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REDUCED REPRODUCTIVE SUCCESS IN *DROSOPHILA MELANOGASTER* FROM RADIOACTIVELY CONTAMINATED REGIONS OF UKRAINE

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Aim. *The goal of our study was to assess the effect of background radiation on the reproductive system of wild Drosophila melanogaster from Ukraine.* ***Methods.*** *In the present study we addressed the fertility and gonadal dysgenesis in the progeny of wild caught females from each of the studied populations. Fertility was measured as the average number of progeny of 30 females using analysis of variance and Tukey's multiple comparisons method. Gonadal asymmetry was assessed in 50 individuals of each sex by the non-parametric Kruskal-Wallis test.* ***Results.*** *Populations from Chornobyl and those collected nearby the Chornobyl Nuclear Power Plant cooling pond demonstrated the lowest fertility. The degree of donadal dysgenesis showed a weak correlation with the level of radioactive contamination.* ***Conclusion.*** *Our results support the hypothesis that chronic radiation leads to aberrant development of gonads and reduced fertility in populations from radioactively contaminated areas.*

Key words: *natural populations, fertility, gonadal dysgenesis, Drosophila melanogaster.*

Introduction. The nuclear disaster at Chernobyl, Ukraine in 1986 released more than 100 times the amount of radioactive material than was generated by the nuclear bombs in Hiroshima and Nagasaki, polluting more than 200,000 km^2 with radionuclides [1, 2]. There has been relatively little research on the impact of this contamination on biological processes [1, 2], and there is no long-term monitoring in place to track key taxa. Recent studies of birds indicate that species richness and abundance in the most contaminated areas were reduced by more than 30% compared to nearby control areas [3]. Information on the reproductive consequences of the radiation due to the Chernobyl disaster is scarce [2]. Long-term studies of reproductive success in barn swallows (*Hirundo rustica*) have revealed dramatic reductions in the incidence of reproduction, decreased clutch

size in breeding pairs, and decreased hatching success [4]. Extensive studies of reproductive success in great tits (*Parus major*) and pied flycatchers (*Ficedula hypoleuca*) showed reduced hatching success in contaminated areas, although this effect differed among habitats [5]. Similarly, studies of the bank vole (*Clethrionomys glareolus*) have found that dramatically elevated rates of embryonic mortality are related to mutation accumulation due to Chernobyl fallout [6]. These studies, and others, suggest that radioactive fallout from Chernobyl has negatively impacted reproduction in a number of vertebrate species.

Radiation-induced mutagenesis has been studied in fruit flies (*Drosophila*) for decades, but uncertainty remains about the cross-generational effects of low dose radiation on natural populations [7]. In the wild, other stressors such as high temperature, starvation and ethanol exposure, can also cause developmental defects, sterility and reduced lifespan. These stressors interact non-additively with radiation exposure [8], further complicating the study of long term chronic exposure to radionuclides in wild populations.

Studies of low dose radiation exposure have had mixed results. Even within a single study, low doses of radiation caused a reduction in fecundity, but an increase in egg-to adult viability in *Drosophila* [7]. *D. melanogaster* populations collected from areas of Belarus and Ukraine showed increased mutational loads [9], increased sterility and decreased viability and lifespan [10]. In another study, the level of sex-linked mutations in Chernobyl *D. melanogaster* populations showed no increase during the years 2005 to 2006 [11]. Further complicating the situation, it is known that ionizing radiation also activates mobile genetic elements, and mobile elements are responsible for about 80% of spontaneous mutations in Drosophila [12]. Aside from increasing the rates of mutation and recombination, active mobile elements can cause gonadal reduction, the complete failure of one or both gonads to develop.

Since the activity of mobile elements continues for two to three generations [13], we hypothesize that there will be a negative correlation between the radiation exposure history of populations and their fecundity, and predict that the higher the background radiation level at the collecting site, the lower the fecundity of the descendents of individuals collected from that site will be. We also hypothesize that the proximate cause of the lower fecundity will be hypogonadism, as measured by the levels of unilateral and bilateral gonadal dysgenesis present in the populations, and we predict that individuals descended from populations with a higher level of radiation exposure will be more likely to exhibit gonadal reduction.

Materials and methods

Collection. *Drosophila melanogaster* flies, which are widely distributed in Ukraine, were collected in the autumn when their population numbers were highest. Flies were trapped across the Ukraine in locations that varied in the degree of background radiation, ranging from 13 uR/h in Kyiv (Kiev) to 2100 uR/h at the shores of the cooling pond of the Chernobyl Nuclear Power Plant (Table 1). The resident populations of flies varied considerably by site. Since the Polesskoe site was a garbage dump for surrounding regions with lower background radiation, data derived from flies captured there were not assignable to a known radiation exposure history.

Fecundity assay. From individuals collected from each site, 30 females were selected at random and placed individually in 50 ml vials with five ml of standard laboratory medium at 26˚ [14], and allowed to lay eggs. Once the offspring of each individual

Table 1. Collection sites with background radiation levels, character of the site, and number of flies trapped

Site	Character	Radiation	Females	Males	Total
Kyiv (Kiev)	Orchard	13 uR/h	469	532	1001
Odessa	Urban	14 uR/h	830	1120	1950
Piryatin	Orchard	15 uR/h	89	162	251
Lubny	Fruit canning factory	16 uR/h	374	789	1163
Uman'	Fruit canning factory	16 uR/h	512	519	1031
Polesskoe	Garbage dump	50 uR/h	80	49	129
City of Chernobyl	Abandoned town	100 uR/h	43	52	95
Cooling pond	Industrial	2100 uR/h	33	17	50

had emerged as adults, one day old daughters and sons were randomly selected and placed individually in a new food vial. After five days, flies were released and all adult offspring that developed in these tubes were scored. Fecundity was estimated as the mean number of grandoffspring for the 30 females from each site. After a log + 1 transform, an analysis of variance was performed, and the populations were compared using Tukey's *post hoc* multiple comparison test.

Gonadal dysgenesis assay. Asymmetry in gonad size was measured in 50 individuals of each gender drawn from the grandoffspring generation from each site. Individuals were scored for gonadal dysgenesis as 0 if both gonads were healthy, 1 if one gonad was visibly smaller or absent, and a 2 if both gonads were reduced or absent. The mean of these 50 individual scores was used as the level of gonadal dysgenesis for each population. A Kruskal-Wallis non-parametric test was used to analyze the gonadal dysgenesis data.

Results and discussion

Fecundity. There was a significant relationship between fecundity and population, with fecundity lowest in the two sites with the highest background radiation, and highest in the Polesskoe garbage dump site, (ANOVA, $F_{8, 261} = 4.64$, $P < 0.0001$, Figure 1).

Gonadal dysgenesis. The gonadal dysgenesis assay revealed a weak association between background radiation level and developmental disruption. Of the 400 male flies examined, only one, from the City of Chernobyl site, had any apparent reduction in testes size. A larger proportion of the 400 females had unilateral (13 individuals) or bilateral (18 individuals) dysgenesis of the ovaries. The two populations with relatively high levels of background contamination (Chernobyl City and the Cooling Pond) had the highest level of dysgenesis, while the remaining populations had no or very low incidences of dysgenesis (Kruskal-Wallis, $\chi^2 = 43.2172$, d.f. = 7, $P < 0.0001$, Figure 2).

The data reported here support the hypothesis that chronic exposure to radionclides results in increased levels of gonadal dysgenesis and reduced fecundity in populations with significant radioactive contamination (Figure 1). Unexpectedly, the population located in a garbage dump had significantly higher fecundities than predicted based on background radiation levels in the area, likely reflecting the importation of flies and food sources from uncontaminated areas distant from Chernobyl. Flies collected from the two other contaminated locations (Chernobyl City and the Cooling pond) were

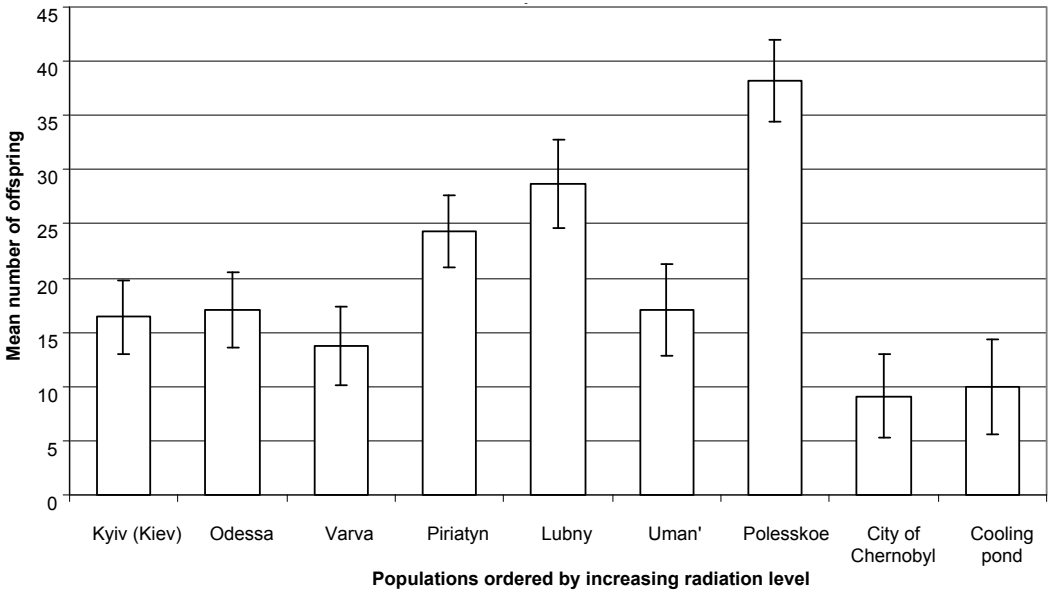

Figure 1. Population variation in fecundity. Polesskoe and the City of Chernobyl populations were significantly different from the other populations by a Tukey *post hoc* multiple comparison test. Polesskoe, a garbage dump site, gathered food waste from across the region and beyond

feeding on indigenous food sources (i.e. fruit from local trees) and were not influenced by human activities. The hypothesis that gonadal reduction, the likely mechanism underlying reduced fecundities, would be more likely in the more contaminated areas was also supported, with the Chernobyl City and Cooling Pond populations displaying the highest incidences of dysgenesis (Figure 2).

In a study of the distribution of P elements in Ukrainian populations of *D. melanogaster*, it was found that their incidence was lower in areas with higher levels of radionuclide contamination [15], in seeming conflict with our results. However, given that ionizing radiation can activate mobile elements [16], and activated mobile elements can cause one or both gonads to fail to develop, it is possible that the radioactive contamination from the Chernobyl disaster is indirectly selecting against the activation of mobile elements in the affected populations.

The observation of non-significant male gonadal dysgenesis is further evidence for the presence of P elements in the study populations. P elements have a critical temperature above which they cause gonadal dysgenesis. The critical temperature for females is 24˚ to 26˚, lower than the critical temperature for males, which is from 27˚ to 29˚ [17]. Had the rearing temperature been set higher, males also would have likely exhibited gonadal dysgenesis.

Environmental stress, including radiation, induces the activity of transposable elements in many other organisms besides *Drosophila* [18]. For example, a small increase in UV-B radiation activates the *Mutator* transposon in *Zea mays*, which goes on to cause a cascade of mutations in subsequent generations [19]. The mutagenic effects of radionuclides from the Chernobyl disaster may be enhanced by this mecha-

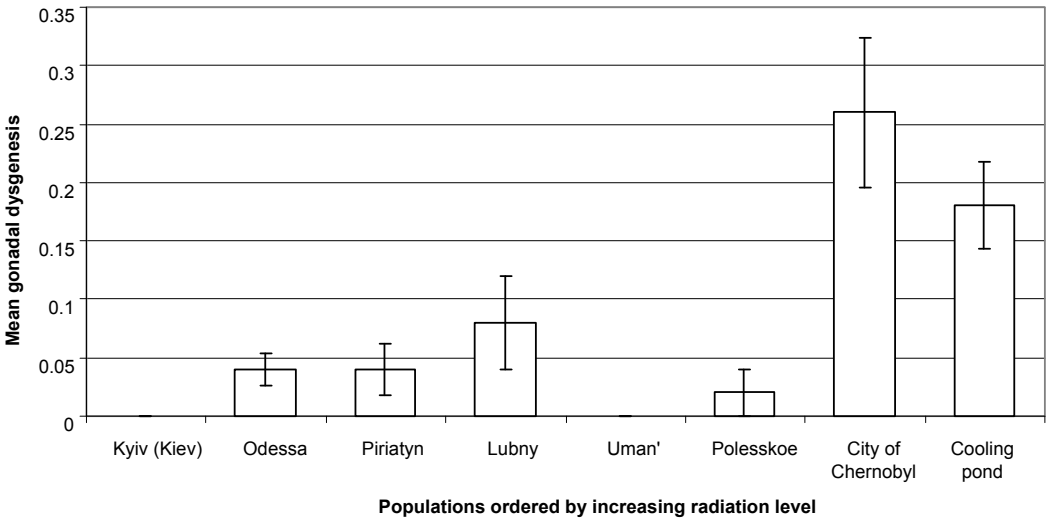

Figure 2. Gonad development was strongly affected by background radiation exposure. Individuals drawn from the two populations nearest Chernobyl were much more likely to have undeveloped gonads

nism. If some species are more affected than others, it may directly or indirectly impact overall species richness and abundance, possibly explaining the loss of biodiversity seen by Moller and Mousseau [3]. The effects of mobile elements need not be confined to the local populations; once a mobile element has been activated, it can spread through a species worldwide, as has been the case with *D. melanogaster*, and is presently happening in *D. simulans* [20].

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ЗНИЖЕННЯ РЕПРОДУКТИВНОГО УСПІХУ У *DROSOPHILA MELANOGASTER* З РАДІОАКТИВНО ЗАБРУДНЕНИХ РЕГІОНІВ УКРАЇНИ

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Мета. Метою роботи було оцінити вплив радіоактивного забруднення на репродуктивну систему представників природних популяцій *Drosophila melanogaster* України. **Методи.** Досліджували плодючість та рівень редукції гонад у нащадків самок із кожної досліджуваної природної популяції. Плодючість оцінювали як середнє число нащадків 30 самок з використанням дисперсійного аналізу та тесту множинного порівняння Тьюкі. Рівень редукції гонад вимірювався у 50 особин кожної статі, для аналізу даних було використано непараметричний тест Крускала – Уолліса. **Результати.** Найнижчу плодючість продемонстрували нащадки популяцій м. Чорнобиля та водойми-охолоджувача ЧАЕС. Аналіз редукції гонад показав слабкий зв'язок між фоновим рівнем радіактивного забруднення і розвитком порушень. **Висновки.** Отримані результати підтверджують гіпотезу про те, що хронічний вплив радіаційного забруднення призво-

дить до порушень у розвитку гонад і зниження плодючості у представників популяцій із радіоактивно забруднених регіонів.

Ключові слова: природні популяції, плодючість, гонадний дисгенез, *Drosophila melanogaster*.

СНИЖЕНИЕ РЕПРОДУКТИВНОГО УСПЕХА У *DROSOPHILA MELANOGASTER* ИЗ РАДИОАКТИВНО ЗАГРЯЗНЕННЫХ РЕГИОНОВ УКРАИНЫ

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Цель. Целью работы была оценка влияния радиоактивного загрязнения на репродуктивную систему представителей природных популяций *Drosophila melanogaster* Украины. **Методы.** Исследовали плодовитость и уровень редукции гонад среди потомков самок каждой исследованной природной популяции. Плодовитость оценивали как среднее число потомков 30 самок с использованием дисперсионного анализа и теста множественного сравнения Тьюки. Уровень редукции гонад измеряли у 50 особей каждого пола, для анализа данных использовали непараметрический тест Крускала – Уоллиса. **Результаты.** Самой низкой плодовитостью характеризовались потомки популяций г. Чернобыль и водоема-охладителя ЧАЭС. Анализ уровня редукции гонад продемонстрировал слабую связь между фоновым уровнем радиоактивного загрязнения и развитием нарушений. **Выводы.** Полученные результаты подтверждают гипотезу о том, что хроническое радиоактивное загрязнение отрицательно влияет на процесс развития гонад и плодовитость у представителей популяций из радиоактивно загрязненных регионов.

Ключевые слова: природные популяции, плодовитость, гонадный дисгенез, *Drosophila melanogaster*.