	0.35	0.15	0.29	0.73
Kyiv	0.22	0.21	0.30	0.26	0.20	0.52
Kharkiv	0.44	0.29	0.53	0.63	0.29	0.75
Chornobyl’ City	0.30	0.46	0.26	0.27	0.26	0.67
Cooling pond	0.19	0.24	0.19	0.10	0.10	0.48

Table 3. **Penetrance (%) of the aberrant C2 vein in mutant flies**

Strain	Female	Male
Kyiv C2	81.15 ± 2.83	65.52 ± 3.6
Odesa C2	55.24 ± 4.85	30.93 ± 4.69
Odesa C2-1 august	95.57± 1.64	93.33 ± 1.86
Odesa C2-2 august	84.62 ± 2.67	80.98 ± 2.89
Uman′	77.11 ± 3.26	41.72 ± 4.01
Uman′ C2	74.21 ± 3.47	46.41 ± 4.03
Varva C2	53.76 ± 3.06	11.07 ± 1.94
Cooling pond C2	9.73 ± 2.79	0.00

Authors thank Dr G. Milinevsky, the staff of Biology Department of Mechnikov National University of Odesa, and the staff of the National Institute of Viniculture and Wine Industry UAAS for their valuable help in material collection. Authors thank Berezhnaya V., Institute of Cell Biology and Genetic Engineering NASU for her valuable help in producing photographs. Authors thank Dr Andrii Rozhok, Department of Biochemistry and Molecular Genetics, University of Colorado School of Medicine, Aurora, *CO, USA for his invaluable assistance, for sharing his knowledge and helpful suggestion regarding the paper preparation.*

ВЫСОКАЯ ЧАСТОТА НАСЛЕДСТВЕННЫХ ИЗМЕНЕНИЙ В ПРИРОДНЫХ ПОПУЛЯЦИЯХ *DROSOPHILA MELANOGASTER* УКРАИНЫ

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Известно, что уровень спонтанных мутаций в природных популяциях конкретных видов – величина постоянная. Неадаптивные мутации регистрируются с низкой частотой и очень быстро подлежат элиминации вследствие генетического дрейфа. Однако влияние факторов окружающей среды может приводить к появлению неадаптивных мутаций в природных популяциях, в частности за счет механизма генетической ассимиляции. В работе приведено описание мутантного фенотипа, который встречается с высокой частотой и проявляется нарушением развития второй поперечной жилки крыла у *Drosophila melanogaster*. Явление имело место одновременно в разных природных популяциях плодовых мух Украины, что делает невозможным появление такого события вследствие природного отбора или генетического дрейфа. Обсуждаются причины описанного феномена, включая роль внешних факторов, таких как температура, в возможной генетической ассимиляции.

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Received 23.03.15