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Population density effects on longevity

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Abstract

Population density, or the number of adults in an environment relative to the limiting resources, may have important long and short term consequences for the longevity of organisms. In this paper we summarize the way in which crowding may have an immediate impact on longevity, either through the phenomenon known as dietary restriction or through alterations in the quality of the environment brought on by the presence of large numbers of individuals. We also consider the possible long term consequences of population density on longevity by the process of natural selection. There has been much theoretical speculation about the possible impact of population density on the evolution of longevity but little experimental evidence has been gathered to test these ideas. We discuss some of the theory and empirical evidence that exists and show that population density is an important factor in determining both the immediate chances of survival and the course of natural selection.

Theory of population density and senescence

The study of the consequences of population density on the longevity of organisms was in fact first explored by scientists interested in problems in evolutionary ecology. In this field there had for some time been interest in understanding those ecological conditions which would favor two alternative life history patterns (Cole, 1954). Semelparity is one of these patterns which is characterized by a burst of reproduction shortly after sexual maturity, followed by rapid senescence and death. Semelparous life histories are observed in annual plants, salmon and black widows, in addition to many other diverse groups of organisms. Iteroparity, which is the pattern displayed by humans, fruit flies and many other organisms, is characterized by repeated episodes of reproduction after sexual maturity and thus a prolonged adult life stage.

One of the early attempts to understand the ecological pressures which may be important to determining which life history pattern might be most advantageous was made by MacArthur and Wilson (1967). In this work, MacArthur and Wilson suggest that natural selection will act in qualitatively different ways for populations kept at very high densities as opposed to those kept at very low densities. Much of the intuition and theory presented by MacArthur and Wilson was later expanded by several people including Pianka (1972). Predictions from these verbal theories were that under low density conditions rapid reproduction and thus early maturity and semelparity would be favored, while at high population density repeated reproduction and thus iteroparity should be advantageous.

Thus, these ecological theories suggest that low population density would generally accelerate senescence while high density would favor delayed reproduction and increased longevity. Many of the logical underpinnings of these verbal theories have been found to be faulty (see Mueller, 1991, for a review). Following the work of MacArthur and Wilson, Pianka, and others, formal theories which specifically took into account population age-structure and density were examined (Charlesworth, 1980). These theories (Charlesworth, 1980) showed that if density simply resulted in a constant increase in the rate of mortality, there would be no change in the form of selection relative to populations living at low density. However, if density-dependent natural selection acted only on pre-adult survival or fecundity, then it is possible that selection would favor increased longevity and delayed reproduction.

Evidence of density effects on longevity

In this section we focus on the experimental literature dealing with *Drosophila melanogaster*, which spans more than 60 years. While the effects we discuss are fairly well known, they have important implications for recent reports of decreasing rates of mortality with age (Carey *et al.*, 1992).

The earliest study with *Drosophila* (Pearl, Miner & Parker, 1927) used a protocol which has been repeated in more recent studies. A fixed number of adults is used to initiate the experiment, and the survival of this cohort of individuals is followed over time. An important aspect of this experimental protocol is that population density decreases over time since dead individuals are never replaced. As a result, the patterns of mortality from these types of experiments show a response which reflects both the aging process (which presumably increases rates of mortality) and declining density (which will presumably decrease rates of mortality).

The 200 control population in Figure 4 is an experiment like the one just described. From this experiment one can see that rates of mortality increase to a peak at age 11 days and then decline. There is a second increase in rates of mortality very late in life. These data late in life are not terribly accurate and result from the last few surviving adults. Obviously, when each of these last few adults die the estimated rate of mortality will be high (reaching 100% in the time interval at which the last adult dies). We have seen similar phenomena in our laboratory. Figures 1-2 show the mortality patterns for two different populations of *Drosophila melanogaster* (called B and O; see Rose, 1984b) which have experienced different selection regimes. More importantly, each population was initiated at two adult densities, 32 and 200.

Figures 1a,b show the mean and standard error of age-specific mortality rate for the five replicate B and O populations at densities of 32 and 200 flies per vial respectively. In figure 1a, at density 32, the initial mean rate of mortality is low for both the B and O populations; this rate begins to diverge at age 20 days when the B population begins to increase sharply. The corresponding sharp increase in the Os

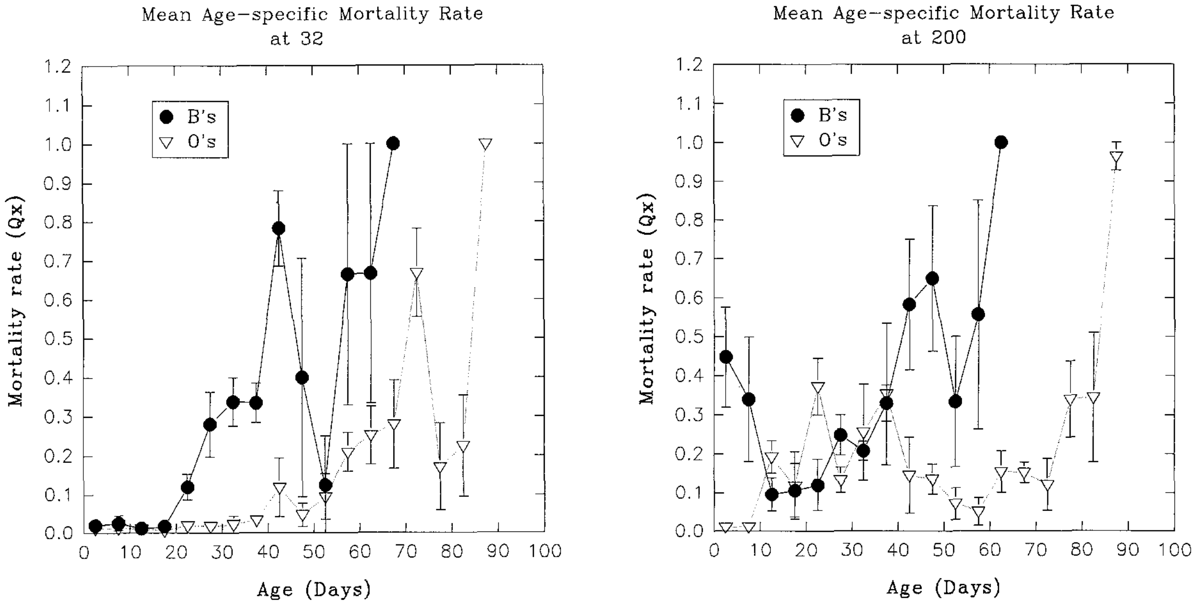

Fig. 1. (a) The fraction of the population dying during each 5 day period are shown for 5 replicated B (early reproduced) and five replicated O (late reproduced) populations kept at an initial density of 32 in a 8 dram vial. Sex ratio was 1:1 and dead individuals were not replaced during the experiment. (b) Similar data as in (a) except the density was initially 200 adults.

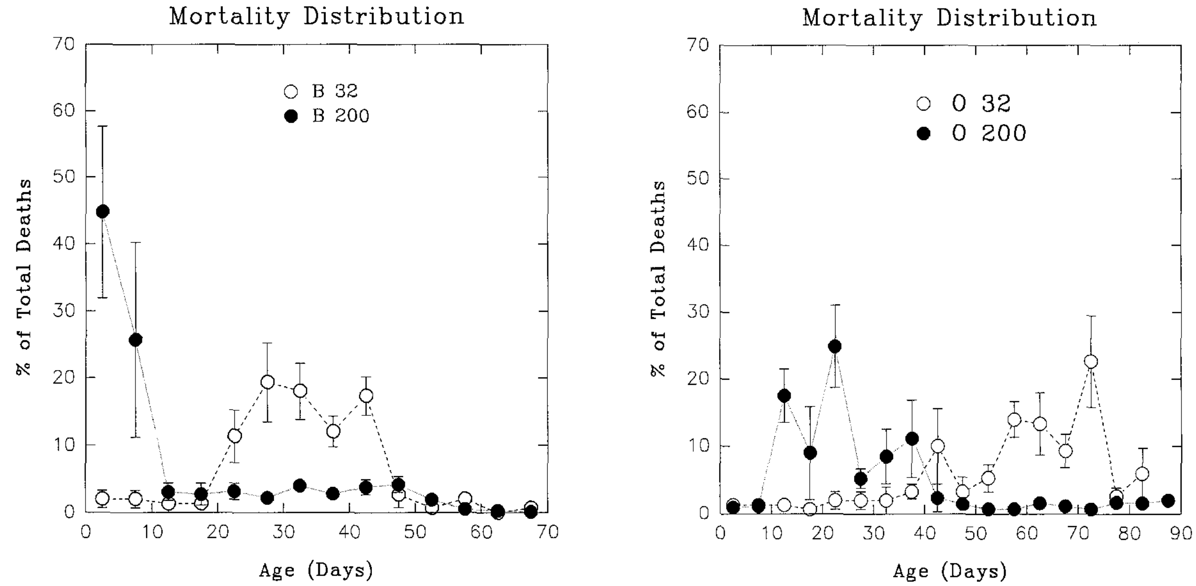

Fig. 2. (a) The same data as in figure 1 but plotted as a fraction of the total deaths for the B populations and (b) for the O populations.

does not occur until after day 40. At later ages the variance in mortality rates between lines becomes so large that accurate assessment of mortality rate is not possible; this is due to the fact that the few individuals left are not always dying in the same intervals for each line. Figure 1b shows the mortality rates for these same populations assayed at 200 flies per vial. In the B population the initial age-specific mortality rates are quite high. These fall after day 10 and then begin to increase afterward until all flies have died (≈ day 60). The Os, on the other hand, show a similar low mortality rate as observed at density 32, a rise and fall between days 10 and 60, and a final increase until all flies have died (≈ day 80).

Figures 2a and 2b are the percent of total deaths that occur in a time interval v. density treatment for the B and O lines respectively. They show that for the Bs at high density (200) that most deaths occur early on (≤ day 10), and then constant percentages are observed until all flies have died, while at low density the distribution of deaths peaks at intermediate ages (days 20-50). The pattern for the Os is qualitatively similar, although the magnitude of death percentages per interval is similar in both the high and low density treatments, and the means of the distributions are considerably to the right of those observed in the Bs. These figures seem to indicate that two processes may be at work to produce the observed mortality distributions in these stocks, both age-specific mortality factors (related to senescence) and density-dependent mortality factors (related to crowding resistance). The selection regime that produced the Os has been shown to have increased a number of stress resistance and physiological performance capacities of these stocks (Service *et al.*, 1985; Service 1987; Graves & Rose, 1990; Graves *et al.*, 1992). It is possible that these mechanisms of general stress resistance have also produced resistance to crowding effects in the Os relative to their B controls.

A third population (called K; see Mueller & Ayala, 1981a) kept at high adult densities (Fig. 3) again shows this same pattern. These data show that the patterns of mortality may also vary between males and females but larger samples would be required to establish this definitively. The mortality of females from 5-10 and 10-15 days was slightly higher than that of males, although not significantly so (t = 1.43, 1.76, with p = 0.288, 0.219 respectively).

Recently, Carey *et al.* (1992) have conducted experiments, using a protocol similar to the one used in the *Drosophila* experiments reviewed here,

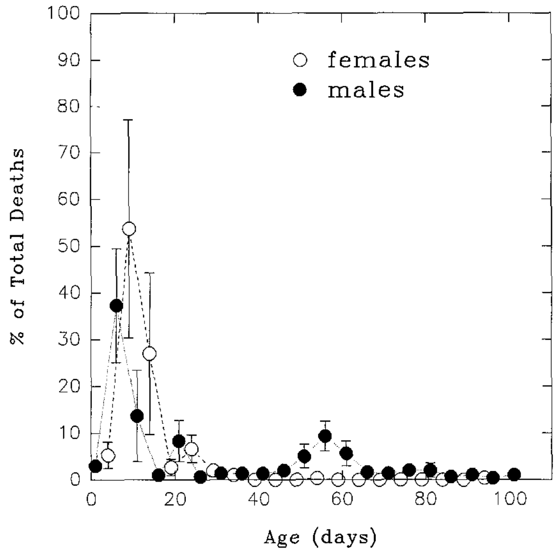

Fig. 3. The fraction of the population that died per 5 day period is shown for the K (high density) populations. Each experiment was initiated with a total of 200 adults, 100 of each sex.

with medflies (*Ceratitis capitata*). Thousands of medflies were put into population cages and the mortality of this cohort was followed over time; dead flies were not replaced, so density declined during the course of this experiment. Mortality reached a peak in middle ages and then fell at later ages. Carey *et al.* took this as evidence that these populations are not in fact senescencing, i.e. rates of mortality actually decreased at later ages rather than increased. However, these populations of medflies were also experiencing declining population density, which would be expected to cause rates of mortality to rise and then decline in a manner similar to what has been observed for the numerous *Drosophila* populations. In this light the Carey *et al.* results are more relevant to density-dependent mortality than they are to the process of senescence (Nusbaum *et al.*, 1993).

Over evolutionary time the manner in which adult populations are affected by density may affect the pattern of selection for either reproduction early or later in life. For example, one would expect that if densities always stayed high, then natural selection would favor earlier reproduction (Charlesworth, 1980). However, if populations faced an early density crisis, and survivors might expect lower overall densities and a renewal of resources, then selection for delayed reproduction might result. The rationale for the second scenario is twofold. First, if all individuals at high density are reproducing early, than larval competition would be fierce. Any individual who could withhold reproduction and lay eggs after the population has crashed may actually reproduce more offspring over their life time. Second, if energy for survival of density stress is produced by shutting down reproduction, then the previous scenario is even more likely. Populations with lower reproductive effort retain greater energy reserves in their tissues (Service, 1987; Graves *et al.*, 1992). The evidence now suggests that stocks with different selection histories, when exposed to density stress, utilize energetic reserves that would otherwise be available for reproduction.

Mechanisms by which density affects longevity

In this section we consider mechanisms which have immediate effects on longevity as opposed to long term evolutionary effects.

Food limitation

One of the most obvious mechanisms by which population density may affect Darwinian fitness and hence longevity is by food limitation. The treatment of food as a limiting factor goes back to the earliest thinking about population density effects in ecological theory. Food as a limiting factor was the cornerstone of Malthus' thinking in the now infamous 'An Essay on the Principle of Population', in which Malthus made the erroneous claim that starvation in Ireland and England was due to the fact that food supplies had been exhausted (Malthus, 1798). Despite Malthus' sociological error, the fundamental logic of food limitation as an agent of selection is sound, and was crucial in the formulation of Darwin's principle of natural selection (Darwin, 1859). Modern theory has reformulated the idea of food limitation as a mechanism of density dependent population growth and in relation to life history trade-offs (e.g. Andrewartha & Birch, 1954; Tanner, 1966; Schoener, 1973; Mueller & Ayala, 1981a; Mueller, 1988; Mueller, Gonzalez-Candelas & Sweet, 1991).

Numerous examples exist in which both invertebrate and vertebrate animal populations have been shown to have their fertility lowered or mortality increased by food scarcity (*Drosophila*: Chiang & Hodson, 1954; insects in general; Klomp, 1964; snails: Eisenburg, 1966; Daphnia: Slobodkin, 1954; Frank, Boll & Kelly, 1957; Coleopteran beetles: Davis, 1945; sea urchins: Levitan, 1989; tits and goldchrests: Gibb, 1960; red grouse: Jenkins, Watson & Miller, 1970; birds and mammals in general: Lack, 1954). The competition for food generally has greater fitness effects on juveniles (fish: Beverton, 1962; voles: Hoffman, 1958; deer: Leopold, Sowls & Spencer, 1956; Mitchell, 1973; *Drosophila*: Prout & McChesney, 1985; moths: Gordon & Stewart, 1988; humans: Kulin *et al.*, 1984).

Dietary restriction

Most of the ecological and life history evolution literature has focused on population density effects in which food limitation causes both a depression in fecundity and an increase in mortality. However, gerontological research on laboratory rats and mice has shown that milder levels of dietary limitation (caloric, not nutrient) may actually extend longevity. This phenomenon has been called dietary restriction (DR).

Dietary restriction (DR) has a somewhat broad phylogenetic occurrence and is particularly powerful in its extension of mammalian longevity. The observation that dietary [caloric] restriction extends mammalian longevity was originally reported in laboratory rats (McKay *et al.*, 1935) and has also been demonstrated in a variety of organisms: flies, water fleas, fish, and mice (Ingram *et al.*, 1990). This has prompted a number of authors to speculate on the evolutionary origin of this response for mammals (Holliday, 1989; Harrison & Archer, 1988; Phelan & Austad, 1989).

The debate between Harrison and Archer (1988) and Phelan and Austad (1989) centered on how dietary restriction proved adaptive. Harrison and Archer (1988) argued for natural selection increasing the reproductive life span of mice that experienced periodic episodes of food shortage or drought. They proposed that dietary restriction benefits might be greater in a species with shorter reproductive life spans (therefore *Mus musculus* should benefit more by dietary restriction than *Peromyscus leocopus*, a species which already has a much greater reproductive life span). Phelan and Austad (1989) suggested that natural selection does not work in the way described above. They propose that life extension by dietary restriction is an incidental consequence of its effect on the timing and amount of reproduction. Thus their prediction was that dietary restriction should have the greatest impact on species with early and copious reproduction as compared to with late sexual maturity and relatively small amounts of energetic investment in reproduction. Comparative tests of the predictions of these hypotheses have yet to be conducted.

Experimental evidence exists to support the assertion that dietary restriction operates by fostering analogous trade-offs between growth, survival, and reproduction in a wide variety of organisms: mosquitoes fed on sucrose (Pena & Lavoipierre, 1960a,b;) spider (Austad, 1989); waterstriders (Kaitala, 1991); carabid beetles (Ernsting & Isaaks, 1991); rotifers (Robetson & Salt, 1981); and rats (Holehan & Merry, 1985a,b). Chippindale *et al.* (1993) have shown that dietary restriction (an environmental manipulation) can have the same phenotypic effect as selection for delayed reproduction (a genetic manipulation) in *Drosophila melanogaster*.

There are several striking results of the Chippindale *et al.* study. The study employed early-reproduced, control (B) lines, late-reproduced, postponed senescent lines (O), desiccation selected stocks (D) derived from Os and their controls (C). All stocks showed significant increases in longevity in the D.R. treatments except the (C) females. Longevity and fecundity in the (B) and (O) stocks that had shown the D.R. responses were negatively correlated. The fecundity of the (B) and (O) stocks recovered when their nutritional regimes were switched. Starvation resistance (positively correlated with longevity) and fecundity were also negatively correlated, as in the case of selection for delayed reproduction treatments (Rose, Graves & Hutchinson, 1990; Rose *et al.*, 1992).

This study is one of the most powerful demonstrations yet of phenotype responses to environmental variation paralleling a genetic response to selection. Similar results were found in Partridge (1987). The failure of Lebourg and Medioni (1991) and David (1971) to find D.R. responses in *Drosophila* is likely due to methodological differences, as described in Chippindale *et al.* These studies used food levels that suppressed both fecundity and longevity, thus causing a positive correlation between longevity and fecndity that would not have existed at higher food levels. It has been demonstrated both theoretically and experimentally that environmental conditions may exist in which phenotypic correlations do not reflect genetic correlations (Service & Rose, 1985).

Thus, the D.R. results suggest that at some adult population densities, in a wide variety of species, longevity may actually be increased in contradiction to ecological theory. The antagonistic pleiotropy hypothesis predicts that this increase in longevity will occur at the expense of some component of early reproductive fitness. Models of density-dependent population growth have not yet taken into account this possibility.

Mechanisms of stress resistance and density in Drosophila melanogaster

High population density may also affect the ability of individuals to withstand stress of various sorts. These effects may then make individuals more susceptible to background causes of mortality, thus reducing longevity. The earliest controlled studies of the impact of crowding on longevity was made by Pearl and his colleagues (Pearl & Parker, 1922; Pearl, Miner & Parker, 1927) on *Drosophila melanogaster*. In one study *D. melanogaster* adults were kept at two different adult densities: 200 and 35. After 16 days some of the replicate tretments at density 200 were reconstituted to total densities of 200 (since many of the initial 200 adults had died). Samples of adult flies which had been placed at density 35 were also reconstituted to a density of 200. From Figure 3 it is apparent that the chances of survival are smaller for adults which had lived their first 16 days at a density of 200 compared to the adults raised at density 35, even when both experienced the same high density late in life. Of course from the control populations it is also clear that high density alone dramatically increases the chances of mortality, especially at young ages when these density differences were most profound.

Table 1. The effects of density on two physiological characters, desiccation resistance and starvation resistance, at two densities (low: 32 adults per vial, high: 200 adults per vial) and in two populations (B and O).

	B Low	B High	O Low	O High
Desiccation (h)	8.28	7.19	13.19	7.22
Standard error	0.78	0.38	1.22	0.33
Starvation (h)	40.37	31.05	58.69	52.75
Standard error	2.07	2.02	2.09	1.53

Table 2 shows the effect of high population density on selected physiological performance characters in *D. melanogaster* stocks created by age-specific selection (B v. O, from Graves *et al.*, in prep). These data were derived from B and O stocks maintained at high density (200 per 8 dram vial) or low density (32 per 8 dram vial) for 24 h. The high density treatments for desiccation resistance, flight duration, and starvation resistance all showed a decline in performance regardless of stock. The O stock showed some density resistance for flight duration at 64; the mean flight duration was slightly higher than 32. The difference in starvation resistance for 24 h was not significant for the O stock.

However, the loss of physiological performance in the postponed senescent, long-lived O lines seemed to be more pronounced for desiccation resistance and flight duration. Graves *et al.* (1992) and Service (1987) demonstrate that the underlying energetic sources for these physiological characters are different. Glycogen has been shown to be the chief reserve accounting for differential performance in flight and desiccation, while lipids are central in starvation resistance. Wigglesworth (1949) has shown for *Drosophila* that during the course of starvation glycogen reserves get mobilized first; Graves *et al.* (in prep.) have confirmed this for these stocks. If density stress at some level involves mobilization of energetic reserves, then it is likely that glycogen reserves should be exhausted before lipids, and those physiological suites dependent on glycogen should be more density-sensitive than lipid suites. These data support that possibility.

Other mechanisms

The effects of density may act in specific ways that

Table 2. The effects of density on flight duration, at three densities and in two populations (B and O).

Density	32	64	200
B (min)	45.5	39.1	0
Standard error	7.94	3.55	0
O (min)	105	116	1.50
Standard error	8.95	14.0	0.42

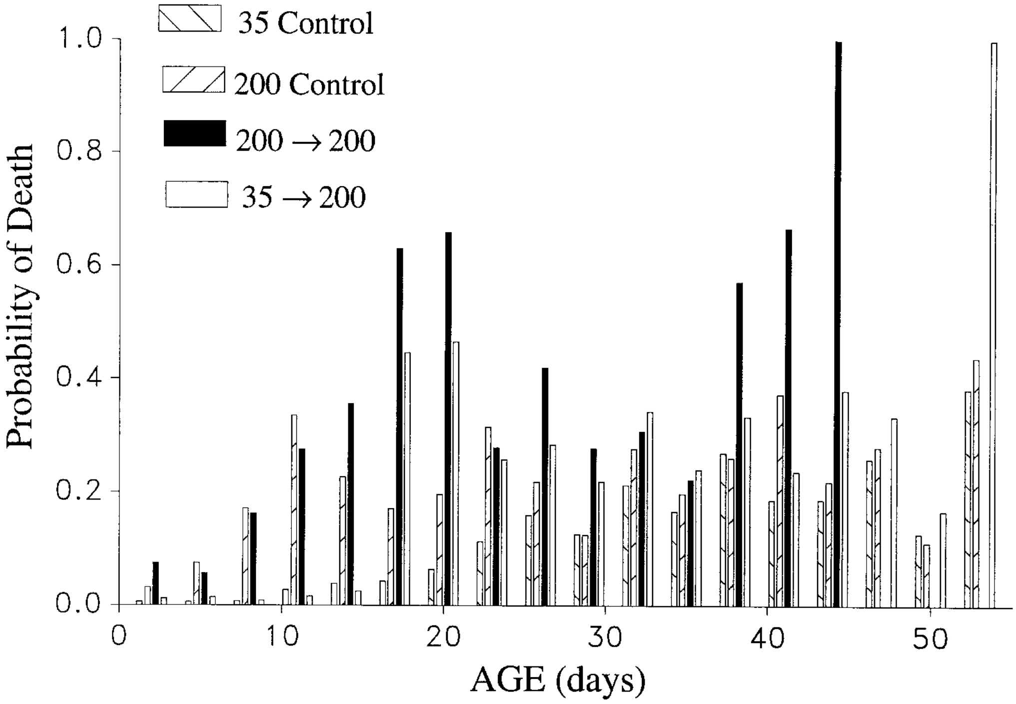

Fig. 4. The chances of death for adult *D. melanogaster* as a function of age and density (Pejarl, Miner & Parker, 1927). Two control populations were started at initial densities of 35 and 200 and survival of these cohorts followed over time. After 16 days adults from the 200 and 35 treatments were sampled and placed at a total density of 200. This dramatically increases the chances of dying but the population raised at 200 for the first sixteen days is more severely affected than the populations raised at a density of 35 during this same period.

are peculiar to certain groups of organisms. For instance, in crowded populations adults of the sea urchin, *Diadema antillarum*, may cannibalize each other (Levitan, 1989). Increased density does not always result in reductions in longevity. For instance, juvenile survivorship of the coral reef fish, *Dascyllus aruanus*, increased with increasing adult density. For this species adults may serve to provide juveniles with predator warning and thus reduce one source of mortality.

Density may also act to modify the environment and affect sources of mortality. In laboratory cultures of *D. melanogaster* there is a dramatic decline in survival rates of adults kept in crowded cultures (Mueller & Ayala, 1981b) and this decline is greater for females than for males. During the course of Mueller and Ayala's experiments (which lasted one week), eggs laid during the first day of the experiment hatched and grew. When the population density is high the large number of larvae gives the food a very soft, sticky consistency. Adults easily get stuck in this material and drown. Females appear to be more adversely affected in these environments since they attempt to oviposit on the food surface while males seem content to live on the sides of the culture away from the food surface. Davis (1945) suggests that declines in longevity with increasing density of the coleopteran *Trogoderma versicolor* may be mediated by changes in the environmental substrate. Communicable disease is also expected to be spread more rapidly in crowded cultures and thus contribute to the decline in longevity (Hassell & May, 1989).

Population density and the evolution of longevity

Juvenile crowding

There are now two population genetic theories of aging with support from different experimental systems. The antagonistic pleiotropy theory (Williams, 1957; Rose, 1991) suggests that genes with beneficial effects on early components of fitness, like fertility and survival, but deleterious effects late in life may nevertheless be favored by natural selection due to the greater weight natural selection puts on early fitness components. The mutation accumulation hypothesis (Medawar) suggests that deleterious mutations occur which may act to reduce survival or fertility late in life, but are only weakly disfavored by natural selection, and hence may accumulate to significant frequencies in most populations, giving rise to senescent phenotypes. A consequence of both of these theories is the prediction that natural populations may harbor genetic variation for senescence which can itself be the focus of selection.

There have now been several successful attempts to select for delayed senescence (Rose, 1984a; Luckinbill *et al.*, 1984; Partridge & Fowler, 1991) in the fruit fly *Drosophila melanogaster*. All of these experiments employed a similar protocol of progressively culturing populations from flies which had lived to a late age and then raising the larvae under a moderate to high density (50 or more larvae per vial). Several attempts at selecting for increased longevity have, however, been unsuccessful (Lints & Hoste, 1974; Lints *et al.*, 1979; Flanagan, 1980). These experiments differed from the previous ones in that the larvae were raised at a low density (10 larvae per vial). Is it possible that the density at which larvae are raised could have an effect on the ability to select for adult longevity?

The previous question can be partitioned into two related questions, one of which is easier to answer than the other. The first is, does the larval density affect adult longevity at the phenotypic level? This question was addressed in early work on *Drosophila* by Miller and Thomas (1958). They showed that larval crowding, which generally reduced the size of the resulting adults, had the effect of increasing adult longevity. An important distinction, then, is when does larval crowding ensue, and can we consider a larval density of 10/vial to be meaningfully different from a larval density of 50/vial? Miller and Thomas showed that larval densities of 5-20 larvae/vial showed no appreciable change in final adult size but at densities of 40, 60, 80 and 100 larvae/vial adult size showed a continuous decline. Thus, the larval densities used by Rose, Luckinbill and Partridge can all be considered to fall in a range of densities in which the adult size is less than its maximum due to larval crowding.

A second, more difficult, question is, does the expression of additive genetic variation for longevity vary depending on the larval environment? The only practical way to test that proposition is to directly measure the additive genetic variation in the two environments or to select for the trait of interest in both environments and observe the change in phenotypic means of the populations due to this selection. In fact the latter experiment has been done by Luckinbill and Clare (1985) with the finding that selection for longevity was not successful when the larval rearing density was 10/vial but was successful at densities of 70, 120 or higher per vial. This effect is known as a genotype by environment interaction and implies that the outcome of selection for longevity will depend critically on the environmental conditions under which the selection is carried out. In this case at least one critical environmental factor is larval density. At this time there is insufficient information about the genetic and physiological mechanisms affecting longevity to offer a detailed explanation for this particular genotype by environment interaction.

Interactions between age-specific and density-dependent natural selection

The theories of density-dependent and age-specific natural selection have developed almost independently of each other (see Charlesworth, 1980, for exceptions). Nevertheless, it is still of some interest to determine if these different types of natural selection affect common sets of genes or physiological processes. Preliminary information is available from a comparison of *Drosophila* populations independently selected by age-specific and density-dependent selection (Mueller *et al.*, 1993). Mueller *et al.* (1993) examined traits which had responded to age-specific selection in populations that had been subject to density-dependent selection and vice-versa. In general there was little evidence that age-specific selection affected the traits that responded to density-dependent selection or vice versa.

Recently, Mueller (unpublished) has found that populations that have evolved at high larval densities are more tolerant to environmental urea than are controls. This is not too surprising since crowded larval cultures can have very high levels of urea. Perhaps more surprising was the observation that populations selected for increased longevity also have an increased ability to resist high levels of environmental urea (Leroi, unpublished), since these populations and their controls are raised at the same low density. These results suggest the possibility that these two types of selection may affect common sets of genes or physiological mechanisms. Further study of the mechanisms of urea tolerance may help determine these connections.

Summary

Increasing population density generally decreases longevity. These effects may be mediated through a variety of mechanisms such as reduction in food resources, alterations of the physical environment, and disease. Longevity is a trait that is molded by natural selection. By changing the manner in which natural selection acts, longevity may be increased in populations. The ability to successfully select for increased longevity in *Drosophila* is affected by the density regime used in the experiment. Current results with *Drosophila* also suggest that common physiological pathways may be involved in the process of adapting to high density and to prolonged lifespan.

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References

Andrewartha, H. G. & L. C. Birch, 1954. Distribution and Abundance of Animals. University of Chicago Press, Chicago.

Austad, S. N., 1989. Life extension by dietary restriction in the bowl and doily spider, *Frontinella pyramitela*. Exp. Gerontol. 24: 83-92.

Beverton, R. J. H., 1962. Long-term dynamics of certain North Sea fish populations, pp. 242-259 in The Exploitation of Natural Animal Populations, edited by E. D. LeCren and M. W. Holgate. Wiley, N.Y.

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.

Charlesworth, B., 1980. Evolution in age-structured populations. Cambridge University Press.

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

Chippindale, A. K., A. M. LeRoi, S. B. Kim & M. R. Rose, 1993. Phenotypic plasticity and selection in *Drosophila* life-history evolution. I. Nutrition and the cost of reproduction. J. Evol. Biol. 6: 171-193.

Cole, L. C., 1954. The population consequences of life history phenomena. Q. Rev. Biol. 29: 103-137.

Darwin, C., 1859. On the Origin of Species. Reprinted by Harvard University Press.

David, J., J. Van Herrewege & P. Fouillet, 1971. Quantitative underfeeding of *Drosophila*: effects on adult longevity and fecundity. Exp. Gerontol. 6: 249-257.

Davis, M. B., 1945. The effect of population density on longevity in *Trogoderma versicolor* creutz (= *T. inclusa* Lec.). Ecology 26: 353-362.

Edney, E. B. & R. W. Gill, 1968. Evolution of senescence and specific longevity. Nature 220: 281-282.

Eisenburg, J. F., 1981. The Mammalian Radiations. The University of Chicago Press, Chicago.

Eisenburg, R., 1966. The regulation of density in a natural population of the pond snail *Lymnaea elodes*. Ecology 47: 889.

Ernsting, G. & J. A. Issaks, 1991. Accelerated aging: a cost of reproduction in the carabid beetle *Notiophilus biguttatus* F. Funct. Ecol. 5: 299-303.

Flanagan, J. R., 1980. Detecting early-life components in the determination of the age of death. Mech. Aging Develop. 13: 41-62.

Frank, P. W., C. D. Bell & R. W. Kelly, 1957. Vital statistics of laboratory cultures of *Daphnia pulex* DeGree as related to density. Physiol. Zool. 4: 287-305.

Gibb, J. A., 1960. Populations of tits and goldcrests and their food supply in pine plantations. Ibis 102: 163-208.

Gordon, R. M. & R. K. Stewart, 1988. Demographic characteristics of the stored product moth *Cadra cautella*. J. Anim. Ecol. 57: 627-644.

Graves, J. L. & M. R. Rose, 1990. Flight duration in *Drosophila melanogaster* selected for postponed senescence. Chapter 5. in Genetic Effects on Aging II., Telford Press, Caldwell, NJ.

Graves, J. L., E. C. Toolson, C. Jeong, L. N. Vu & M. R. Rose, 1992. Desiccation, flight, glycogen, and postponed senescence in *Drosophila melanogaster*. Physiol. Zool. 65: 268-286.

Hamilton, W. D., 1966. The moulding of senescence by natural selection. J. Theor. Biol. 12: 12-45.

Harrison, D. E. & J. R. Archer, 1988. Natural selection for extended longevity from food restriction. Growth Dev. Aging 52: 207-211.

Hassell, M. P. & R. M. May, 1989. The population biology of host-parasite and host-parasitoid associations. Chapter 22, in Perspectives in Ecological Theory, edited by J. Roughgarden, R. M. May and S. A. Levin. Princeton University Press, Princeton, N.J.

Hoffman, R. S., 1958. The role of reproduction and mortality in population fluctuations of voles (Microtus). Ecol. Monog.

28: 79-109.

Holehan, A. M. & B. J. Merry, 1985a. Modification of the oestrous cycle hormonal profile by dietary restriction. Mech Aging and Dev. 32: 63-76.

Holehan, A. M. & B. J. Merry, 1985b. The control of puberty in the dietary restricted female rat. Mech Aging and Dev. 32: 179-191.

Holehan, A. M. & B. J. Merry, 1986. The experimental manipulation of aging by diet. Biol. Rev. Camb. Phil. Soc. 61: 329-368.

Holiday, R., 1989. Food, reproduction and longevity: is the extended lifespan of calorie-restricted animals an evolutionary adaptation? Bio Essays 10: 125-127.

Ingram, D. K., R. Weindruch, E. W. Spangler, J. R. Freeman & R. L. Walford, 1987. Dietary restriction benefits learning and motor performance of aged mice. J. Gerontol. 42: 78-81.

Luckinbill, L. S. & M. J. Clare, 1985. Selection for life span in *Drosophila melanogaster*. Heredity 55: 9-18.

MacArthur, R. H. & E. O. Wilson, 1967. The theory of island biogeography. Princeton University Press.

Malthus, T., 1798. An Essay on the Principle of Population. Reprinted by MacMillan, New York.

McKay, C. M., L. A. Maynard, G. Sperling & L. L. Barnes, 1939. Retarded growth, life span, ultimate body size and age changes in the albino rat after feeding diets restricted in calories. J. Nutr. 18: 1-13.

Medawar, P. B., 1952. An unsolved problem in biology. H. K. Lewis, London.

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

Mitchell, B., 1973. The reproductive performance of wild Scottish red deer, *Cervus elaphus*. J. Repro. Fert. 19: 271-285.

Morris, J. G., 1991. Nutrition, in Environmental and Metabolic Animal Physiology. Wiley-Liss, New York.

Mueller, L. D., 1987. Evolution of accelerated senescence in laboratory populations of *Drosophila*. Proc. Natl. Acad. Sci. USA. 84: 1974-1977.

Mueller, L. D., 1988. Density-dependent population growth and natural selection in food limited environments: the *Drosophila* model. Am. Nat. 132: 786-809.

Mueller, L. D., 1991. Ecological determinants of life-history evolution. Phil. Trans. R. Soc. Lond. B 332: 25-30.

Mueller, L. D. & F. J. Ayala, 1981a. Trade-off between r-selection and K-selection in *Drosophila* populations. Proc. Natl. Acad. Sci. U.S.A. 78: 1303-1305.

Mueller, L. D. & F. J. Ayala, 1981b. Fitness and density dependent population growth in *Drosophila melanogaster*. Genetics 97: 667-677.

Mueller, L. D., J. L. Graves & M. R. Rose, 1993. Interactions between density-dependent and age-specific selection in *Drosophila melanogaster*. Functional Ecology (in press).

Mueller, L. D., F. Gonzalez-Candelas & V. F. Sweet, 1991. Components of density-dependent population dynamics: models and tests with *Drosophila*. Amer. Nat. 137: 547-475.

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

Partridge, L., 1987. Is acclerated senescence a cost of reproduction? Funct. Ecol. 1: 317-320.

Partridge, L. & Fowler, 1992. Direct and correlated responses to selection on age at reproduction in *Drosophila melanogaster*. Evolution 46: 76-92.

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*. Amer. 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*. Amer. Nat. 61: 289-318.

Pena de Grimaldo, E. & M. M. J. Lavoipierre, 1960a. Efecto de la fertilazacion sobre la ovopostura de los mosquitos *Aedes aegypti* variedad queenlandensis, con algunas observaciones sobre anomalias y viabilidad de los hevos retindos por los mosquitos esteriles fecundizados a diferentes intervalos despues de la comida de sangre. Rev. Iberica Parasitol. 20: 163-176.

Pena de Grimaldo, E. & M. M. J. Lavoipierre, 1960b. Longevidad de los mosquitos *Aedes aegypti* variedad queenlandensis fecundados y no fecundados, alimentados con sangre o privados de ella; y dejados en qyuno; o preveidos de aqua; de solucion de azucar; o de aqua y solucion de azucar. Rev. Iberica Parasitol. 20: 39-52.

Phelan, J. P. & S. N. Austad, 1989. Natural selection, Dietary Restriction, and Extended longevity. Growth, Dev. and Aging.

Pianka, E., 1972. *r*- and *K*-selection or *b* and *d* selection? Am. Nat. 106: 581-588.

Prout, T. & F. McChesney, 1985. Competition among immatures affects their adult fertility: population dynamics. Am. Nat. 126: 521-558.

Robertson, J. R. & G. W. Salt, 1981. Responses in growth, mortality, and reproduction to variable food levels by the rotifer, *Aspolanchia girodi*. Ecology 62: 1585-1596.

Rose, M. R., 1984a. Laboratory evolution of postponed senescence in *Drosophila melanogaster*. Evolution 38: 1004-1010.

Rose, M. R., 1984b. Evolutionary route to Methusalah. New Scientist 103: 15.

Rose, M. R., 1985. Life history with antagonistic pleiotropy and overlapping generations. Theor. Popul. Biol. 28: 342-358.

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

Rose, M. R., J. L. Graves & E. W. Hutchinson, 1990. The use of selection to probe patterns of pleiotropy in fitness characters. Chapter 2. In: Insect Life Cycles: Genetics, Evolution, and Coordination. Springer-Verlag. Berlin.

Rose, M. R., L. N. Vu, S. Park & J. L. Graves, 1992. Selection on stress resistance incrases longevity in *Drosophila melanogaster*. Exop. Gerontol. 27: 241-250.

Schoener, T., 1973. Population growth regulated by intraspecific competition for energy or time. Theor. Popul. Biol. 4: 56-84.

Service, P. M., 1987. Physiological stress mechanisms of increased stress resistance in *Drosophila melanogaster*. selected for postponed senescence. Physiol. Zool. 60: 321-326.

Service, P. M., 1989. The effect of mating status on life span, egg laying, and starvation resistance in *Drosophila melanogaster*. in relation to selection for longevity. J. Insest Phys. 35: 447-452.

Service, P. M., E. W. Hutchinson, M. D. MacKinley & M. R. Rose, 1985. Resistance to environmental stress in *Droso*-

phila melanogaster. selected for postponed senescence. Physiol. Zool. 58: 380-389.

Service, P. M. & M. R. Rose, 1985. Genetic covariation among life-history components: The effect of novel environments. Evolution 39: 943-945.

Slob, A. K., S. J. M. Vreeburg & J. J. van der Werff ten Bosch, 1979. Body growth, puberty and under nutrition in the male guinea pig. Br. J. Nutr. 41: 231-237.

Slobodkin, L. B., 1954. Population dynamics in *Daphnia obscura* Kurz. Ecol. Monog. 24: 69-88.

Tanner, J. T., 1966. Effects of population density on growth rates of animal populations. Ecology 45: 733-745.

Wigglesworth, V. B., 1949. The utilization of reserve substances in *Drosophila* during flight. J. Exp. Biol. 26: 150-163.

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