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Longevity in the *Drosophila virilis* Species Group. I. The *D. virilis* Phylad[1]

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ABSTRACT. Adult longevity of *Drosophila* is dependent upon many factors. In this study, the differences in longevity due to species, strain, and sex were examined for members of the *D. virilis* species phylad: *D. virilis, lummei, texana, americana,* and *novamexicana*. Newly eclosed adults from 12 laboratory strains representing diverse geographical localities were analyzed as to their longevity on standard cornmeal medium in order to discover interspecific, intraspecific, and sexual differences. The Texmelucan strain of *D. virilis* lived the longest. Males of this strain had an average longevity of 69 days, but females survived nearly 90 days. In contrast, the Chinook strain of *D. americana* was the shortest-lived with males surviving for 24 days, whereas the females lived 34 days. The other strains had mean longevities between these two extremes with there being significant interspecific differences. *Drosophila virilis* and *D. lummei* were not significantly different from each other but both lived significantly longer than the other three species; however, *D. texana, D. americana,* and *D. novamexicana* were not significantly different from each other. Significant intraspecific variation was found within *D. virilis, D. texana,* and *D. americana*. Females usually lived longer than males although the differences were not significant. Variation in the adult longevity of members of this phylad may be at least partially due to, although not limited to, differences in species, strain, and sex.

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INTRODUCTION

Adult longevity and aging in *Drosophila* have been the focus of studies by many researchers (see review by Lamb 1978), resulting in a wealth of information about longevity and what influences it. Since longevity can be a component of fitness in different organisms (Hamilton 1966) including mammals (Sacher 1978), scientists have tried to assess the genetic characteristics of this trait. Longevity has been reported to be an adaptive trait and genetically controlled by minor genes with epistatic interactions in *Drosophila* (Bourgois and Lints 1982). Hiraizumi (1985) reported no significant cytoplasmic effects on longevity of *D. melanogaster,* although some significant chromosomal-cytoplasmic interaction effects were observed.

One of the most obvious reasons for differences in longevity would have to be the rearing conditions both before and during adulthood. Various authors have reported effects of environmental factors on longevity in *Drosophila*. Temperature effects have been reported by

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Burcombe and Hollingsworth (1970), Hollingsworth (1969), and Lamb (1968). The role of nutrition in longevity is known (David, et al. 1983; Hollingsworth and Burcombe 1970). The effects of ultraviolet and ionizing radiation, as well as that of photoperiod, have been studied (Allemand, et al. 1973; Atlan, et al. 1969; Felix and Ramirez 1967). Miller and Thomas (1958) reported that larval crowding influenced adult longevity.

Sex differences in longevity have been reported although the results are mixed (Maynard Smith 1959; Gonzales 1923). In addition, longevity may also depend on the mating status of the fly, since differences have been reported for mated and virgin males and females (Bilewicz 1953; Maynard Smith 1958).

Species and strain differences in longevity have also been reported, although there is still a lack of data for many species (Felix and Ramirez 1967; Maynard Smith 1959; Spiess, et al. 1952). A compounding problem may be whether or not laboratory strains are similar to wild populations with respect to longevity. One would expect some differences although actual studies are lacking. An excellent system for this type of study exists with the *D. virilis* group, as laboratory strains of all known species from diverse geographic localities are maintained in culture.

The *virilis* species group is one of the best studied groups of *Drosophila*. Throckmorton (1982) has written an excellent review that incorporates cytology, morphology, biochemistry, and additional information into a possible evolutionary scheme for this group. The group now consists of 14 species and subspecies, holarctic in distribution. Species are known to be endemic to the Palearctic Region, the Nearctic Region, and at least one species is endemic to both (Throckmorton, 1982). The species group is divided into two phylads, *virilis* and *montana* based upon cytological, morphological, and ecological differences. In this study only the *virilis* phylad is considered. It consists of five species: *D. virilis, D. lummei, D. novamexicana, D. americana texana* (referred to later as *texana*), and *D. americana americana* (referred to as *americana*).

The purpose of this study was to determine the longevity of 12 strains of the *virilis* species phylad. Both interspecific and intraspecific differences were studied as well as differences between males and females. The information collected contributes to the results of current life-cycle and cytological studies being conducted to further our understanding of evolution in these insects.

METHODS AND MATERIALS

Twelve strains of the five species of the *D. virilis* phylad of the *D. virilis* species group were studied (Table 1). These strains were obtained from the National *Drosophila* Species Resource Center, Bowling Green State University. They were chosen for their geographic diversity, and also because they were being used in current cytological studies. Stock vials of each strain contained approximately equal numbers of flies so that rearing conditions were similar for all strains. These stocks were maintained under the same conditions as the experimental vials.

Newly eclosed adults of each strain were removed from the stock vials daily for one week, anesthetized with just enough ether to allow sexing, and placed in new vials containing fresh cornmeal medium (Yoon 1985). Each vial was labeled as to species, strain, sex, and date of eclosion. Each vial contained approximately 10 flies (to avoid crowding) of the same strain and sex collected on that day. The flies were maintained in a room with a temperature of 23 ± 1°C, relative humidity of 45-50%, and a 12D:12L photoperiod. The surviving flies were switched to new vials with fresh cornmeal medium every six weeks, the same schedule used for the stock vials. Vials were checked daily for the number of survivors. Visual inspection of the vials was usually adequate, although sometimes the vials were shaken lightly to determine whether a fly was dead or just inactive. However, these disturbances were kept to a minimum in order to reduce the possible effects on longevity.

Longevity was measured as the difference between eclosion date and date of death. The results were analyzed by species, strain, and sex with the Kruskal-Wallis and Wilcoxon statistical tests (Zar 1984).

RESULTS

Adult longevity in the *D. virilis* phylad differs with respect to species, strain, and sex (Table 1). Females of the Texmelucan strain of *D. virilis* lived the longest (approximately 90 days), whereas the Chinook strain of *D. americana* males survived only about 24 days. Significant differences among species were also found when males and females of all strains for each species were combined (Table 2). *Drosophila virilis* lived the longest (70 days), followed by *D. lummei* (68 days), *D. texana* (47 days), *D. novamexicana* (47 days), and *D. americana* (42 days). The results of the Kruskal-Wallis statistical test are shown in Table 2. Two divisions are clearly seen: *D. virilis* and *D. lummei* were not significantly different from each other, but both were significantly different from the other three species. None of the latter were significantly different from each other. Care must be taken though not to interpret these results incorrectly, since significant intraspecific differences were observed and may be more important in determining longevity (Table 1).

Intraspecific differences were more variable. No significant strain differences were found within *D. lummei* or *D. novamexicana;* in each of the other three species, one strain lived significantly longer. (Table 1). The greatest difference was found in the two strains of *D. americana.* The Chinook strain lived a much shorter time than any other strain or species (Table 1). There was also an increased number of early deaths observed for this strain. More than 25% of these flies died within 10 days of eclosion. These early deaths caused the mean longevity of this strain to be lower than any strain (Table 1).

In addition, there was a general tendency for females to survive longer than males; however, the difference was significant in only two strains (Table 1). Females tended to live from one to two weeks longer on the average. In three strains males had longer lifespans; however, none of these differences were significant (Table 1).

DISCUSSION

The evolutionary relationships of the *D. virilis* species group proposed by Throckmorton (1982) are based on biochemical and morphological evidence as well as cytological studies of polytene and metaphase chromosomes. Experimental results consistently reflect this same pattern of evolution and speciation for this group. The results of our longevity studies are in agreement as well. The proposed phylogeny has *D. virilis* and *D. lummei* more closely related to each other than either is to any other species in this group. This relationship is also supported by our results. Whereas both of these species were significantly different from the other three species, the two species did not have significantly different longevities (Table 2). Since *D. texana, D. americana,* and *D. novamexicana* failed to show any significant differences

TABLE 1
Longevity of species of the D. virilis *species phylad.*

Species	Strain	Sex	N	Longevity (days)*	Range (days)
D. virilis	U.S.S.R.	♂	23	65.4 ± 4.4	17-96
		♀	9	77.1 ± 6.5	38-98
	Texmelucan, Mexico	♂	23	69.3 ± 5.4**	12-97
		♀	16	89.5 ± 0.6**†	85-94
	Pasadena, California	♂	20	63.4 ± 3.5	25-87
		♀	32	69.9 ± 0.8	52-74
D. lummei	Moscow, U.S.S.R.	♂	16	75.1 ± 4.1	27-93
		♀	25	70.3 ± 2.3	45-85
	Japan	♂	25	68.9 ± 3.8	21-89
		♀	26	69.7 ± 3.5	15-92
	Finland	♂	21	57.4 ± 5.1	7-95
		♀	8	68.5 ± 11.1	8-94
D. novamexicana	Moab, Utah	♂	8	42.9 ± 3.8	19-53
		♀	11	49.1 ± 4.8	7-63
	Antlers, Colorado	♂	10	51.2 ± 6.0	19-70
		♀	19	44.9 ± 4.2	17-69
D. texana	New Orleans, Louisiana	♂	15	44.7 ± 3.7**††	7-59
		♀	31	56.2 ± 2.0**	17-58
	Morrilton, Arkansas	♂	12	42.0 ± 7.4	7-67
		♀	13	34.4 ± 5.0	10-57
D. americana	Anderson, Indiana	♂	12	58.5 ± 7.2†††	12-84
		♀	12	71.4 ± 5.8	30-92
	Chinook, Montana	♂	23	24.2 ± 4.6	3-56
		♀	19	34.3 ± 4.9	4-61

*Mean ± standard error of the mean
**Significant difference between sexes at $P \leq 0.005$
†Significantly different from Pasadena strain at $P \leq 0.05$
††Significantly different from Morrilton strain at $P \leq 0.05$
†††Significantly different from Chinook strain at $P \leq 0.05$

TABLE 2
Species differences in longevity of the D. virilis *phylad.*

Species	Longevity*	(R)**
D. virilis	70.8 ± 3.1	277.74
D. lummei	68.2 ± 4.7	263.38
D. texana	47.4 ± 3.9	138.54***
D. americana	42.3 ± 5.2	138.23***
D. novamexicana	46.9 ± 4.3	132.82***

*Mean ± standard error of the mean for all strains and both sexes combined for each species
**Mean rank scores based on Kruskal-Wallis Test
***Significantly different from *D. lummei* at $P < 0.001$

from each other, one might conclude that they are more closely related. This is also in agreement with the proposed phylogeny. However, the importance of this may be overstated since there were significant intraspecific differences in *D. virilis, D. texana,* and *D. americana* (Table 1).

Felix and Ramirez (1967) reported the mean longevity for *D. virilis* to be 54 days. All of our strains of *D. virilis* lived longer than this; however, the differences could probably be explained by different rearing conditions or strain differences. The longevity data for other species vary. For *D. melanogaster* (wild type), mean adult longevity is approximately 38 days (Yoon 1985), 39 days (Gonzales 1923), 35 days (Ramel and Eiche 1960) or 49 days (Doane 1960). For *D. persimilis,* a median lifespan has been estimated to be 110 days (Spiess, et al. 1952). This is nearly twice as long as that for *D. subobscura* (Maynard Smith 1959). For *D. repleta,* a species somewhat distantly related to *D. virilis,* the longevity is approximately two months (Sohal 1970). Comparisons for the other species in the *virilis* group are difficult to analyze, as this literature is not readily available, or else the tests have not been completed for these strains or species. There is a large difference in the range in longevity shown by some strains. The lower end of the range may reflect overetherization of the flies or the flies sticking to the medium before reawakening. In the present study, however, the vials were kept horizontal for one to two hours after the flies were anesthetized. *Drosophila virilis* has also been reported as difficult to etherize (Alexander 1976). This may also have been important. The Chinook strain of *D. americana* is one strain that may be easy to overetherize. Since over 25% of these flies died within 10 days of eclosion, there may be additional factors or possibly some type of lethal effect that will require further study. The very high mean longevity for the Texmelucan strain females of *D. virilis* is due partly to the total lack of early deaths. No female of this strain survived for less than 85 days, but none lived longer than 94 days. The reason for this is not readily apparent.

The literature on differences in longevity due to sex differences and mating status is contradictory. Although in this study only virgins were used, various authors have reported that virgins have either a longer or shorter lifespan than non-virgins (Bilewicz 1953; Maynard Smith

1958). On the other hand, Kidwell and Malick (1967) found no differences between mated and unmated female *D. melanogaster.* Differences due to sex were present in this study, but their significance appeared to be minimal. Although females outlived males in 75% of the strains, the difference was significant in only two of the nine strains. In three strains the male had the longer lifespan, but once again the differences failed to be significant. The longer female lifespan may possibly be due to the presence of two X chromosomes in the female. The second X chromosome may compensate for any lethal genes that may be present on the first X chromosome, and are expressed in the male.

In summary, one can conclude that adult longevity differences in members of the *D. virilis* phylad may be due in part to factors such as species, strain, and sex, but cannot be explained by these alone.

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