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Population density effects on longevity revisited

A note in response to 'Density and age-specific mortality' by J.W. Curtsinger

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J.W. Curtsinger, in his short communication in this volume entitled 'Density and Age-Specific Mortality', states that our suggestion that population density might has been a confounding variable affecting the demonstration of declining late-age mortality rates observed in the Medfly experiments in Carey *et al.* (1992) is specious. His claim results from four lines of argument: first that Medflies are not *Drosophila*, second that our experiments investigating density effects in *D. melanogaster* were small in size, third that we used extremely high densities to show significant effects, and finally that the density effects we showed only could have had significant impacts on early mortality rates. He continues in this piece to ask if the declining late-life mortality rates observed when utilizing large initial populations are real, and if so, what do they tell us about the fundamental processes underlying senescence.

Medflies are not *Drosophila*

Graves and Mueller (1993) reviewed a wide variety of data concerning density effects on longevity in a variety of species. This paper implicitly recognized that while all species seemed to be sensitive to density in some way, the nature of and mechanisms determining these effects were not well known. Thus, nothing in our paper asserted that particular observations of density effects seen in *Drosophila* or any other taxon must be in operation in *Ceratitus* (Medflies). The point that was made, that Curtsinger fails to acknowledge, is that the existence of these types of density effects in *Drosophila* argue for the plausibility of this phenomenon in Medflies. This of course argues for the construction of experimental techniques which would not confound density and aging as in Carey *et al.* (1992). It is curious that after asserting the difference between *Drosophila* and Medflies, Curtsinger then goes on to cite his own research with *Drosophila*, which to him suggests that density should not be an important confounding factor in Carey's Medfly experiments. At this point, we suggest that Curtsinger take his own advice. In the end no amount of post-hoc arguing will rescue a badly designed experiment. The only way to really address the question of density effects in Medflies is to carry out experiments with that species to show how these might be manifested in that species. It can be noted that Curtsinger cites a new experiment in his comment (Vaupel, Carey and Liedo, submitted) designed to examine the density question.

Population density effects

The adult and larval densities utilized in our experiments in *Drosophila* are consistent with those used in classical studies of this problem in this species (see e.g. Pearl & Parker, 1922; Pearl, Miner & Parker, 1927; Chiang & Hodson, 1954; Miller & Thomas, 1958; Graves & Mueller, 1993; Mueller, Graves & Rose, 1993). The studies of Pearl and his colleagues utilizing these same densities in similar volumes found that there were strong effects of density on mortality rates. They showed that these effects were non-linear across densities, and generally manifested earlier in

life (Pearl, Miner & Parker, 1927). This study utilized 13,000 wild type flies, with a total of 4,750 assayed, in the three highest density treatments. This study also showed some evidence for the deceleration of mortality rates at the latest ages, but the authors mentioned that their sample of flies surviving to later ages was too small to give any statistical validity to this claim.

The *Drosophila* data indicate that stocks with different selection histories relative to age-specific and density effects can exhibit radical differences in response to density. The NDCLA long-lived stocks produced by Luckinbill *et al.* (1984) utilized a combination of age-specific and strong density dependent larval selection actually increase their survivorship with density (at densities extremely high on Curtsinger's chart; Graves, 1988). This is also shown in the data from Pearl, Miner and Parker (1927) and in the r- and K-stocks produced by Mueller and Ayala (1981) (see also Mueller, Graves & Rose, 1993). This all argues that the impacts of density-dependent and age-specific selection on mortality patterns are complex and cannot be ignored, *a priori*, as in Carey *et al.* (1992) (it will be shown below that the authors of that study considered this a potential problem). It does not argue, as Curtsinger suggests, that we think that these functions are identical in *Drosophila* and Medflies.

The data seem to indicate at present, that at least in *Drosophila*, initial population density does seem to have its greatest impact on the mortality of young flies (Pearl & Parker, 1922; Pearl, Miner & Parker, 1927; Curtsinger *et al.*, 1993; Graves & Mueller, 1993; Mueller, Graves & Rose, 1993). Thus we do not disagree with this point made by Curtsinger. The experimentation performed thus far does not eliminate the possibility that there is no impact of declining densities on the mortality of older flies, and certainly the evidence indicates that all density functions are sensitive to the past evolutionary history of the stocks surveyed. In the case of the Medflies utilized in Carey *et al.* (1992) it was impossible to say anything about these possibilities, due to absence of any detailed knowledge concerning these characteristics in their Medfly stocks.

In addition, we made no contention that our experiments were not small relative to Carey *et al* (1992). They were not originally designed to make precise measurements of mortality rates, as made possible by the much larger experiments cited by Curtsinger. These experiments were designed to examine the physiological mechanisms by which density affects longevity in genetically differentiated stocks. Thus we do not agree with the statement that the results of our studies are irrelevant to understanding sources of mortality encountered by flies placed under density stress. Mueller, Graves and Rose (1993) reports changes in stress resistance and performance characters, in genetically differentiated stocks, relative to density-dependent and age-specific selection, which are known to be related to survival in *Drosophila* in these laboratory conditions. These levels of physiological detail are nowhere demonstrated in the studies cited in Curtsinger (this volume) used to claim the irrelevance of our work.

Philosophy, method and the deceleration of aging

We sense that, in fact, there is far more to this controversy. The disagreement is not just concerning the details of individual experimental design:

> 'At issue is whether the observation on Medflies, specifically the deceleration of mortality at older ages, reveals a fundamental property of aging with important genetic and evolutionary implications, or is just an artifact of experimental procedure mediated by density'.
>
> J.W. Curtsinger [this volume]

We would agree, but we would rephrase the question even more sharply. Let us assume that the deceleration of mortality observed at later ages in these experiments are real; does this tell us anything useful concerning genetic and evolutionary principles relates to senescence?

To understand this problem, we must return to the central questions that Carey *et al.* (1992) was designed to address. These questions were formulated by 'classical' gerontological thinking and may be listed as follows:

1. That senescence can be operationally defined by and measured from the increase in mortality rates with age.
2. That the basic pattern of mortality at adult ages in nearly all species follows the same unitary pattern described by the Gompertz model (exponential increase).
3. That species can be characterized by the species-specific life-span as measured (i) the oldest age attained, even in a relatively small population of 100 or fewer individuals, or (ii) a pattern of age-

specific mortality tending toward unity at the maximal age.

Carey *et al.*, 1992, p. 460.

The Carey *et al.* (1992) study examined mortality rates from large cohorts of Medflies kept under different conditions to address these questions. The treatments were experiment 1, males flies kept alone in cups (N = 21,204), experiment 2 in which male flies were kept individually in tissue cells (N = 27,181) and finally experiment 3 in which male flies were kept in cages (7,200 per cage, with a total cohort of 1,203,406 flies assayed). The results of this study were inconsistent with the classical demographic predictions outlined above. It found that in all cases that mortality rates decelerated at later age, that the mortality rates thus did not follow the Gompertzian prediction, and finally that the patterns of age-specific mortality observed were sensitive to the population size assayed.

Figure 2 from Carey *et al.* (1992) shows the age-specific mortality rates measured in the three different experiments. Two of the experiments give qualitatively similar results (the experiment with cups and cells), although it should be noted that a large fluctuation in mortality appeared centered around day 40 in the cell experiment that was not exhibited in the cup treatment. The third experiment gives a very different pattern of age-specific mortality, e.g. the mortality rates show a steady and marked decline from ages 55 days until the end of the experiment (about 100 days). In contrast the results from the experiment with cups shows an increase in mortality rates from 80–90 days and then fluctuates erratically after that time. The experiment with cells shows a more gradual increase in mortality rates from age 50–90 days followed by fluctuation and an eventual decline in the mortality rates.

The importance of the results from the cages is emphasized by the inclusion of the detailed data from this experiment alone in Table 2 of the Carey *et al.* paper. Certainly one should already be concerned that such different results were obtained for the same organism in different experiments. The authors, however, did not see these results as being qualitatively different, even though they pointed to the possibility of differential density effects existing between the treatments:

> 'In contrast, flies held in groups of 7,200 were subject to conditions that increase mortality risk—large cage size for flying, mating, some egg-laying, mechanical wear, *and considerable stress due to crowding* (our emphasis).
>
> Carey *et al.*, 1991, p. 459.

Graves and Mueller (1993), after Nusbaum *et al.* (1993), suggested one obvious explanation for the difference in these experiments and the dramatic decline in mortality seen in the cage experiments is the concomitant change in density that was uncontrolled in but present in the cage experiment and not the others. Consequently, that paper concluded that the interpretation of these results may be more relevant to density-related phenomenon than to aging.

In part, our suspicion of these experiments is conditioned by our utilization of evolutionary theory as the chief intellectual tool governing our thinking concerning phenomena related to senescence. The evolutionary theory of senescence is predicated on the declining force of natural selection acting on soma at advanced ages. The population genetic mechanisms consistent with that theory are antagonistic pleiotropy (trade-offs between alleles beneficial to early v. late-life) and mutation accumulation (alleles with neutral early-life negative late-life effects; Medawar, 1952; Williams, 1957). Evolutionary theorists concerned with aging never would have made the predictions that senescence is operationally defined by increasing rates of mortality with age; instead our view is that senescence is more properly defined by:

> a persistent decline in the age-specific fitness-components of an organism due to internal physiological deterioration.
>
> Rose, 1991, p. 20.

The pattern of mortality at later age is thus determined by the balance of age-specific actions of alleles governed by the antagonistic pleiotropy and mutation accumulation mechanisms. It then follows that evolutionary biologists would not have thought that there should be any *a priori* reason why mortality rates should follow the Gompertzian or any other specific functional relation. Thus, evolutionary theories of senescence do not necessarily require that mortality rates increase at all ages. For instance, the mutation accumulation hypothesis would assert the mortality rates at age x + 1 should be greater than those at age x because deleterious alleles have a greater impact on fitness at age x than at age x + 1. However, at ages that are many standard deviations beyond those normally found in the wild such differential effects

on fitness would not be expected, nor would a pattern of increasing mortality. For example, Hughes and Charlesworth (1994), utilizing male *Drosophila* from lines with different third chromosomes, found that there was increasing genetic variance in mortality rates with age. The genetic variation for mortality rates in these lines was not significant in early life, but was significant at the later ages. They suggested that this might account for the possibility of deceleration of mortality rates at the oldest ages. This phenomenon is a direct prediction of the mutation accumulation hypothesis, consistent with the overall evolutionary theory of senescence (Medawar, 1952; Mueller, 1987). In addition, unlike Carey *et al.* (1992), this study held density and mating effects constant.

Conclusion

Density effects are ubiquitous and their effects on life history characters are not well understood in insects. Thus experiments which purport to study these phenomena should take heed of these problems in their construction. In addition, the evolutionary theory of aging calls into question the relevance of the results of Carey *et al.* (1992) in regards to the fundamental processes that govern senescence. For these reasons we stand by the statements of Nusbaum *et al.* (1993) and Graves and Mueller (1993) in regards to Carey *et al.* (1992) that:

> 'these results may be more relevant to the density-related phenomena than to aging'.

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References

Curtsinger, J.W., H.H. Fukui, D.R. Townsend & J. Vaupel, 1993. Demography of genotypes: Failure of the limited life-span paradigm in *Drosophila melanogaster*. Science 258: 461–463.

Curtsinger, J.W., 1994. Density and age specific mortality. Genetica [this volume].

Carey, J.R., P. Liedo, D. Orozco & J.W. Vaupel, 1992. Slowing of mortality rates at older ages in large medfly cohorts. Science 258: 457–461.

Chiang, H.C. & A.C. Hodson, 1954. An analytical study of population growth in *Drosophila melanogaster*. Ecol. Monog. 20: 173–206.

Graves, J.L., 1988. The effect of selection for life span on life history characters and response to population density in *Drosophila melanogaster*. Ph.D. Thesis, Wayne State University. U.M.I. order #8910327.

Graves, J.L. & L.D. Mueller, 1993. Population density effects on longevity. Genetica 91: 99–109.

Hughes, K.A. & B. Charlesworth, 1994. A genetic analysis of senescence in *Drosophila*. Nature 367: 64–66.

Luckinbill, L.S., R. Arking, M.J. Clare, W.C. Cirrocco & A. Buck, 1984. Selection for delayed senescence in *Drosophila melanogaster*. Evolution 38: 996–1003.

Medawar, P., 1952. An Unsolved Problem in Biology. H.K. Lewis, London.

Miller, J.S. & J.L. Thomas, 1958. The effects of larval crowding and body size on the longevity of adult *Drosophila melanogaster*. Ecology 39: 118–125.

Mueller, L.D., 1987. Evolution of accelerated senescence in laboratory populations of *Drosophila*. PNAS USA 84: 1974–1977.

Mueller, L.D. & F.J. Ayala, 1981. Trade-off between r- selection and K- selection in *Drosophila* populations. PNAS USA 78: 1303–1305.

Mueller, L.D., J.L. Graves & M.R. Rose, 1993. Interactions between density-dependent and age-specific selection in *Drosophila melanogaster*. Functional Ecology 7: 469–479.

Nusbaum, T.J., J.L. Graves, L.D. Mueller & M.R. Rose, 1993. Letters to Science. Science 260: 1567.

Pearl, R. & S.L. Parker, 1922. Experimental studies on the duration of life. IV. Data on the influence of density of population on duration of life in *Drosophila*. Am. Nat. 56: 312–321.

Pearl, R., J.R. Miner & S.L. Parker, 1927. Experimental studies on the duration of life. XI. Density of population and life duration in *Drosophila*. Am. Nat. 61: 289–318.

Rose, M.R., 1991. Evolutionary Biology of Aging. Oxford University Press, New York.

Vaupel, J.W., J.R. Carey & P. Liedo, submitted. Medfly mortality decline not due to declining density.

Williams, G.C., 1957. Pleiotropy, natural selection, and the evolution of senescence. Evolution 11: 398–411.