Natural Selection, Dietary Restriction, and Extended Longevity, by John P. Phelan and Steven N. Austad, Harvard University, Department of Organismic and Evolutionary Biology, Cambridge, MA 02138.

In a recent editorial, Harrison and Archer (1988) speculated that the observed life extension associated with dietary restriction in laboratory mice, *Mus musculus,* was a byproduct of natural selection working to extend reproductive life beyond periods of food shortage in the wild. They further hypothesized that in the longer-lived (in the laboratory) white-footed mouse, *Peromyscus leucopus,* natural selection has favored a longer life, which secondarily extended reproductive life, thus creating a fundamentally different adaptation to periodic food shortage.

We feel that these hypotheses raise intriguing issues about the linkage between reproduction and longevity. The evidence for such a linkage is overwhelming, not only for rodents but throughout metazoans. We feel however that a consideration of the details of natural selections's operation, combined with an appreciation of these rodents' lives in nature, argues against their hypotheses and for an alternative — life extension by dietary restriction is an incidental consequence of its effect on the timing and amount of reproduction.

Our first point is that because Darwinian fitness is *measured* by the relative number of breeding offspring produced by an adult, it may be thought of most usefully as always working on the timing and magnitude of reproduction. Other things being equal, increased reproductive longevity will always be favored, as will increased fecundity. The fact that other things (e.g. probability of accidental death, genetic tradeoff between current reproduction versus future expected reproduction) are not equal is what determines population and species differences in reproductive schedules and longevity. Therefore describing two evolutionary mechanisms, one affecting reproductive life span, the other total life span, seems to us an inaccurate representation of the evolutionary process.

An important related point is that reproductive senescence is largely irrelevant to life in nature. It is very rare to find postreproductive individuals in nature. If reproduction is considered to last until the end of parental care after the last reproductive episode, even human survival past the age of last reproduction is a recent common phenomenon in evolutionary terms. In the case of wild-type *Mus musculus* maximum reproductive age is about 670 days (Sacher & Hart, 1978). A variety of field studies (Brown, 1953; Berry 1968; Newsome, 1969; Tomich, 1970) indicates *M. musculus* median life span to be 130 days and average 90% mortality to be 279 days. Even if some finite fraction of the adult population live long enough to experience reproductive senescence, natural selection to extend reproductive life will be very weak. Analogously, field studies of *P. leucopus* show that in spite of their laboratory longevity, they have a short life in nature (median = 62 d, 90% mortality at 174 d — Snyder, 1956). Thus, if extending reproductive life requires delaying sexual maturity, life extension could easily be maladaptive. For instance, if sexual maturity occurs in *P. leucopus* at about 45 days and a single reproductive episode (gestation + lactation) takes about another 50 days, then an average mouse produces less than one litter in nature, and any delay in sexual maturity would substantially increase the probability of dying before reproduction.

We propose that dietary restriction extends life as a secondary consequence of its effect in delaying age at maturity and decreasing the subsequent rate of reproduction. If our hypothesis is valid, then dietary restriction should extend life in a wide variety of species irrespective of whether sporadic bouts of resource shortage are part of the species' ecology. Indeed, life extension by dietary restriction has been demonstrated in a wide range of invertebrate and vertebrate taxa. In addition, our hypothesis predicts that dietary restriction will have the largest impact on species with early and copious reproduction and the smallest on species with late sexual maturity and comparatively small energetic investment in reproduction. We note in passing that *P. leucopus* matures somewhat later than *M. musculus* (45 vs 35 d — Sacher & Hart, 1978), and that total weight of a weaned litter is about the same in both species (34 vs 32% of maternal weight, respectively (Svihla, 1932; Pelikan, 1981). If both species produce the same number of litters per year, we predict that the life-extending effect of dietary restriction will be roughly equivalent of wild-type *M. musculus* and *P. leucopus.*

Harrison and Archer's consideration of the impact of natural selection in the context of the ecology of a species in the wild is a laudable attempt to facilitate thinking about comparative animal longevity. Fortunately, their hypothesis and ours make mutually exclusive predictions about the effect of dietary restriction in Peromyscus. We hope the requisite research to distinguish between them is forthcoming.

References

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Response 1 by D.E. Harrison and J.R. Archer.

Phelan and Austad clearly show how basic Darwinian fitness operates. We did not mean to imply that there are two separate evolutionary mechanisms, one affecting reproductive lifespan and the other total lifespan. Due to the fact that evolution operates only on "passing along genes", we hypothesize that there may be important linkages between the *physiological and biological* mechanisms controlling female reproductive lifespan and those controlling longevity.

We agree that reproductive senescence is usually irrelevant to life in nature, but we argue that this is not always true. In fact the main point of our ideas is that occasional events, catastrophes, can give a temporary but enormous selective advantage to females able to reproduce later in life.

The comparison of life spans in the wild indicates that wild *Mus musculus* normally live at least as long as wild *P. leucopus*; their ages of sexual maturity and time required for a reproductive episode also appear similar. These data show the importance of a question that we tried to answer: why does *P. leucopus* have the potential to live more than twice as long as *Mus musculus*?

Finally, we agree that the correct test of our ideas lies not in argument, but in the laboratory. We agree that it is critically important to determine whether dietary restriction retards aging equally well in long and short-lived species. The suggestion that dietary restriction extends life as a secondary consequence of delaying growth and development was made by McCay *et al.* (J. Nutr. 10:63-79, 1935); this is similar to the proposal by Phelan and Austad that dietary restriction extends life as a secondary consequence of delaying age of maturity and decreasing the subsequent rate of reproduction. This idea has been challenged by findings that dietary restriction begun at 6 months of age was as effective as that begun at 6 weeks in rats (Yu *et al.*, J. Gerontol. 40: 657-70, 1985), and that there were substantial benefits in mice from dietary restriction begun at 12 months of age (Weindruch and Walford, Science 215: 1415-8, 1982).

We end with a practical consideration. Genetically defined mice (inbred strains and F_1 hybrids of two inbred strains) are convenient in aging studies, as their life spans, pathologies, and responses to food restriction are reproducible. They are readily available for *Mus musculus*, and are being produced for several Peromyscus strains. Is there any reason why genetically defined Peromyscus mice should not be used in testing whether their aging rates are retarded by food restriction? We believe that this would be satisfactory as long as several different genotypes were tested.

Response 2 by S.N. Austad and J.P. Phelan.

The occasional events or catastrophes which you posit give a temporary, but enormous, selective advantage to females able to extend their reproductive lives seems plausible in a general sort of way, even if females only rarely live long enough for reproductive senescence to be a factor. But our point was that unless such an adaptation is "cost-free", that is it negatively affects no other life history parameters, then the "occasional events" would have to be of a rather special sort (in terms of their frequency, severity, and duration, for instance), for the adaptation to spread. To choose one parameter, if the catastrophes were too infrequent (or too frequent!), the adaptation would be disadvantageous. If the life extending effect of dietary restriction is a general mammalian phenomenon, it strikes us as unlikely that such special sorts of catastrophes occur across all species.

Second, we have strong feelings about the notion of using genetically-defined Peromyscus (or F_1 hybrids) to test hypotheses about the adaptive nature of any trait. Inbreeding, as you know, often severely decreases reproductive capacity and physiological efficiency, and breaks down (or even reverses) genetic correlations among traits. Therefore even though F_1 hybrids of inbred strains may outlive either parent strain or even approach the longevity of wild-types, such hybridization does not restore lost linkage groups which were the product of natural selection. To the extent, then, that reproductive capacity, genetic correlations, and coadapted gene complexes affect the phenomenon in question (as we posit they might), the study of inbred strains might be seriously misleading. After all hybrids are not adapted to any environment. Inbreeding may even alter the age-specificity of fundamental physiological processes. For instance, did you notice the pair of papers by Cohen, *et al.* (J. Gerontol. 42:295ff), in which C57BL/6J mice show more rapid wound healing when young than when mature or old, whereas two species of wild-type Peromyscus show most rapid wound repair in aged individuals?

This second point is not a suggestion to abandon work on inbred mice, of course. The experimental utility of genetically uniform strains has been proven many times

over. However, for evaluating adaptive hypotheses, animals recently subject to natural selection seem to us by far the most suitable.

Response 3 by D.E. Harrison.

As you correctly point out, the catastrophe hypothesis that Jon and I suggested would not be needed if food restriction extends mammalian lifespans in a similar fashion regardless of the longevities of the mammals concerned. However why would such a response to food restriction have evolved? Probably it would not be a direct result of evolution, but a secondary result of some basic mammalian biology.

This is what many gerontologists think, and some even suggest that food restriction as an antiaging treatment in man. However efforts to define how food restriction retards or resets a fundamental aging clock have thus far been inconclusive. We suggsted an alternate hypothesis because it demonstrated the importance of determining whether food restriction actually extends longevities in long lived species of mammals. To our delight, the hypothesis also explained the different lifespan potentials between Mus and Peromyscus, despite their similar lifespans in the wild. Your cogent criticisms caution us not to become too fond of our hypothesis; indeed the purpose of any hypothesis is to be rigorously tested. I wish that testing it wasn't such a slow and expensive process.

Your argument seems sound that animals recently derived from the wild should be used to test adaptive hypotheses, since they retain the "adapted" gene groups. Yet beneficial effects of food restriction have been repeatedly demonstrated in many different inbred and F_1 hybrid Mus mice. Furthermore, I am not aware of any data supporting the point that wild type Mus mice outlive healthy, long-lived inbred strains like C57BL/6J (B6) and CBA/CaJ. In one laboratory in which both were simultaneously compared (George Sacher's), B6 mice had greater mean and maximum longevities than wild mice, as well as much slower collagen aging. I suspect that in wild-derived mice, gene complexes that allow quick reactions to avoid predators cause stress due to the small cages and inability to escape. Thus laboratory mice outlive wild-derived ones since the former are "domesticated" — adapted to live and breed in laboratory conditions. However the evidence seems to indicate that such adaptation does not remove the effects of food restriction in extending longevity. Perhaps maximum longevities will only occur in domesticated mice. Are you aware of evidence disproving this?

Response 4 by S.N. Austad

I hope it is obvious that I am very sympathetic to your introduction of the ecology of various species into discussions about their longevity. I just question whether intermittent food shortage of the requisite sort is as widespread in nature as the presumed ubiquity of dietary restriction would suggest. Of course, I also suspect the ubiquity of the phenomenon is exaggerated, and I would be curious to know to what extent dietary restriction impacts species with low reproductive rates (like bats, arboreal phalangeroid marsupials, most large mammals, and humans). All these species are exceptionally long-lived and so the work will probably not be done, which is a shame.

My point about using "wild" animals to test hypotheses about adaptation had not so much to do with them being longer lived than various inbred strains (although I didn't know that some laboratory strains outlive wild-types in captivity), but that we don't know to what extent they are the same animal, physiologically, due to incredible homozygosity and unusual linkage groups. There is a body of evidence from natural populations that Darwinian fitness in organisms from spruce trees to white-tailed deer to oysters is positively correlated with individual heterozygosity level. Certainly that could have major consequences for maximum longevity. All-in-all, then, combining the ecology of wild animals with the physiology of inbred captives to evaluate adaptive hypotheses worries me.