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THE EVOLUTIONARY ECOLOGY OF SENESCENCE IN *DAPHNIA*

by

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ABSTRACT

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The evolutionary theory of senescence predicts that high extrinsic mortality in natural populations should select for accelerated reproductive investment and shortened lifespan. I examined the theory with natural populations of the *Daphnia pulex-pulicaria* species complex, a group of common freshwater crustaceans that spans an environmental gradient of habitat permanence. I document substantial genetic variation in life history traits among populations of this complex. Populations from temporary ponds have shorter lifespans, earlier and faster increases of intrinsic mortality risk and earlier and steeper declines in fecundity than populations from permanent lakes. I also examine the age-specific contribution to fitness, which declines faster in populations from ponds than those from lakes. Pond *Daphnia* also exhibit faster juvenile growth and higher early fitness, measured as population growth rate (r). I observed negative genetic correlations between r and indices of life history timing, further suggesting tradeoffs between early- and late-life performance.

Understanding the evolution of senescence in nature requires knowledge about genetic variation and ecological relevance of senescence plasticity because senescence is responds to factors such as food and temperature. Therefore, I also examined plasticity of senescence and variation of that plasticity within and between two species complexes of *Daphnia*. I quantified senescence in four environments (crossed high and low temperatures and food levels), to evaluate the plasticity of mortality and fecundity with respect to the ecology of the species' habitats. Senescence was highly plastic, but species were plastic to

different degrees. Population growth rate (r) was most responsive to food, but senescence was most responsive to temperature. Temperature strongly influenced the degree to which genetic differentiation within complexes was expressed, with little differentiation under temperature stress. In the *D. pulex-pulicaria* complex, whose species use markedly different habitats, genetic variation of senescence was strong and robust across environments. Species of the *D. mendotae-dentifera* complex, which inhabit stratified lakes, had similar senescence patterns. Most genetic variation of senescence occurred within, rather than between, species complexes, indicating that divergence of senescence in *Daphnia* is recent.

I further tested for a relationship between extrinsic mortality risk and ecological distribution of senescence variation by measuring natural demographic rates (growth, birth and death) in twelve populations of D. pulex-pulicaria. This work showed a negative relationship between ecological mortality risk and investment in late-life fitness. By constructing a phylogeny of these populations based on mtDNA, I was able to show that variation of senescence is not easily attributed to simple genetic history. My results cannot be explained by a tradeoff between survival and fecundity, nor by non-evolutionary theories of senescence. Instead, the data support the evolutionary theory of senescence because the genetic variation in life histories we observed is congruent variation of death rates experienced in the wild.

to a fry-cook on Venus and
a young man who didn't think he'd
seen anything good that day

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CHAPTER ONE: INTRODUCTION

MICROCRUSTACEA AS A MODEL OF AGING

The science of geriatric medicine is centrally concerned with the process of human aging, with the goal of alleviating its detrimental effects. Crustaceans may not seem to be an obvious choice as a medical model, but I will argue that there are important fundamental questions for which they are ideal. In this chapter, I discuss the evolutionary biology of aging and how certain crustaceans could be used to integrate it with our understanding of the physiological effects of aging. This is necessarily a "looking forward" chapter, because currently little work with crustaceans is related to the biology of aging, let alone geriatric medicine.

Any sensible approach to science begins with clearly identifying questions we wish to answer. Considering what we wish to know about aging and why we wish to know this is the first stage of choosing a model organism. How does the desired information mesh with the already substantial body of knowledge which we have accumulated? These considerations lead to the potential for crustaceans, particularly aquatic microcrustaceans, to be added to the relatively narrow group of model organisms used to study the process of aging.

The first part of this chapter is devoted to defining some questions about the evolutionary biology of aging which are either wholly unanswered or which could profit from taxonomic expansion beyond current model systems. A good general overview of the evolution of aging can be found in Rose (1991). The second part addresses the relative merits and demerits of a few microcrustaceans in the context of these questions. I pay particular attention to introducing aspects of the basic biology of these organisms with which the reader may not be familiar and the relevant aspects of basic evolutionary biology. The third part

briefly reviews the scant direct knowledge of microcrustaceans and aging. I close with a discussion of fundamental tools which should be developed for microcrustacea and the research potential for microcrustaceans in the field of aging.

What is the relationship between nature and the evolution of aging?

This is a broad, overarching question that is largely unaddressed by empirical information. Substantial theory exists to explain the evolution of aging (Williams 1957, Hamilton 1966, Edney and Gill 1966, Charlesworth 1993), and many key predictions and assumptions have withstood laboratory tests. However, the careful control of the laboratory is a far cry from the complexities of nature. Examining the evolution of aging in nature is important because it will reveal whether or not there are critical details which have not been considered in laboratory and theoretical work. Finding a discrepancy between nature and current knowledge makes possible a search for the cause of that discrepancy. Finding no discrepancy reassures us that we are on the right track, a particularly important reassurance for geriatric scientists since their ultimate subject is a naturally evolving species.

A broad component of the relationship between nature and the evolution of aging is *how aging evolves in nature,* which itself has several subsidiary questions. How much genetic variation is generally present for patterns of aging? How closely is the natural selection on patterns of aging coupled to the phenotypic expression of aging? What are the dominant forces (e.g., predation, disease, food availability, habitat permanence, mate choice, parent-offspring conflict) exerting selection on aging? How do these forces operate? What are the relative impacts of fecundity selection and mortality selection? What are the

trade-offs associated with patterns of aging, and how does selection act on those trade-offs (thus indirectly influencing aging)? What constraints are there on the evolution of aging patterns? How does the aging pattern evolve if there is temporal or spatial variation in the strength, direction, or type of selection?

It may be difficult to see the medical applications of answers to these questions, and indeed they may be subtle and indirect. However, this is not to say they are unreal or unimportant. In a human context, consider attempts which may be made to mitigate the detrimental effects of aging. Understanding the trade-offs which selection has created may suggest focused research on potential unwanted side-effects, particularly those which appear only in subsequent generations, either via genetic or maternal effect. Or, it could be that humans have substantially altered the influence of selection on their own patterns of aging, in which case it may be difficult to modify a phenomenon which is in a state of evolutionary flux. Exploring how natural selection acts on the integrated whole of an organism to shape the evolution of aging will afford us a greater understanding of how the different aspects of bodily deterioration are interrelated; a better understanding of the potential repercussions on intervening in aging will be the result.

Natural selection is not the only component of the relationship between nature and the evolution of aging: aging could also affect an organism's ecological interactions. Ecologists have largely (though not entirely, and it is changing) assumed aging to be a minor force in ecological interactions and have rarely looked for it, and have even more rarely explored its impacts. This is not wholly unreasonable, given the extraordinary difficulty of observing aging in the wild, and the observation that most populations are largely unsheltered from extrinsic mortality — hence, few wild animals die of old age. Some aspects of

ecological interactions, however, such as mating behavior and thermotolerance, have received some attention in a laboratory context.

Historical attitudes notwithstanding, it is still true that ecological aspects of aging are worthy of study, and that their study in conjunction with evolutionary biology is especially apropos. Good evolutionary biology is intertwined with ecology, just as good ecology is intertwined with evolutionary biology. The ecological context of aging is critical, though largely unknown from an empirical perspective, because it is primarily responsible for the natural selection exerted on aging. It is also important because humans are involved in ecological interactions, even though this concept is rarely emphasized. Understanding our own aging, therefore, requires an understanding of the ecological impacts and the ecological context of aging. For example, as organisms age, they may become more susceptible to disease, in turn having an impact on their ability to provide for offspring. This is an extension of the expression of aging beyond that which occurs in the body. The fact that we humans are part of an ecological system is probably relevant to many other areas of human biology. It is particularly relevant in terms of aging because we are the one species most likely to experience substantial aging, thus we are also the species in which aging is likely to have the most substantial impact on our ecological interactions. However, a variety of reasons, particularly the ability of researchers to control the accumulated experiences of an experimental subject, force us to seek model organisms.

How does the relationship between aging and the environment integrate into our physiological understanding of aging?

Current well-developed models of aging have been focused on understanding the genetic and developmental mechanisms involved in aging (e.g., *Drosophila, Caenorhabditis elegans*) or an understanding of particular physiological processes (e.g., mice). Due to limitations of these models, such as an inability to evaluate their ecological context or a generation time which inhibits thorough demographic work, it is unlikely that they will be useful for integrating our understanding of the genetic, physiological and developmental aspects of aging with the perspective of ecology. This integration is important, because through it we will be able to understand the causes of the complex web of interactions and trade-offs underlying physiological, developmental, biochemical and other types of traits which degrade as an organism ages. Therefore, if we are choosing a new model system for aging specifically to integrate the ecological perspective, it is imperative that we are still able to evaluate the traits of the organism that are the foundation of our interest in aging.

Essentially, this question is about bridging the interests of the gerontologist to the interests of the evolutionary biologist. To the gerontologist, aging is a property of individuals, a deterioration of the functioning of the body. To the evolutionary biologist aging is a property of populations, a demographic phenomenon embodied by declining average fecundity and survival probability. A clear understanding of how these two levels, the individual and the population, are linked is important for a thorough understanding of either level.

The merits and demerits of microcrustaceans

Consider the general properties that make an organism amenable to laboratory studies in evolutionary biology. First, the organism needs to be fairly

easy to maintain in the lab. This means that the organism should be small, so that many individuals can be packed into limited lab space. It also means that the environmental conditions required need to be well known and straightforward. Furthermore, for evolutionary biology, it is desirable to have high fecundity (enabling the researcher to generate adequate sample sizes). Second, the organism should have a short generation time. This is because many types of investigations will be improved by a multigenerational outlook. Also, it is becoming more and more apparent that maternal effects (aspects of the maternal environment/genotype which have an influence on the phenotype of offspring) must be controlled for, and preferably incorporated into studies. Thus, several generations of lab acclimation (and perhaps also acclimation to specific experimental conditions) may be important. A short generation time is usually correlated with a short lifespan, a feature which will be particularly useful to studies of aging. Third, it is always helpful to choose a study organism for which there is a substantial amount of accumulated knowledge. This allows one to proceed more directly to questions of interest, and allows one to support reasonably the assumptions of an experiment and to define the conditions across which results can be generalized.

Another property of model organisms that would be important for studies of aging in particular is the ability to assay aging. This is incorporated in the general properties if we want to use a demographic index of aging (which is the main interest of the evolutionary biologist). However, particular physiological features usually are of interest, such as oxygen consumption, stress tolerance, disease resistance, or locomotive ability. This is especially true from the gerontologist's perspective. In developing a model system for aging, these should be taken into account, because they allow relating the aspects of bodily deterioration directly to evolution. In fact, we would ideally want an organism

that has an analog for as many of the characteristics of aging in humans that are important as possible. A property which will facilitate assays of aging is iteroparity (multiple reproductive events per lifetime). This is because the portion of lifespan over which aging can be expected in animals which reproduce once and then die is so compressed that making meaningful observations of the process would be difficult.

Crustaceans represent an opportunity to more fully integrate ecology into our knowledge of aging (Reznick 1993), something that would be very difficult with currently widespread model systems. There are several candidate crustaceans for research in the evolutionary biology of aging. The basic considerations of laboratory amenability, short generations and high fecundity lead to the microcrustaceans, aquatic (fresh and saltwater) crustaceans of the groups Copepoda and Cladocera. I focus on the family Daphniidae (Cladocera) because they are the family for which there is the most substantial body of knowledge which is pertinent to investigations of the biology of aging.

Microcrustaceans are small (usually <4mm), poikilothermous arthropods with well-developed circulatory, respiratory, neurological, digestive, reproductive and muscular systems. In addition, there is tremendous diversity in these physiological systems, necessary to cope with the diversity of habitats in which they are found. For example, herbivores, omnivores and detritivores can be found in the family Daphniidae; digestive systems must vary to assimilate the different types of food. Variation in respiratory function is necessitated because the oxygen concentration in water can vary widely. *Daphnia pulex*, a common North American species, is known to have reproductive modes including obligate asexuality, hermaphroditism, and sexuality in the wild; this presents an unparalleled (and unexplored) opportunity for genetic control. Members of the family Daphniidae are particularly well suited to physiological and

developmental biology because they are transparent and many intraorganismal processes can be observed with nothing more than a light microscope. A large body of physiological and developmental information has been generated (Peters and de Bernardi 1987), although it has not been applied to aging.

Daphniidae have been the subject of research (ecological, developmental and physiological) since the mid-1800's. In that time, they have become as much a model organism in ecology as there exists. In particular, they have been the subject of work on population biology, community ecology and life history studies. Culture methods are well-established (Goulden, et al 1982, Peters 1987), based on a good understanding of nutrition (Lampert 1987), and have been defined with sufficient precision for *Daphnia* to become one of the standard bioassay tools of ecotoxicology. They (and all other microcrustaceans) grow in a series of instars, developmental stages punctuated by molting of the carapace. The infrastructure required to support culture of daphniids is not significant, and maintenance of cultures is fairly inexpensive. Source populations can be found in most lakes and many ponds. They have a short generation time (first reproduction can occur as early as 5 days) and their lifespan can probably range from 20-200 days, depending on species, population and environmental conditions. The importance of having a model organism for which there is a great deal of variation in lifespan (and, presumably, rates of aging) cannot be overemphasized: it is the examination of variation that permits scientific explanation of causes and consequences.

In addition to their general acceptability, Daphniidae have a number of particular qualities which affect their usefulness in the evolutionary biology of aging. First, and vital in terms of the questions delineated earlier in this chapter, is the tremendous background of ecological knowledge about Daphniidae and the potential to link it to a substantial body of physiological knowledge. This is

absolutely critical for understanding how natural selection has shaped aging, and is not a feature of animals which are commonly used as models of aging, such as *Drosophila* or *C. elegans*. Importantly, the type of ecological knowledge about daphniids we have is appropriate to selection on aging. The forces of selection which shape aging are those which alter the relative fitness value of particular age classes. This means that the natural risk of mortality must be understood, and furthermore, that understanding the age-specificity of the forces of mortality will enable a more refined approach to studying aging. For daphniids, the factors which cause extrinsic mortality are well known (Threlkeld 1987): impacts of numerous predators (and their size-specificity) have been quantified, the temporal changes of abiotic factors which kill daphniids (anoxia, pond-drying) are easily measured, and there is currently a resurgence of interest in the microparasites (i.e., diseases) of *Daphnia* which should prove important.

There are approximately 40 species of *Daphnia* in North America, and perhaps 125 world-wide; this is the most well-studied genus of daphniids, and one of the most well-studied microcrustaceans. Not all species are equally well known, nor has one particular species become the leading research subject, although there are a handful of species which receive the bulk of attention. This taxonomic diversity should not be viewed as a drawback, though, because it is accompanied by ecological diversity. This, combined with emerging knowledge of their evolutionary relationships, makes the genus a good candidate for comparative studies of aging. Different species of *Daphnia* exist along a range of selection pressures on aging, and should exhibit different patterns of aging. This would then be the raw material for comparing particular aspects of the physiological and biochemical mechanisms of aging. These comparisons can be supported by the growing knowledge of the evolutionary relationships among the species, genera and families of Cladocera.

One of the most valuable ecological tools for understanding the evolution of aging is not our knowledge of what might kill *Daphnia*, but our ability to quantify mortality easily in a natural population (Threlkeld 1987). Slightly more complicated, but also well-developed, are methods of determining size (≈ age) specific mortality risk. This allows us to move beyond simple descriptions of how selection should be influencing aging in a population, to measuring the selection a population is currently experiencing, thus freeing one from the nagging feeling that something else important is being overlooked. If we are willing to make the assumption that current selection reflects past selection, we can draw conclusions about how the current phenotype has evolved. This critical assumption can be considered in light of our knowledge of the factors which cause *Daphnia* death in order to evaluate it.

The second major aspect of daphniid biology that is relevant to research in the evolutionary biology of aging is their unusual mode of reproduction. Daphniids are cyclic parthenogens, animals which typically reproduce by ameiotic parthenogenesis (cloning of females) punctuated by generations of sexual reproduction (Hebert 1987). This is the basis of the ease of working with them in the lab, because large numbers of isogenetic females can be naturally produced. Thus, it becomes easy to segregate environmental and genetic influences on a particular trait. In the lab, it is usually simple to set conditions under which parthenogenesis is the sole reproductive mode. Juveniles develop directly into adults, and the timing of development can be adjusted with food and temperature. Historically, breeding has been difficult due to a required period of diapause for sexually produced eggs. Now, though, this presents only a delay in hatching offspring, opening up the potential for elaborate breeding designs involving cloning, selfing, inbreeding, outbreeding — even interspecific hybridization is possible.

The unusual reproductive mode creates opportunities unavailable in other organisms (How does maleness or femaleness influence aging? With daphniids, genetically identical males and females can be produced to explore this), but it also could be problematic for some types of questions. One unique advantage is that the naturally occurring genetic diversity can be captured and maintained in the lab, without resorting to methods which may induce inbreeding depression or substantial change of variation. An equally important advantage is the ability to explore the effects of a particular agent (a vitamin, a temperature, a hormone, a predator) on a particular genotype. A potential difficulty is that some natural lineages undergo large numbers of asexual generations (particularly obligately asexual lineages) leading to generalized mutation accumulation, a complication if one is interested in the genetic mechanisms by which aging evolves in *Daphnia.*

There is one glaring aspect of *Daphnia* biology that makes them less than wonderful for aging research. This is the lack of a strong body of molecular genetic and biochemical knowledge about this system. Although there is a wide literature on the population genetics of *Daphnia*, based on allozyme characters, and soon to be DNA characters, *Daphnia* simply have not been used in molecular genetics. Historically, this probably was due to the inability of early geneticists to find any phenotypic traits of *Daphnia* which have simple mendelian inheritance (this was not for lack of trying) in combination with the difficulty of breeding them. To my knowledge there are no known genes in *Daphnia*, they have not been transformed, and are undeveloped as a system for genetic manipulation. Even the number of chromosomes present in certain species is in dispute. Furthermore, although there is a reasonable amount of whole-organism physiological knowledge, this has not led to extensive exploration of molecular biology and biochemistry. These have been the general directions aging research is proceeding in other organisms, and *Daphnia* may not be able to catch up. This

is not to say they are not worth developing, though. *Daphnia* are more suitable than current models for the major questions outlined above, and there is greater potential for developing a robust molecular genetic knowledge of *Daphnia* than there is of developing a robust ecological knowledge of, say, *Drosophila*.

One other, relatively minor, drawback to working with *Daphnia* is that as ideal for lab studies as they are, sample sizes of 100,000 animals (which have recently been used in *Drosophila*) are unrealistic for *Daphnia*. Not only would this be very labor intensive, but space would be a problem. This is because as *Daphnia* grow (from 0.5 to 3.5 mm in some species, but only to 1.2mm in others), the volume of water in which they are kept must increase exponentially (due to their feeding behavior) in order for the juvenile and the adult to experience the same environment. Thus, not only are they slightly larger than other model organisms, but the mechanism by which density affects them requires a greater-than-linear increase in space relative to their growth.

Knowledge about aging in microcrustaceans

There have been rather few studies examining aging in daphniids, and those that do exist typically report average lifespans of several clones under the same conditions, or of a single clone under several conditions. The most complete studies are from the 1930s, when A. M. Banta and his associates made several careful studies of aging in *D. longispina*. These studies are all the more intriguing because they occurred before the theoretical explanation of aging was developed, and before modern *Daphnia* culture methods became established (they used Banta's Manure Infusion as a food source).

In one study (Wood, et al 1939a), they were simply observing growth and reproduction over the lifespan of a single clone, at 25°C and "unlimited" food.

But their *hourly* observations over the ≈30 day lifespan illuminate life history changes that occur during *Daphnia* senescence. While "young" (until the ninth instar), instars typically lasted 38 hours, and ended with the release of young followed within minutes by molting. As animals aged, instar duration lengthened and became irregular, and was decoupled from the release of offspring (by up to 2 days). Clutch size also decreased as animals aged, and a post-reproductive sterile period roughly equal to 1 instar was observed. Furthermore, they report that animals "gradually become sluggish in their movements and may finally rest on the substratum," a phenomenon I have observed in four additional species.

Another study examined growth patterns of 6 different clones (inbred in the lab), and is probably the first to report genetic variation of lifespan (Wood, et al 1939b). In general, slow growth was associated with longer lifespan, but all 6 clones had shorter lifespans and were smaller (and usually slower growing) than the standard, natural, clone used in the previous study. Other studies by the same group showed that semi-starvation increased longevity, lowered heart rate and reduced fecundity (Ingle 1933), but that semi-starvation followed by good feeding increased longevity further while restoring lifetime fecundity to that of individuals well-fed throughout their life (Ingle, et al 1937).

A separate lab worked on the influence of temperature on lifespan in *D. magna*, showing that a 10° increase approximately halved lifespan (MacArthur & Baillie 1929a) and that metabolic rate (as indexed by heart rate) was inversely proportional to lifespan (MacArthur and Baillie 1929b). Thus, by the end of the 1930s, temperature and food level, two of the most important environmental influences on lifespan had been demonstrated in *Daphnia*. These early studies are the only microcrustacean studies focused on aging, and only rarely since has lifespan even been reported in the large body of zooplankton life history

research. Korpelainen's (1986) data confirms the effect of temperature in *D. magna*, and that there is naturally occurring genetic variation in the response to temperature among 5 clones. She also includes data on males of the same 5 clones, and the variation among their temperature response differs from that of the females. Furthermore, she shows a similarly complex pattern of influences of photoperiod on lifespan. None of these studies have explored the environmental conditions which would have selected for the observed phenotypes. Very recent experiments are starting to change that. Substantial genetic variation among populations and species has been shown for four species, which, at least at the species level, appears to be related to natural selection (Dudycha, unpublished data).

Prospectus: Developing microcrustaceans as a model of aging

The current utility of microcrustaceans for the evolutionary biology of aging is primarily in the comparative method. Comparative biology has not been an important component of evolutionary aging research, but it presents the best opportunity for understanding the process in nature. Daphniids appear to be an ideal organism for this research, because there is a substantial range of ecological variation within the family, including situations where evolutionary acceleration and deceleration of aging is predicted. They also present a unique opportunity where one can quantify naturally-experienced mortality risks (selection) and rates of aging, as assayed by a wide variety of indices, under specified conditions in a genetically controlled fashion. Relevant ecological variation also occurs within species among populations, but comparisons may not be straightforward due to factors such as gene flow or plasticity, both of which tend to dampen evolutionary change. Overall, by applying the

comparative method to daphniids, we should be able to explore whether natural patterns of aging are associated with particular environmental influences. More specifically relevant to geriatrics, the subsequent ability to disentangle the suite of traits which make up bodily deterioration will be enhanced through an understanding of the components of natural patterns of aging and the mechanisms by which they are influenced.

The future utility of *Daphnia* could include manipulative experiments if a "genetics toolbox" is built as a foundation. The power of *Daphnia* as a model organism would become truly impressive: it would encompass genetics, molecular biology, development, physiology, demography, ecology and evolution. The ability to identify particular loci that affect aging in *Daphnia* would lead to the potential for describing natural allelic variation and its relationship to the selective environment. The ability to manipulate particular loci would enable experiments that investigate the mechanisms whereby aging affects ecological interactions. These two distinct avenues of research could greatly illuminate how aging of individuals in nature affects and is affected by interactions with other organisms. Finally, we would have a model system where aging could be explored throughout the biological hierarchy, guiding us in our attempts to understand the aging of humans.

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CHAPTER TWO: NATURAL GENETIC VARIATION OF LIFESPAN, REPRODUCTION AND JUVENILE GROWTH IN *DAPHNIA*

with A. J. Tessier

Senescence is a post-maturation decline in the physiological state of organisms as they grow older. From an evolutionary perspective, this decline is most relevant as a decrease in age-specific rates of survivorship and fecundity. The evolutionary theory of senescence (ETS) argues that the ultimate cause of senescence is a decline in the force of selection as organisms age (Medawar 1952; Williams 1957; Hamilton 1966; Charlesworth 1980). The force of selection declines because phenotypic effects at later ages influence smaller and later portions of reproduction relative to early phenotypic effects. Furthermore, these later effects are not exposed to selection in individuals who die before expression. Populations facing a greater risk of extrinsic mortality have a stronger decline in the force of selection and greater senescence should evolve in them. ETS has substantial support from laboratory experiments that have tested predictions about the response to selection on late-life performance (e.g., Rose & Charlesworth 1980; Mueller 1987; Partridge & Fowler 1992; Zwaan, et al. 1995a). Tests of critical assumptions, including the existence of mutations with age-specific effects (Pletcher, et al. 1998) and age-specific patterns of genetic variance in mortality (Promislow, et al. 1996) and fecundity (Tatar, et al. 1996) also support ETS. Research has focused on understanding and discriminating among the genetic mechanisms that permit the evolution of senescence (e.g., Rose and Charlesworth 1981; Rose 1984; Partridge and Fowler 1992). However, ascertaining the relative contributions of the non-mutually exclusive genetic mechanisms will not illuminate whether the general theory adequately explains

the wide diversity of senescence patterns found in nature. Relatively little work has examined the ecological context of senescence evolution (Reznick 1993; Tatar, et al. 1997; Dudycha, in press).

Tests of ETS by artificial selection on stock laboratory populations have dealt with situations where selection is direct and constant. Natural systems may be more complicated due to indirect and changing selection pressures (Abrams 1991, 1993), but in general, we expect higher extrinsic mortality to select for greater senescence (Williams 1957; Hamilton 1966; Edney and Gill 1968; Charlesworth 1980, 1993). However, if the mortality falls predominantly on juveniles, reduced senescence is expected because adults are evolutionarily more valuable (Hamilton 1966; Charlesworth 1980; Abrams 1993). Although there is no general reason to expect that the relative magnitude of juvenile versus adult mortality is correlated with the absolute magnitude of extrinsic mortality in nature, they are often confounded in selection experiments (Clark 1987). Spatial or temporal changes in factors exerting mortality in nature may shift the net direction of selection on senescence through changes in the age-specificity of mortality or changes in the total level of mortality. It is not clear that the success of ETS in explaining senescence evolution in relatively straightforward laboratory settings will translate into success in explaining senescence as it has evolved in the wild.

Comparisons among taxa have suggested that maximum or mean lifespan, which represent only one aspect of senescence, are consistent with ETS. The ability to fly, a trait that presumably reduces extrinsic mortality risk, is associated with longer maximum lifespan in mammals (Prothero and Jurgens 1987) and birds (Holmes and Austad 1995). Keller & Genoud (1997)

demonstrated a cross-taxon association between social system and estimated mean lifespan of female ants. They argued that in captivity, queens live longer than reproductive females from solitary taxa because queens' lifespans have evolved in a relatively protected colonial environment. A recent analysis of avian and mammalian mortality schedules (Ricklefs 1998) moves beyond lifespan and shows, under reasonable extrinsic mortality assumptions, that acceleration of adult mortality rate is positively related to the extrinsic mortality risk faced by young adults. These studies used organismal traits to approximate vulnerability to extrinsic mortality and estimate risk based on those approximations. One study (Tatar, et al. 1997) has looked at an environmental axis to make predictions based on exposure to mortality risk. Tatar, et al. (1997) showed that, when raised in a common environment, male grasshoppers from low elevations (i.e., a long season) live longer and have slower increases in age-specific intrinsic mortality than those from high elevations.

Complete life table data are difficult to acquire, particularly when quantifying senescence, because one normally needs to shield individuals from extrinsic mortality (but see Promislow 1991; Ricklefs 1998). This difficulty places limits on the interpretation of comparative studies, which have usually relied on summary statistics such as the maximum or mean lifespan. First, senescence is a non-linear change over time whose trajectory can vary in shape and magnitude. Simple summary statistics may not capture this variation. Second, mortality is only part of the fitness equation. Testing predictions of ETS has often yielded supportive data, but many empirical reports (e.g., Curtsinger, et al. 1992; Carey, et al. 1992; Tatar, et al. 1993) have revealed patterns of mortality not completely predicted by ETS. The unexpected patterns may be due in whole or in part to shortcomings of the theory (Blarer, et al. 1995; McNamara and Houston 1996;

Pletcher and Curtsinger 1998), or it may simply be that the expected senescence pattern was expressed in unmeasured traits. Reproduction must be included for an accounting of fitness reduction due to senescence because age-dependent changes in a tradeoff between survival ability and reproduction may cause declines in either ability (Partridge and Barton 1993, 1996). We are unaware of comparative work specifically incorporating reproductive declines that evolved in nature.

Objectives of this Study

We compare senescence patterns in closely related taxa living across a known gradient of mortality risk. First, we quantify the magnitude and distribution of natural genetic variation in senescence using full mortality and fecundity data collected under standard conditions that shield the animals from extrinsic mortality. Second, we look for tradeoffs (negative genetic correlations) between composite indices of senescence and early-life performance. Such tradeoffs are a mechanism by which rapidly senescing genotypes could persist in the face of invasion by long-lived genotypes and provide a framework for understanding how senescence is integrated into the total life history. Third, we consider the potential for further evolution of senescence in nature by examining differentiation of senescence among populations within species. We focus on multiple populations of the species complex *Daphnia pulex-pulicaria*, a group of freshwater microcrustaceans well-suited to studies of senescence and ecology (Bell 1984; Reznick 1993; Dudycha, in press).

Daphnia *and the Pond-Lake Gradient*

The taxonomic status of *Daphnia pulex* and *D. pulicaria* is controversial. Two recent mtDNA phylogenies conclude that *D. pulex* and *D. pulicaria* are not distinct clades (Lehman, et al. 1995; Crease, et al. 1997). However, the most recent taxonomic revision (Hebert 1995) considered them separate species, based on differences in habitat occupied (pond or lake) and fixed allelic variation at the LDH allozyme locus. Because we are mainly interested in the habitat differences among taxa, here we discuss them as distinct species. Our view is that the nominal species are ecologically distinct, but extremely close genetic relatives. Naturally occurring hybrid (LDH heterozygotic) populations are also fairly common and so are included in our study.

Daphnia pulex and *D. pulicaria* are small (< 3.5mm) crustaceans, which appear to segregate along an environmental gradient of habitat permanence (Deng 1997). *Daphnia pulex* is typically found in temporary ponds, while *D. pulicaria* is found in deep lakes. Populations of *D. pulex* must recruit from diapausing eggs in spring and undergo a variable period of reproduction via immediately developing eggs before producing diapausing eggs. Anoxia or desiccation kills individuals that survive to early summer. Variation in pond size, local hydrology and annual weather patterns create variation in the length of time any one pond is habitable by *D. pulex*, but all of our study ponds dry by summer. Hence, maximum lifespan of *D. pulex* is constrained to a few months by the temporary nature of their habitat. In contrast, *D. pulicaria* lives in a permanent and environmentally more stable habitat, and can persist year-round in deep lakes (Geedey, et al. 1996; Geedey 1997). In the absence of any strong abiotic constraints, resources and predators regulate *D. pulicaria* population

dynamics, generating predictable peaks of habitat quality in the spring and fall (Hutchinson 1967). Planktivorous fish constitute the most important predator, but *D. pulicaria* can effectively avoid them in deep water (Tessier and Welser 1991). Populations of *D. pulicaria* display low birth and death rates even at the time of peak planktivory (Leibold and Tessier 1998). Perennial populations of *D. pulicaria* also produce both immediately developing and diapausing eggs, but we have observed that diapause investment is much less than in *D. pulex* (Geedey 1997; Dudycha, unpubl. data).

Hybrids of *D. pulex* and *D. pulicaria* occur in a broader range of waterbody type than either parent, including both shallow ponds and lakes. In some cases they co-occur with one of the parents, but they typically occupy habitats poorly exploited by either parent, such as permanent fishless ponds. Little is known about hybrid population dynamics, however a few local populations become undetectable in late summer. (C. Steiner, pers. comm., 1998)

Gradients of habitat permanence cause strong differences in extrinsic mortality faced by different populations if individuals have no mechanism to escape habitat loss (e.g., migration). As habitat duration lengthens, the time until certain death is extended, and therefore the duration of potentially useful adult lifespan is also extended. Based on an expected lifespan driven by abiotic and biotic factors regulating population dynamics, we predicted the evolution of faster senescence in pond *D. pulex* than in lake *D. pulicaria*. We made no *a priori* predictions regarding the senescence of hybrids, due to uncertainty regarding how the parents' genomes would combine and to the lack of any reasonable expectation for adaptation to their place on the habitat duration gradient.

METHODS

Clonal Isolation and Lineage Establishment

Daphnia will reproduce in the lab by strictly ameiotic parthenogenesis, allowing us to capture the genetic variation in nature by randomly sampling a population and establishing isogenetic lines. We isolated five randomly chosen clones from each of three populations of *D. pulex*, three populations of *D. pulicaria*, and two populations of naturally occurring hybrids. We did not choose populations randomly; rather, we chose populations of the parent species to represent the extremes of the habitat permanence gradient. This consideration did not have much effect on the choice of *D. pulex* populations, but we chose *D. pulicaria* populations based on data indicating they were perennial populations (Geedey 1997). We chose hybrid populations from permanent waters free of fish planktivory. All populations are in SW Michigan, except one of *D. pulex*, which is in Michigan's Upper Peninsula. Clones were isolated in April and May 1996, except for a few clones isolated in spring 1995. Collecting clones at this time of year maximizes the genetic variation we sample due to recent hatching of sexually produced diapausing eggs (Lynch, 1984; Tessier, et al. 1992; Geedey, et al. 1996). We established lineages from all clones and acclimated them to lab conditions (20-22°, satiating food) for ≥ 3 generations prior to life history trait measurement (Tessier and Consolatti 1989, 1991). Routine monitoring and microscopic examination (at 460X) verified that lineages were free of parasites.

Life History Trait Measurement

We started life tables with neonates whose mothers were all raised simultaneously from birth for several weeks at low density (1/50 mL) to minimize variable maternal effects. Life tables began concurrently with neonates (~12 hr old) from the third or later clutches of offspring. For each population, 3 cohorts consisting of 2 neonates from each of the 5 clones (30 neonates/population) were set up. Some minor deviations from this ideal occurred because a few clones failed to produce an adequate number of female neonates on the day the experiment started. Animals were changed to fresh water every 2 d, fed a satiating food level (20,000 cells/mL *Ankistrodesmus falcatus* daily), and incubated at 20° on a 16:8 L:D cycle. Water volume was adjusted as animals grew and died such that a cohort could not filter more than 50% of their water between feedings (increased from 10 mL/neonate to 300 mL/animal at the largest size; Knoechel and Holtby 1986). We recorded mortality daily and fecundity every 2 d until all animals died.

Daphnia have two modes of reproduction, parthenogenetic reproduction that produces immediately developing offspring, and (usually) sexual reproduction that produces diapausing eggs. Only immediately developing offspring were included in our estimates of age-specific daily fecundity (m_x). When a female produced an ephippium, the easily-identified structure into which diapausing eggs are deposited, we adjusted fecundity values by considering that female to be unavailable for reproduction until the next instar (Lynch 1989). Thus, m_x was calculated by dividing the number of directly developing offspring produced by the number of live females that were not producing an ephippium. Normally, no eggs will be deposited in the ephippium

when males are not present. We assumed that individual females did not engage in continuous ephippial production.

Juvenile survival was nearly perfect for most populations. As an additional measure of juvenile performance, we estimated juvenile growth rates of 50 clones of *D. pulicaria*, 11 clones of *D. pulex* and 16 hybrid clones. These clones were isolated as part of another project from a broader array of Michigan populations than those for the life tables were. Mothers were raised as in the life tables. For each clone 18-24 randomly chosen neonates were immediately harvested; an additional 10 neonates were raised to maturity (here, defined as the day eggs were released into the brood chamber) under the same conditions as in the life tables, except they were fed a higher food level (40,000 cells/day). This food level is functionally equivalent (satiating) to the food level used in the life tables (Lampert 1987). Neonates and mature animals were dried overnight at 55°, then weighed (to within 0.1 μg) on a Cahn microbalance. We used time to maturity in conjunction with neonatal and mature mass to calculate juvenile specific growth rate ($\mu g\ \mu g^{-1}\ d^{-1}$; Tessier and Goulden 1987).

Quantifying Aspects of Senescence

Mortality data reflects the capability of the average individual to maintain its body under the daily rigors of life. Any degradation in that capability appears as an increase in the age-specific *conditional* probability of mortality (given survival to the beginning of the age class), commonly called the hazard function. Even if maintenance capability is constant, survivorship will still decline if it is imperfect. Hence, it is an increasing hazard that signifies decreasing maintenance capability, and is considered evidence of senescence.

Fecundity data reflects the capability of the average individual to produce progeny. Any degradation of this capability is manifest simply as a decline in the age-specific fecundity rate, and is considered evidence of senescence.

Observing a decline in either survival ability or fecundity alone may be evidence of senescence, but it may instead reflect a reallocation of effort between these two fitness components. As an organism ages, it may trade off mortality risk (i.e., somatic maintenance) with fecundity. Therefore, when comparing rates of senescence among taxa it is important to examine age-specific patterns of survival and fecundity jointly. We use two general approaches in our comparison of populations of the *Daphnia pulex-pulicaria* complex.

We first analyze age-specific patterns of survivorship and fecundity as separate fitness traits. Separately comparing the degradation of each trait across taxa is straightforward since each reflects a component of age-specific fitness (mortality hazard or per-capita fecundity) for the average individual. However, only when both traits lead to the same conclusion will this approach address senescence satisfactorily. Therefore, we also compare the taxa by combining survival and fecundity into single measures of age-specific fitness.

One of the most widely used summary measures of age-specific fitness is reproductive value, which specifies the contribution of each age class to r, the rate of population increase. Reproductive value (v_x) has been suggested as a useful measure for senescence studies (Partridge and Barton 1996). However, interpretation of v_x with respect to senescence as a process of physiological degradation is not at all straightforward. Because v_x weights each age class's expected fecundity by its contribution to r, early reproduction has greater

"value" than equal reproduction at a later age. Therefore, as an alternative measure of age-specific fitness, we also examine the unweighted contribution of each age class to R_0, the total lifetime reproduction. We refer to this age-specific measure as the intrinsic value (i_x).

Because our newborns enter age-class 1, we calculate reproductive value, v_x, with this equation (Goodman 1982):

$$v_x = \frac{e^{r(x-1)}}{l_x} \sum_{j=x}^{\infty} e^{-rj} l_j m_j$$

where l_x (cumulative survivorship) and m_x (per-capita fecundity) are taken directly from the life table data and r is iteratively calculated by Newton-Raphson approximation. Intrinsic value, i_x, is calculated as the ratio of current and expected future reproduction to the expected lifetime reproduction of an individual:

$$i_x = \sum_{j=x}^{\infty} l_j m_j \Big/ \sum_{j=0}^{\infty} l_j m_j$$

We scale by expected lifetime reproduction (R_0) to simplify comparison across taxa that differ in overall capability. The scaling by R_0 causes intrinsic value to reflect relative changes in age-specific fitness rather than absolute changes. In some cases, comparison of absolute changes may be appropriate, however some tradeoffs (e.g., between offspring size and number) may bias an absolute comparison.

Statistical Analyses

To evaluate whether there was potential evidence for senescence in the mortality data alone, we pooled the data for each taxon and fit it to a Weibull model. Weibull models normally express age-specific mortality (μ) at age x as $\mu_x = \lambda\gamma(\lambda x)^{\gamma-1}$ (Lee 1980). When γ, the dimensionless "shape parameter," equals one, the hazard function is constant and there is no evidence of senescence. If $\gamma > 1$, the hazard function increases with age; since this indicates that the conditional per-capita mortality rate increases with age, it is potentially (pending analysis of fecundity) evidence of senescence. The "scale parameter," λ, scales the model to a baseline rate of mortality, but is not indicative of senescence. We estimated γ with the SAS v. 6.09 Lifereg procedure.

We applied two types of regression model to the mortality data to test for differences among taxa in the pattern of mortality hazard. First, we used an accelerated failure-time (AFT) model to test for differences in the timing of high mortality hazard. AFT models assume that a factor (in this case, taxon) affects *failure time* (lifespan) multiplicatively, shifting hazardous periods along the timeline (Kalbfleisch and Prentice 1980; Fox 1993). Analyses of failure-time data in other fields normally address shifts in the time-period when failures occur (Kalbfleisch and Prentice 1980; Lawless 1982; Fox 1993), but this has rarely been done in investigations of senescence. Instead, lifespan is commonly examined due to its inherent interest, but it serves to identify only the endpoint of senescence. To apply the AFT model, we chose an underlying gamma distribution because its flexibility allows, but does not require, the theoretical expectation of monotonically increasing age-specific mortality (see Kalbfleisch and Prentice 1980 or Fox 1993 for a general discussion of survival distributions).

We also ran comparisons using alternative biologically plausible underlying distributions, including the Weibull distribution, and found our results were robust regardless of distribution. Second, we used a proportional-hazards (PH) model to test for differences among taxa in the magnitude of mortality hazard increase. PH models assume that a factor affects *hazard functions* multiplicatively, changing the magnitude of hazard in a given period (Kalbfleisch and Prentice 1980; Fox 1993). Juvenile mortality was excluded from analyses. All survivorship analyses were run in SAS v. 6.09 with the Lifereg and PHreg procedures.

We used repeated-measures ANOVA to test for differences among taxa in the pattern of age-specific fecundity. A significant time × taxon interaction indicates differences in age-specific changes of fecundity profiles and may be evidence of differences in senescence if horizontal (age) compression of one taxon's profile relative to the other's is evident. If there is a significant interaction and mortality analyses show the same relative contrast, then we can conclude there is evidence of differences in overall senescence. We also used repeated-measures ANOVA to test for differences among taxa in the shapes of their reproductive value and intrinsic value curves. For the combined measures, a significant time × taxon interaction indicates differences in the rate of change of age-specific fitness with respect to r (reproductive value) or R_0 (intrinsic value).

We tested for correlations between r and two indices that summarize the major differences among populations in temporal distribution of age-specific fitness. The first index was the age when v_x peaks, which we determined by fitting cubic polynomials, the simplest equations allowing asymmetry, to each reproductive value curve. The second index was the age when only 25% of

intrinsic value remained. We chose 25% to allow most of the divergence between taxa to occur, while minimizing any influence of "tails" in the data.

RESULTS

Mortality and Fecundity

Daphnia pulex had a substantially shorter intrinsic lifespan (median = 38d, max = 54d) than *D. pulicaria* (median = 62d, max = 99d; Fig. 1A). Both species showed decreasing ability to maintain their bodies as they aged. This was evident from both the increasing mortality hazard functions (Fig. 2) and Weibull shape parameters > 1 (*D. pulex* $\gamma = 7.7$, 95% CI = 5.1, 15.5; *D. pulicaria* $\gamma = 2.9$, 95% CI = 2.4, 3.6). The timing of high mortality hazard was significantly earlier in *D. pulex* than in *D. pulicaria* (AFT model; $p < 0.0001$, $\chi^2 = 566.2$, df = 1). The magnitude of mortality hazard increase was also significantly greater in *D. pulex* than in *D. pulicaria* (PH model; $p < 0.0001$, $\chi^2 = 57.6$, df = 1). Hence, intrinsic adult mortality increased both more and earlier in *D. pulex* than in *D. pulicaria*. The survivorship curve of hybrids (median lifespan = 58 d, max = 91 d; Fig. 1B) had a roughly similar shape to *D. pulex* (hybrid $\gamma = 6.1$, 95% CI = 5.1, 7.7), yet it was delayed, similar to *D. pulicaria* (Fig. 2). Within both parent species, there was also significant among-population variation in the timing of high mortality hazard (*D. pulex*: $p < 0.0003$, $\chi^2 = 16.3$, df = 2; *D. pulicaria*: $p < 0.0001$, $\chi^2 = 33{,}125.1$, df = 2). Similarly, variation in the magnitude of mortality hazard increase was significant (*D. pulicaria*: $p < 0.0001$, $\chi^2 = 18.7$, df = 1) or marginally so (*D. pulex*: $p = 0.066$, $\chi^2 = 3.4$, df = 1; Fig. 1A).

The shape of the fecundity curve for *D. pulex* was significantly compressed horizontally (changes occurred faster) relative to *D. pulicaria* (time × taxon interaction; $F = 4.12$, $p < 0.0001$, $df = 1, 46$; Fig. 3A). The difference between the species in their degradation of reproductive output was striking, because over the entire time that *D. pulex* was sharply declining (ages 35-55 d), *D. pulicaria* continued to improve its daily fecundity, declining moderately only after reaching 65 days old. Hybrids had a greater overall fecundity rate than either parent (Fig. 3B). The shape of the hybrid curve was strongly peaked, like that of *D. pulex*, but extended to much later ages like that of *D. pulicaria*. There was also significant variation in the shapes of the fecundity curves among populations within *D. pulicaria* and marginally significant variation within *D. pulex* (*D. pulicaria*: $F = 1.94$, $p < 0.0001$, $df = 2, 94$; *D. pulex*: $F = 1.38$, $p < 0.069$, $df = 2, 54$).

Combined Fitness Values

Despite exhibiting an earlier and steeper increase of mortality hazard and a more rapid decline of fecundity, *D. pulex* had a higher *r* than *D. pulicaria* ($t = 2.654$, $df = 2$, $p = 0.057$). This was a consequence of *D. pulex*'s more rapid increase in fecundity early in life (Fig. 3). There was no obvious pattern of difference in expected lifetime reproduction (Table 1).

The shape of the reproductive value profile for *D. pulex* was significantly compressed horizontally relative to *D. pulicaria* (time × taxon interaction; $F = 7.43$, $p < 0.0001$, $df = 1, 46$; Fig. 4A). Note that by the age when *D. pulicaria* peaks in its ability to contribute to population growth, *D. pulex* has lost essentially all of its ability, even though both species achieved similar maximal age-specific

contributions. Therefore, *D. pulex* lost its reproductive value faster. The reproductive value curve of hybrids (Fig. 4B) rose rapidly like *D. pulex*, peaked midway between the parents, and declined at late ages similar to *D. pulicaria*. There was also significant variation within each parent species in the shapes of the reproductive value profiles (*D. pulex*: $F = 4.87$, $p < 0.0001$, $df = 2, 54$; *D. pulicaria*: $F = 1.45$, $p = 0.010$, $df = 2, 94$).

Intrinsic value is defined to be 1 at age = 0 and it remained 1 until animals matured, then declined roughly linearly until about 95% of intrinsic value had been lost. However, *D. pulex* lost its ability to contribute to R_0 substantially faster than *D. pulicaria* (time × taxon interaction; $F = 23.24$, $p < 0.0001$, $df = 1, 46$; Fig. 5A), creating a difference > 30 days between the ages when 25% of intrinsic value remained. The hybrid populations, again, were intermediate to the parental taxa (Fig. 5B).

There was a strong, significant negative correlation between r and the age when v_x peaks ($r = -0.923$, $p = 0.01$) and a weaker correlation between r and the age when 25% of intrinsic value remained ($r = -0.679$, $p = 0.064$; Table 1 and Fig. 6). These correlations both suggest a genetic tradeoff between early life fitness (which maximizes r) and late life fitness (which extends the age when v_x peaks and when 25% i_x remains). Since r is used in the calculation of v_x, we looked for any mathematical dependence between r and the age when v_x peaks by running simulations with hypothetical life history data (combinations of 4 l_x and 28 m_x profiles). In all life histories, reproduction began at age 11 and continued until age 50. With these hypothetical life histories, we generated points that evenly filled the range of parameter space (r: 0-0.5; age when v_x peaks: 10-50 d) defined

by our empirical data (Dudycha, unpublished data). This was true for both non-senescent (constant or decreasing age-specific mortality rate and constant or increasing fecundity) and senescent (increasing age-specific mortality and/or decreasing fecundity) life history profiles. These simulations also confirmed that the age when v_x peaks is substantially affected by late-life traits, whereas r is not. Thus we are certain that the tradeoff we inferred is biological, not mathematical, in nature.

Juvenile Size and Growth

In our comparison of juvenile performance, we observed that *D. pulicaria* matured in 6.4 ± 0.1 SE, *D. pulex* in 5.4 ± 0.2 SE and hybrids in 6.0 ± 0.2 SE days. These differences are small relative to lifespan, but are a substantial portion of the juvenile period. One-way ANOVA revealed that per-offspring investment, measured as neonate mass, varied among the taxa ($p < 0.0001$, $F = 11.8$, $df = 2, 77$). *D. pulex* had the smallest neonates (2.4 ± 0.2 SE μg), followed by hybrids (2.9 ± 0.2 μg) and *D. pulicaria* (3.5 ± 0.1 μg). Post-hoc pairwise comparison showed that *D. pulicaria* was significantly different from *D. pulex* ($p < 0.0001$) and from hybrids ($p = 0.0243$). Differences in per-offspring investment between the parents are even more obvious when comparing clones with similar mass at maturity (Fig. 7). *Daphnia pulicaria* had a significant correlation between neonatal and adult mass ($r = 0.83$, $p < 0.001$; Fig. 7). The relationship was isometric (reduced major axis regression slope = 1.0 ± 0.08), showing that *D. pulicaria* clones had the same juvenile specific growth rate (0.378 ± 0.003 SE μg μg^{-1} d^{-1}), regardless of their mass at maturity. Neonatal and adult mass of hybrids were also correlated ($r = 0.68$, $p = 0.004$) and isometric (r. m. a. regression slope = 0.98 ± 0.19). However, the elevation of the hybrids' slope was distinctly below that of *D. pulicaria*

(ANCOVA: $F = 88.2$; df = 1, 63; $p < 0.001$) and they matured slightly earlier, contributing to an overall faster juvenile specific growth rate in hybrids (0.514 ± 0.009 SE $\mu g\ \mu g^{-1}\ d^{-1}$). Our sample of *D. pulex* clones spanned a limited range of adult sizes, and there was no significant correlation between neonate and adult mass ($r = 0.3$, $p = 0.37$). These *D. pulex* clones largely fell within the 95% probability ellipse for the hybrids (Fig. 7), and because they had an even shorter maturation time, their juvenile specific growth rates were faster (0.613 ±0.015 SE $\mu g\ \mu g^{-1}\ d^{-1}$) than *D. pulicaria* ($F = 611.9$, $p < 0.0001$, df = 1, 59).

Discussion

We conclude that senescence occurs in all of our study populations because both the hazard functions increased and fecundity rates declined over later life. Our analyses of mortality and fecundity combined also showed a degradation of age-specific fitness in later life, excluding the possibility that the separate degradations of survival and reproductive capabilities create an illusion of senescence via a changing tradeoff between them. Furthermore, our data show that *D. pulex*, a resident of ephemeral habitats, experiences greater senescence than *D. pulicaria*, a resident of permanent habitats, as expected by the evolutionary theory of senescence. Specifically, *D. pulex* has a shorter lifespan, an earlier and steeper rise in mortality rate, an earlier and sharper decline of fecundity, a horizontally compressed reproductive value and a more rapidly declining intrinsic value than does *D. pulicaria*.

Our approaches to analyzing life history data with respect to the concept of senescence are complementary. The separate mortality and fecundity analyses document that both survival and reproductive abilities degrade in concert,

supporting a common assumption (Partridge and Barton 1993). Our analysis of reproductive value specifies how much the combined degradation of fitness components affects age-dependent loss of the ability to contribute to population growth. This view of senescence highlights how the contribution of alleles to phenotypic evolution changes with age, and may be especially important in studies that seek to measure selection on senescence. Finally, our analysis of intrinsic value gives a summary picture of biological abilities, showing the overall degradation with age of an average individual. Under laboratory conditions, where survivorship declines primarily due to intrinsic breakdown, this measure is closer to a physiological view of senescence than reproductive value.

Studies that examine traits under a single set of laboratory conditions, but deduce predictions from field conditions, are potentially biased by genotype-by-environment interactions. We conducted our life tables under abiotic conditions that are benign and at a satiating food level. These conditions are not unusual for any of our populations in the field. However, the average temperature *D. pulex* experiences in ponds is warmer and closer to 20° than the temperature *D. pulicaria* experiences in lakes, because *D. pulicaria* spends summer in cold deep water (Leibold and Tessier 1998). Resource levels can be high in lakes or ponds, but are usually lower in lakes than in ponds (Leibold and Tessier 1998; Tessier, unpubl. data). Therefore, the lab environment resembles the pond habitat of *D. pulex* more closely than the lake habitat of *D. pulicaria*. It is unlikely that an even closer match to the pond environment would improve late-life fitness in *D. pulex* enough to reverse the fundamental differences between the species' senescence patterns through a genotype-by-environment interaction.

Assuming that the lab environment is sufficiently similar to natural conditions to make an unbiased extrapolation to the field, we can ask to what degree the observed life history differences are functionally significant in the field. The lifespans of *D. pulex* seen in the lab are shorter than the duration of their habitats (typically, snowmelt occurs in late February/early March and the ponds dry in mid-June, making ponds habitable by *D. pulex* for about three months). An exact match is not expected because there is interannual variation in the duration of ponds, and most individuals are not born on the earliest possible day, therefore potential lifespan is less than a pond's average duration.

In contrast, lakes may be a permanent habitat, but habitat quality is not uniform over the year. *Daphnia pulicaria* population density is often greatly reduced in summer and under ice in winter due to low resource availability in the former and the combination of low resources and very cold temperature in the latter. Spring and fall are predictable periods of high algal (resource) availability, and consequently *Daphnia* population growth is also high (Hutchinson 1967; Sommer 1989). Individual daphnids who can survive from spring to fall (or vice-versa) may have a considerable advantage over genotypes who rely on diapause to get through summer and winter. Diapause is disadvantageous because these eggs depend on imperfect environmental cues to decide when to leave diapause. The lifespans we saw in the lab suggest that substantial numbers of *D. pulicaria* may live long enough to cross the seasonal troughs of habitat quality, because the average temperature and resource level they experience in the field is lower than lab conditions. Daphnid lifespans have been shown to lengthen in colder temperatures (MacArthur and Baillie 1929; Ingle 1933) and under reduced resources (Ingle, et al. 1937). When resources increase in spring and fall, a population of *D. pulicaria* will be composed mainly

of large adults that persisted from the last good season. These adults can now resume a high level of reproduction, which had necessarily declined during the poor resource period (Leibold and Tessier 1998). Because this "persistent" demography will be more rapidly responsive to resource change than a "diapause" demography, the population can exploit the resource flush better than a population constrained to hatch from diapausing eggs or even one whose age-structure is relatively juvenile.

We have argued that the differences in senescence between *D. pulex and D. pulicaria* have evolved as a result of differences in their habitats. However, we do not present data to show that mortality imposed by habitat loss (or shielded by habitat maintenance) is the direct mechanism responsible for the genetic differences in senescence. Other differences between the habitats may influence senescence evolution. For example, temporary habitats force *Daphnia* populations to use diapause. Senescence differences could evolve as a correlated effect of selection on investment in diapause through costs of reproduction. This selective pathway may contribute to the different senescence patterns expressed by *D. pulex* and *D. pulicaria* in our study because *D. pulex* produced more diapause structures than did *D. pulicaria* (1.2 vs. 0.3 ephippia per female per lifetime). Size-selective predation may also vary between temporary ponds and permanent lakes. Because size and age are correlated, size-selective predation could exert selection on senescence. We do not know whether size-selective predation systematically varies among our study populations. If predation falls mainly on large individuals in temporary ponds and on small individuals in permanent lakes, this could contribute to the evolution of the senescence differences we found.

Tradeoffs

What allows rapidly senescing organisms to persist despite the existence of long-lived genotypes? One elaboration of ETS is based on the hypothesis of genetic tradeoffs: that poor late-life fitness is genetically linked to good early-life fitness through genes that are antagonistically pleiotropic (Hamilton 1966; Rose 1991; Stearns 1992). In this scenario, selection for a good early-life phenotype will indirectly select for a poor late-life phenotype. Optimality approaches to senescence inextricably tie senescence to development (Hamilton 1966; Partridge and Barton 1993, 1996), making ETS also a theory of *early*-life performance. The developmental theory of senescence hypothesizes that pleiotropic genes benefit development rate at the expense of extended adult survival and reproduction (Zwaan, et al. 1991, 1995b). The simple prediction is that juvenile performance should be better in taxa that have faster senescence.

Life history theory focusing on offspring quality also predicts a link between early- and late-life traits. Winkler and Wallin (1987) modeled the relationship between total reproductive effort and per-offspring investment. They predicted that organisms investing heavily in reproduction would have smaller optimal per-offspring investment. Their model explicitly assumed a tradeoff between reproduction and adult survival. Therefore, their model also predicts a positive relation between optimal per-offspring investment and lifespan.

We demonstrated genetically faster juvenile specific growth in *D. pulex* than in *D. pulicaria*, confirming the developmental theory's hypothesized relationship between early performance and senescence. We also showed

greater per-offspring investment, measured as neonate mass, in *D. pulicaria* than in *D. pulex*, supporting the Winkler and Wallin (1987) model. Hybrids are intermediate to the parental species for both of these traits, and for senescence. *D. pulex* produced relatively small neonates who rapidly grew large, whereas *D. pulicaria* produced large, slowly growing neonates. *D. pulex* evidently gains its advantage in *r* by producing cheap, rapidly growing offspring. This negative relationship between per-offspring investment and juvenile performance is contrary to the usual expectation that greater investment in each offspring produces juveniles who are more fit. However, through joint consideration of the developmental theory and the per-offspring investment model, this negative relationship becomes intelligible, because juvenile performance and per-offspring investment are both mediated by a tradeoff with the senescence of adults.

In a recent review of empirical research on growth rates, Arendt (1997) argued that research rarely addresses tradeoffs between juvenile growth rate and adult fitness (reproduction or maintenance) despite the possibilities that organisms may be unable to reallocate resources perfectly from growth to adult fitness or that rapid growth may build a less sturdy body. In other words, rapid juvenile growth may be advantageous to early adult fitness, yet cause poor late-age fitness. Arendt (1997) also emphasized that genetic variation in growth rate is often overlooked on the assumption that there is no fitness advantage to submaximal growth rates. Our juvenile growth experiment clearly shows that there is variation in specific growth rate among these physiologically similar taxa. In combination, our data showing associations between genetic variation in the packaging of reproductive effort, senescence and juvenile growth suggest that exploration of the mechanisms linking them will be a strong approach to

understanding life history. A large literature discusses optimal life histories from the separate perspectives of senescence and per-offspring investment, but these perspectives rarely intersect. Our results suggest that empirical work in other taxa would benefit from a joint consideration of senescence and per-offspring effort.

The tradeoffs we discovered would prevent invasion of temporary ponds by long-lived genotypes because their reduced *r* places them at a disadvantage when the pond dries, and genotypes can locally persist only by generating a diapausing stage. At this critical accounting period, the number of individuals of high *r* will be greater than those of low *r*. Assuming that the genotypes have equivalent diapause, the long-lived will never catch up because the cycle is reset every year. This rationale may explain the puzzle of rapid senescence, but it highlights a more general problem in evolutionary biology: why genotypes with low *r* are not displaced by genotypes with high *r*. Although it is speculative, we suspect that the inability of short-lived, high-*r* genotypes to invade lakes may be related to their inability to persist across habitat quality troughs. If an individual's lifespan precludes survival through an extended period of poor habitat quality, its genotype can persist only through offspring production or through diapause. Neither strategy is likely to be effective in lakes. Offspring cannot survive and grow well in comparison to adults during poor resource conditions, and diapause is complicated by the need for a reliable, accurate cue of improving habitat conditions. Furthermore, a diapause strategy involves a time lag, delaying the ability of diapausing genotypes to respond to the habitat improvement compared to genotypes that persist as surviving adults. In sum, the ability of long-lived individuals to span summer or fall may outweigh the advantages of high lab-based *r*, because high *r* is genetically linked to other traits,

i.e. short lifespan and small offspring, that are disadvantageous during low resource periods in lakes.

We focused our study on several populations of a single species complex in order to make detailed life history comparisons under common conditions. At this time, we do not have a phylogenetic estimate for these populations and cannot say definitively whether our comparison includes independent evolutionary divergences or the differences we found may be partly due to historical constraints. However, the local existence of interspecific hybrids, which occupy an intermediate position along tradeoffs between *r* and senescence, suggests that the species are genetically very similar and historical constraint on life history evolution may be minimal. Even if our populations prove to be phylogenetically non-independent, the principal alternative theory for explaining senescence, that degradation is caused simply by metabolic exhaustion (Comfort 1979), cannot explain the variation we found. A review of the rather extensive work on metabolic rate in *Daphnia* (Peters 1987) found no evidence for taxonomic differences; metabolism scaled negatively with size. Therefore, if senescence were ultimately caused by metabolic exhaustion, we would have seen no variation between the species, or faster senescence in *D. pulicaria*, because *D. pulicaria* adults are on average smaller than *D. pulex* (Fig. 4; Hebert 1995).

Our results indicate that there is potential for continued evolution of senescence in these taxa because we observed non-trivial extant genetic variation for age-specific fitness and lifespan within gene pools. Moreover, at the macroevolutionary level, these species are not reproductively isolated, so one could argue that the variation throughout the species complex is available for

further evolution in a single gene pool. However, since we only examined variation at the level of populations (and only 3 populations per species), we are unable to generate reliable estimates of the heritability of senescence.

A recent study by Lynch, et al. (1998) proposes an alternative interpretation to standing genetic variation of life history traits. By comparing the genetic variation of life history traits in a natural population of *D. pulex* to that created by a laboratory mutation accumulation experiment, they concluded that much of the natural genetic variation may be due to continually arising, transient deleterious mutations. Our study differs by examining among-population variation (rather than within) and by emphasizing late-life traits, whose mutations will be less transient than the early-life traits studied by Lynch, et al. (1998). Variation in late-life traits will be less transient than in early-life traits because selection is less effective at late ages. Furthermore, among our populations senescence trades off with early life history, a pattern that cannot be explained by uniformly deleterious mutations.

Prior tests of ETS have included laboratory selection on senescence, which give a detailed picture of senescence, and broad comparisons of many taxa, usually addressing only mean lifespan. Our report complements these tests by extending the understanding of senescence evolution to detailed life history changes in the face of contrasting patterns of natural selection, and by jointly addressing mortality and fecundity. We conclude that the pattern of senescence exhibited by *Daphnia pulex-pulicaria* is consistent with ETS. This result is limited, though, in that the selection pressures are historical, and thus cannot be directly quantified, and that our desire for thorough senescence data limited the number of populations we could study concurrently. It will be possible to expand our

findings, because there are many instances of *Daphnia* sister species inhabiting different parts of the habitat duration gradient. Our results additionally suggest a relationship among senescence, offspring investment and juvenile fitness (specific growth rate). This raises the questions of what physiological traits proximately cause the large differences in life history, whether the different life history traits are affected by the same physiological traits, and what ecological consequences emerge from that life history variation. A finer understanding of genotype-environment interactions and of age-specific mortality risk in *Daphnia* are the next steps to making the link.

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Table 2-1. Values of fitness measures and the timing of fitness contribution in *D. pulex* and *D. pulicaria*. See text for explanation of variables.

	D. pulex			*D. pulicaria*			Hybrid	
Population	**WIS**	**OL3**	**MCP**	**WAR**	**PIN**	**LAW**	**WHT**	**WIN**
r	0.54	0.45	0.50	0.41	0.41	0.29	0.43	.47
R_0	108	58	180	232	218	34	240	245
v_x peak age (days)	18.5	29.5	23.5	48.5	44.5	62	34.5	42
i_x25 (days)	29.5	35	34	63	62	54.5	50	51

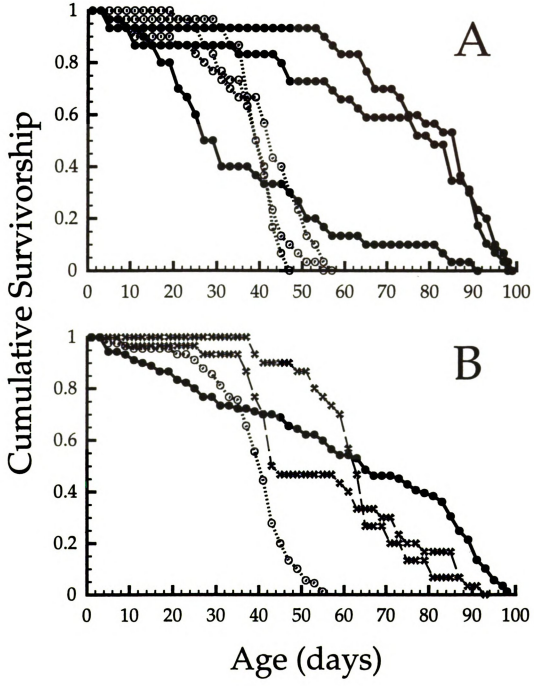

Figure 2-1. Survivorship of *Daphnia pulex-pulicaria*. Median lifespan is the age where survivorship equals 0.5. *A*: 3 replicate populations of *D. pulex* (open circles and dotted lines) and 3 of *D. pulicaria* (closed circles and solid line). *B*: Data in top panel pooled by species (dotted and solid lines), and 2 replicate populations of naturally occurring hybrids (crosses and dashed lines).

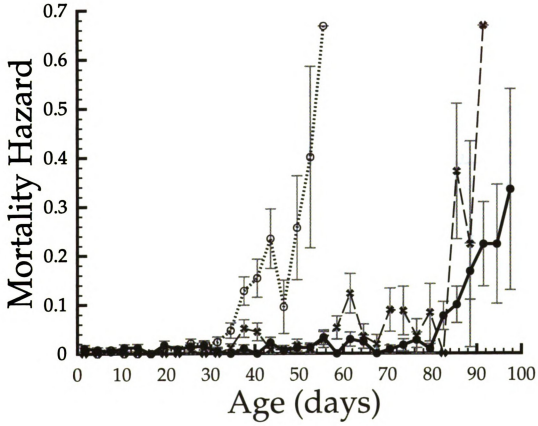

Figure 2-2. Mortality hazard function of *D. pulex*, *D. pulicaria* and hybrids. Symbols as in Figure 1. Standard error bars are based on replicate populations.

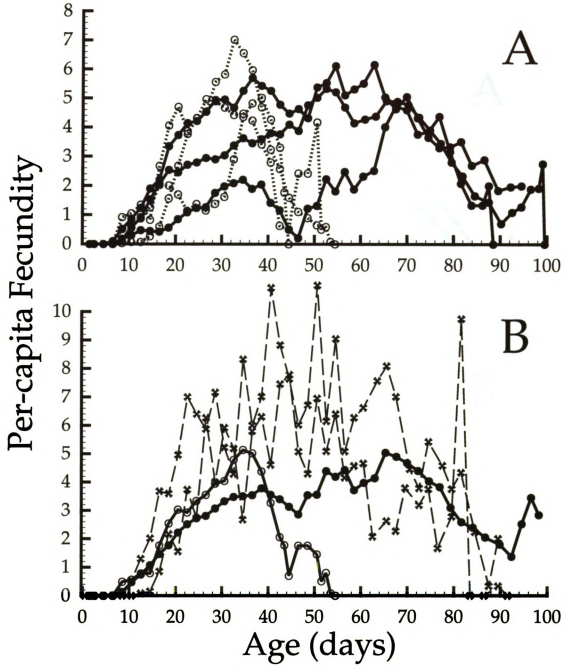

Figure 2-3. Per-capita fecundity of *D. pulex*, *D. pulicaria* and hybrids. *A*: 3 replicate populations each of *D. pulex* and *D. pulicaria*. *B*: Data in top panel averaged by species, and 2 populations of hybrids. Symbols as in Figure 1, except in *B*: *D. pulex* and *D. pulicaria* have solid lines.

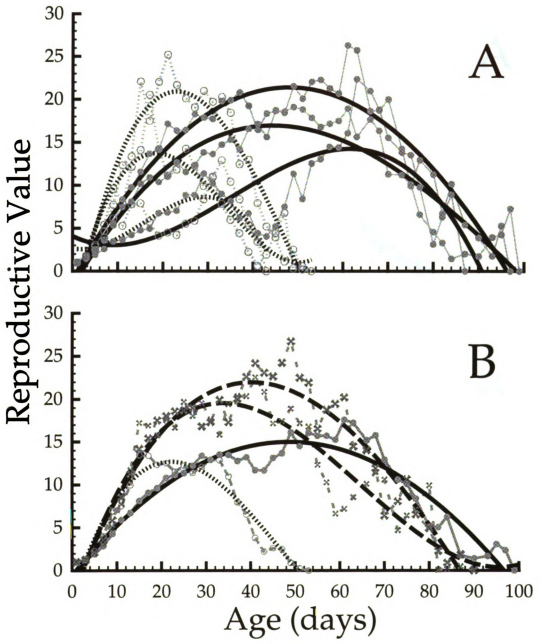

Figure 2-4. Reproductive value of *Daphnia pulex-pulicaria*. Grey symbols and lines are reproductive value data calculated as described in text. Black curves are cubic polynomials fit to each population. *A*: 3 replicate populations each of *D. pulex* and *D. pulicaria*. *B*: Mean values by species of data in top panel, and 2 populations of hybrids. Symbols as in Figure 1.

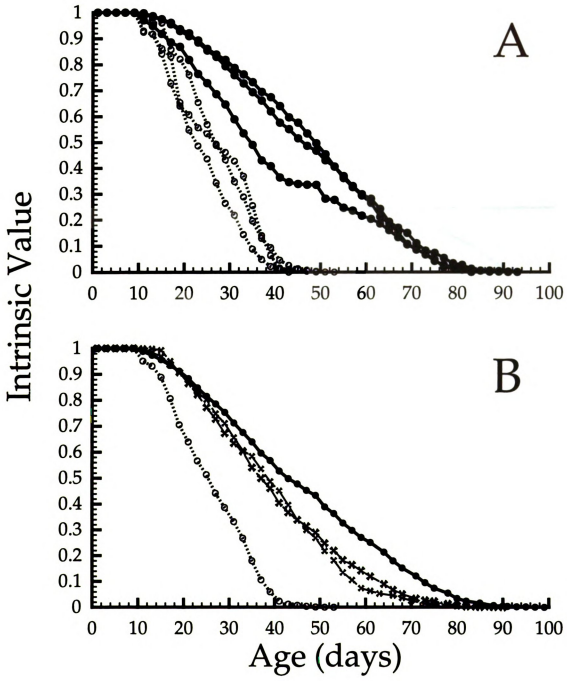

Figure 2-5. Intrinsic value of *Daphnia pulex-pulicaria*. See text for explanation of intrinsic value. *A*: 3 replicate populations each of *D. pulex* and *D. pulicaria*. *B*: Mean values by species of data in top panel, and 2 populations of hybrids. Symbols as in Figure 1.

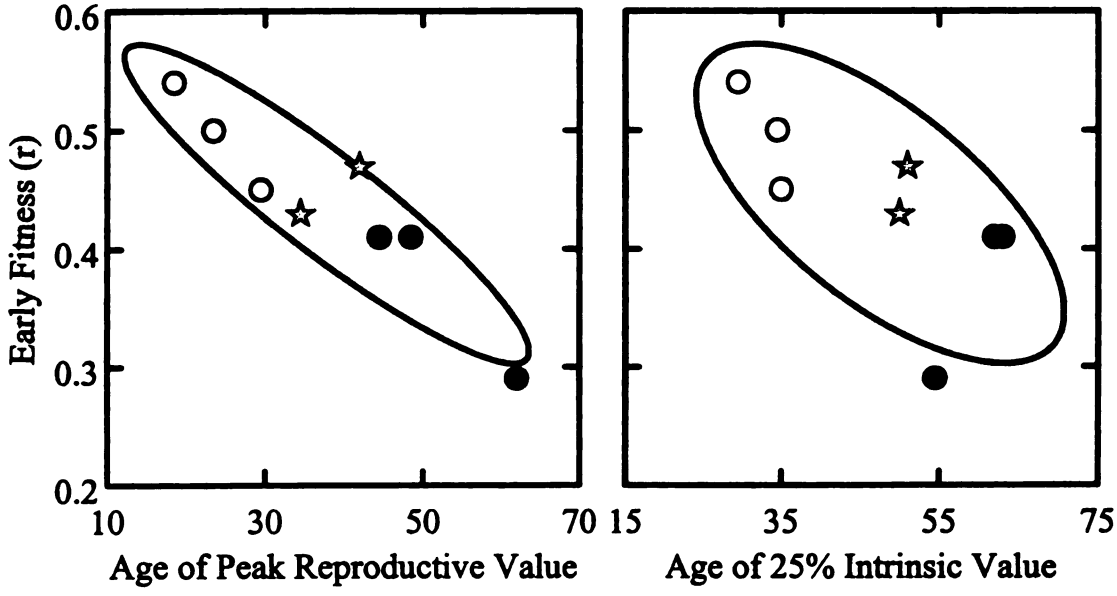

Figure 2-6. Relationship between early fitness and life history timing in *Daphnia pulex-pulicaria*. Age of peak reproductive value is the age when a cubic equation fit to reproductive value peaks. Age of 25% intrinsic value is the age when only 25% of the intrinsic value remains. Black circles are *D. pulicaria*, open circles are *D. pulex* and stars are hybrid. Each symbol represents a replicate population. One standard deviation ellipses are drawn around the data.

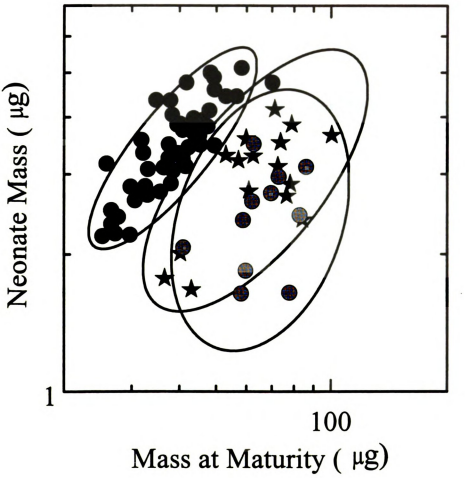

Figure 2-7. Neonate and adult mass (in µg) of clones in the *Daphnia pulex-pulicaria* complex. Each symbol represents a separate clone. 90% confidence ellipses are drawn around each taxon. Dark circles are *D. pulicaria*, light circles are *D. pulex* and stars are hybrid.

CHAPTER THREE: A REACTION NORM APPROACH TO THE EVOLUTIONARY ECOLOGY OF SENESCENCE.

INTRODUCTION

One goal of evolutionary ecology is to understand how genetic differences among taxa produce ecological differences in the way taxa respond to environmental variation. In this paper, I examine how genetic variation of age-specific fitness components is expressed in different resource environments under benign and stressful temperatures. Despite the widespread importance of food and temperature to life history, their joint consideration is surprisingly uncommon (see Sarma and Rao 1991; Butler and Burns 1991; Orcutt and Porter 1994 for examples). Furthermore, investigations of age-specific fitness components often lack an ecological context (Partridge and Harvey 1988; Reznick 1993). In placing age-specific fitness into an ecological context, I show how this approach is useful for understanding the distribution of senescence patterns in nature.

The evolutionary theory of senescence offers an understanding of life history variation through the effects of extrinsic mortality risk on the strength of natural selection. Traits appearing early in an organism's life have a greater effect on total fitness than those appearing late, causing the late traits to be less exposed to selection than early traits (Medawar 1952; Williams 1957; Rose 1991). If an allele is beneficial early in life, but detrimental later in life

(antagonistic pleiotropy), selection will favor it despite its deleterious effects (Williams 1957). Deleterious late life traits can also be evolutionarily sustained through mutation accumulation (Hamilton 1966; Charlesworth 1980). Because extrinsic mortality shapes age-specific potential contribution to fitness, variation in extrinsic mortality risk among populations should lead to the evolution of variation in investment in longevity and potential late-life reproduction (Williams 1957; Hamilton 1966; Edney and Gill 1968; Charlesworth 1980).

Closely related taxa that experience different mortality risks provide an opportunity to study the evolution of senescence in nature (Reznick 1993; Austad 1993a). By comparing their adult performance under controlled conditions, one can test the hypothesis that higher mortality risk leads to the evolution of a faster decline in intrinsic physiological fitness as organisms age (the rate of senescence). Comparison of populations exposed to different mortality risks has been used to support the evolutionary theory of senescence in opossums (Austad 1993b), grasshoppers (Tatar, et al. 1997), ants (Keller and Genoud 1997) and Daphnia (Dudycha and Tessier, in press). When comparing populations, it is useful to measure senescence as declines in the age-specific probability of survival and age-specific fecundity, because demographic measures synthesize the physiological manifestations of senescence and reflect the essential components of evolutionary fitness (Partridge and Barton 1993, 1996).

A problem arises in comparative studies of genetic variation because it is usually impractical to mimic variable natural conditions precisely, and the environments inhabited by different genetic lineages are likely to differ. Rates of senescence measured in a single controlled environment may not adequately reflect senescence as it occurs in nature. Comparisons between taxa may be misinterpreted if the chosen environment substantially favors one taxon, when a different environment could have favored the other (Schlichting and Pigliucci 1998). A possible solution is to compare life histories in a variety of environments, spanning a range that reflects natural environmental variation.

Two environmental variables have been repeatedly shown to influence senescence of animals: food level and temperature (Finch 1990; Rose 1991). The extension of lifespan under dietary restriction has long been known in a wide variety of organisms, including Daphnia (Ingle et al. 1937), rotifers (Fanestil and Barrows 1965), rodents (McCay et al. 1939, 1956), spiders (Austad 1989) and others (reviewed in Comfort 1979; Weindruch and Walford 1988; Rose 1991; Yu 1994). Longer lifespan is also clearly associated with cooler temperature based on evidence from fruit flies (e. g., Hollingsworth 1966; Miquel et al. 1976), Daphnia (MacArthur and Baillie 1929; Korpelainen 1986), rotifers (Meadow and Barrows 1971), grasshoppers (Tatar et al. 1997) and nematodes (Klass 1977). Few of these studies have examined

naturally evolved differences in the influence of the environment on senescence (but see Harrison and Archer 1988; Tatar et al. 1997) or included fecundity senescence. Most have been primarily concerned with understanding the proximate mechanisms of senescence and consequently give only cursory attention, if any, to natural genetic variation or the ecological relevance of the responses.

Perhaps the ecological relevance has been overlooked because of an enduring perception that few wild animals die of old age. While this may be true, it is not true that senescence occurs in few wild animals; senescence is widespread and may substantially interact with ecological dynamics (Rose 1991; Promislow 1991; Ricklefs 1998). For example, senescence likely influences the ability of animals to compete, to avoid predation and to find mates. Senescence evolution may also be influenced indirectly by ecological factors. If extrinsic mortality risk covaries with other aspects of the environment, we might see the evolution of a plastic senescence pattern that responds to detectable environmental changes. To build a useful bridge between senescence in the laboratory and the wild, we need to address the relationship between senescence and traits that determine evolutionary success. Optimality approaches to theory developed for understanding declines of late-life performance necessarily incorporate early-life events (Hamilton 1966; Charlesworth 1980; Partridge and Barton 1993, 1996). This suggests that r, an evolutionary fitness measure that is very sensitive to early

life performance, is a worthwhile component of investigations on the ecological relevance of senescence.

In this paper, I report on the plasticity of senescence patterns of the freshwater crustacean Daphnia in response to food and temperature. These environmental factors vary widely in nature and can strongly influence daphnid life history (e.g., MacArthur and Baillie 1929; Ingle et al. 1937; Korpelainen 1986; Orcutt and Porter 1994; Boersma et al. 1998). In previous work, Dudycha and Tessier (in press) demonstrated substantial genetic variation in senescence patterns in the D. pulex-pulicaria species complex in a single environment. I extend this work by quantifying the senescence of members of two species complexes in multiple environments. My primary goal here is to compare the senescence patterns of sister species in environments representing a natural range of variability. I use these comparisons to address the influence of environment on evolved differences in senescence and to ask whether plasticity of senescence substantially alters our understanding of the evolutionary theory of senescence in nature. I also ask how consistent the patterns of senescence plasticity are at different levels of biological differentiation. Secondarily, I am interested in how gross ecological differences between the two species complexes could explain any macroevolutionary differences in senescence.

Study System

Four ecologically distinct species of Daphnia were chosen for this study because their ecology in Michigan is well known. D. pulex and D. pulicaria are sister species in the daphnia subgenus; D. mendotae (formerly called D. galeata mendotae) and D. dentifera (formerly D. rosea) are sister species in the hyalodaphnia subgenus (Hebert 1995). Each pair of sisters form a complex composed of two parental (nominal) species and naturally occurring hybrid populations (Taylor and Hebert 1992; Hebert 1995; Colbourne and Hebert 1996). It is therefore difficult to demarcate evolutionarily discrete gene pools at the species level. Despite a low degree of divergence and common hybridization within each complex (Lehman et al. 1995; Crease et al. 1997; Taylor and Hebert 1992, 1993; Taylor et al. 1996), each parental species differs in habitat use and seasonal phenology.

Daphnia pulex is found in temporary ponds, while D. pulicaria is found in deep lakes (Hebert 1995; Deng 1997). Populations of D. pulex recruit from diapausing eggs in spring and undergo a variable period of parthenogenetic reproduction via immediately developing eggs before producing diapausing eggs. Anoxia or desiccation kills individuals who survive to early summer. The length of time any one pond is habitable by D. pulex is variable, but all of my study ponds dry by summer. Therefore, maximum lifespan of D. pulex is constrained to a few months by the

temporary nature of their habitat. In local ponds, water temperature varies from 6°-28°, with potentially substantial diel fluctuation over the period that D. pulex populations are active. Food levels for D. pulex are typically very high, but can reach low levels when the population peaks and diapausing egg production begins (A. J. Tessier, unpublished data).

In contrast, D. pulicaria lives in permanent and environmentally more stable deep lakes, and typically persist year-round. (Geedey et al. 1996; Geedey 1997). With no strong abiotic constraints, resources and predators regulate D. pulicaria populations, generating predictable peaks of habitat quality in spring and fall (Hutchinson 1967). During much of the year food availability is low (A. J. Tessier, unpublished data). Deep lakes are a heterogenous thermal environment for D. pulicaria. In winter, lakes are uniformly cold (<4°), but in summer, temperate lakes thermally stratify, with upper waters reaching 20-28° and bottom waters remaining near 10°. Although D. pulicaria generally remain in the cool bottom waters during summer, individuals can migrate into the warm surface waters at night (Leibold and Tessier 1991; personal obervation). Because D. pulex and D. pulicaria occupy strikingly different habitats, the species face different extrinsic mortality risk (e.g., due to habitat ephemerality) and we have documented substantial genetic variation of senescence (Dudycha and Tessier, in press).

Daphnia mendotae and D. dentifera are ecologically less distinct than D. pulex-pulicaria, because both species inhabit thermally stratified lakes. D. mendotae appears to be perennial (like D. pulicaria; Hall 1964; A. J. Tessier, personal communication 1999) and typically remains in the warm surface waters of large lakes (Taylor and Hebert 1992) during both day and night. In contrast, D. dentifera is found in smaller lakes, and appears to have a summer annual phenology. In these lakes, fish planktivory may be higher than that experienced by D. mendotae, and consequently D. dentifera typically displays strong diel vertical migration (Hall 1964; Dodson 1972; Threlkeld 1979, 1980; Leibold and Tessier 1991, 1998, personal observation). Thus, both species experience wide temperature variation, albeit at different temporal scales. Unlike the D. pulex-pulicaria contrast, it is unclear which species faces greater adult mortality.

The differences in phenology and habitat use mean that the four species differ in both relative mortality risk and average experienced temperature. Overall, D. mendotae-dentifera experience greater extrinsic mortality than D. pulicaria (Hall 1964; Threlkeld 1979, 1980; Leibold and Tessier 1998). This is because D. mendotae-dentifera make greater use of the risky upper water habitat in lakes (high fish predation) and have a smaller body size, making them more vulnerable to invertebrate predators (Hall et al. 1970; Dodson 1972; Branstrator 1998). It is unclear whether the periodic catastrophic mortality risk faced by D. pulex or the consistently high predation

risks faced by D. mendotae-dentifera will result in a higher average mortality risk. It is clear, however, that D. mendotae-dentifera experiences the warmest average environment because they use the warm epilimnetic habitat extensively. D. pulex experiences an intermediate average temperature, ranging from the cold water of late winter to the warm water of late spring. D. pulicaria has the coldest average temperature, as it typically opts to remain in the cold hypolimnetic habitat during summer.

METHODS

Life Tables

Life tables were obtained for 16 populations of Daphnia to estimate genetic variation of life histories. Three replicate populations of each parental species and two populations of each hybrid type were used. Daphnia will reproduce clonally in the lab, allowing one to sample the genetic variation in nature by establishing multiple isogenetic lineages. Populations of D. pulex-pulicaria were chosen to reflect the extremes of the habitat permanence gradient, and although these are the typical habitats of the species, they are not a random sample of available populations. Species and hybrid identity was confirmed with the diagnostic LDH allozyme (Hebert 1995). Populations of D. mendotae-dentifera were chosen that showed the greatest relative abundance of the target species, as determined by the AO allozyme locus (Taylor and Hebert 1992, 1993). Sister parental species do not

coexist in any of the chosen populations. All populations are in SW Michigan, except one of D. pulex, which is in Michigan's Upper Peninsula. I isolated five clones from each population, obtained in early spring (D. pulex-pulicaria) or early summer (D. mendotae-dentifera) to maximize the genetic variation sampled (Lynch 1984; Tessier et al. 1992; Geedey et al. 1996). Lineages were established from all clones and acclimated to the lab environment (20-22°, satiating food) for ≥ 3 generations prior to life tables (Tessier and Consolatti 1989, 1991).

Mothers of experimenal individuals were all raised from birth for several weeks at a low density (1/50 ML) to minimize the role of maternal effects in offspring trait variation. Life tables started with newborn individuals ~12 hr old from the third or later clutches. For each population, 30 neonates were followed. Neonates were not measured as individuals, but rather as 10-member cohorts consisting of 2 individuals of each of the 5 clones from a population. A few clones did not produce an adequate number of female neonates at the start of a life table; I compensated for this by using additional neonates from other clones in as balanced a fashion as possible. Cohorts were placed in fresh, filtered (1 μm) lakewater every 2 d and maintained on a 16:8 L:D cycle. Water volume was adjusted as animals grew and died to prevent a cohort from filtering more than 50% of their water between feedings (increased from 10-300 mL/ animal as they grew; Knoechel and Holtby 1986). A small amount of cetyl alcohol was placed on the water

surface to minimize surface film mortality (Desmarais 1997). Mortality was recorded daily and fecundity every 2 d until all animals died.

Life history variation was assayed under four different environmental conditions: 20° or 26° at high or low food (20,000 or 3000 cells/mL *Ankistrodesmus falcatus* fed daily), abbreviated 20°H, 26°L, 20°H and 26°L below. Pilot experiments showed that cohorts died in a few days as juveniles at 28° or at only 2000 cells/mL/day food (J. L. Dudycha, unpublished data). The environments represent a benign and stressful temperature and satiating or minimal food levels. All species studied experience a range of variation including these temperatures and food levels in the wild, despite differences in their mean environment (personal observation; A. J. Tessier, unpublished data). In the most stressful environment, 26°L, one population of D. pulicaria failed to reproduce and I was therefore unable to calculate age-specific fitness measures for it.

Space availability precluded conducting all life tables in all combinations of food and temperature concurrently. I timed life tables primarily to prevent any bias in comparisons across populations of one species complex within any given environment (i.e., all populations of a species complex were run together for each environment). This design allows comparison of life history plasticity across taxa without bias. Secondarily, I wanted to minimize bias in comparing the two species

complexes. To do this, life tables for the two complexes were run over roughly the same time period (D. pulex-pulicaria: early June 1996 through March 1997, D. mendotae-dentifera: mid July-1996 through mid-June 1997). Temporal variation in neonate quality or lakewater chemistry, although likely to have minor influence on senescence pattern, limits my ability to assign causation of differences in life history specifically to temperature or food because not all environments were used concurrently.

Estimation of Age-Specific Fitness

Reproductive value specifies the contribution of each age class to *r*, the rate of population increase, and has been suggested as a useful measure for senescence studies (Partridge and Barton 1996). However, it discounts age-specific fitness with the population growth rate (*r*), creating difficulties in interpreting it as a measure of individual performance (Dudycha and Tessier, in press). Nonetheless, it usefully combines mortality and fecundity in a way that shows age-dependent changes in the potential strength of selection. I also estimate age-specific fitness as the expected contribution of each age class to R_0, the total lifetime reproduction. This has a more straightforward relationship to individual performance, because it has no dependence on the passage of clocktime, a factor extrinsic to the biology of organisms. I refer to this measure as the intrinsic value (Dudycha and Tessier, in press).

The form of the reproductive value equation depends on whether newborns are defined as age-class 0 or 1. I defined them as age = 1, and therefore calculated reproductive value as:

$$v_x = \frac{e^{r(x-1)}}{l_x} \sum_{j=x}^{\infty} e^{-rj} l_j m_j$$

(Goodman 1982). I took l_x (cumulative survivorship) and m_x (per-capita daily fecundity) are taken from the life tables. I used Newton-Raphson approximation to calculate r. Intrinsic value, i_x, is the sum of current and expected future reproduction as a proportion of the expected lifetime reproduction of an individual:

$$i_x = \sum_{j=x}^{\infty} l_j m_j \Big/ \sum_{j=0}^{\infty} l_j m_j$$

(Dudycha and Tessier, in press). The numerator of this equation describes the expected fitness of an age-class. Taking this as a proportion of expected lifetime reproduction (R_0) simplifies comparison across taxa that differ in overall capability by causing intrinsic value to reflect relative changes in age-specific fitness rather than absolute changes. Because this measure incorporates both age-specific survival and fecundity, and reflects only the

intrinsic attributes of the average individual, it is an index of age-specific fitness in both the evolutionary and physiological senses.

Statistical Analyses

Two types of regression model were used to test for differences between parental species in their adult survivorship patterns. I used an accelerated failure-time (AFT) model to compare differences in the *timing* of mortality senescence. This model assumes a factor (here, taxon) affects failure time (lifespan) multiplicatively, shifting the timing of hazardous periods (Kalbfleisch and Prentice 1980; Fox 1993). I specified a gamma distribution in the AFT model because its flexibility allows, but does not require, the theoretical expectation of monotonically increasing hazard (Kalbfleisch and Prentice 1980; Fox 1993). Alternative underlying distributions yielded qualitatively concurrent results that are not reported here.

Second, I used a proportional-hazards (PH) model to test for differences among taxa in the magnitude of mortality hazard increase. This is biologically interpreted as testing for differences in the *rate* of mortality senescence. PH models assume that a factor affects hazard functions multiplicatively, changing the magnitude of hazard in a given period (Kalbfleisch and Prentice 1980; Fox 1993). Juvenile mortality was excluded from all analyses (AFT and PH) by estimating maturation age of each species

in each environment and excluding lifespans shorter than that age. All survivorship analyses were run in SAS v. 6.09 with the Lifereg and PHreg procedures.

Repeated-measures ANOVA was used as a split-plot, univariate test for differences among taxa in their patterns of age-specific fitness. This approach accounts for the correlation between repeated measurements of a singel experimental unit. A significant time × taxon interaction indicates differences in age-specific changes of fitness and is evidence of differences in senescence if horizontal (age) compression of one taxon's profile relative to the other's is graphically evident. Statistical tests for the interaction of a factor with time in r-m ANOVA draw degrees of freedom from both the number of replicate experimental units (here, populations) and from the number of times a trait is measured (von Ende 1993). Therefore, the reported degrees of freedom may seem overly large, but they are appropriate because they reflect the high resolution in time at which I measured traits.

Twice I excluded a population from statistical comparisons between species in a particular environment. One was the D. pulicaria population that did not reproduce at 26°L. Because it did not reproduce, I could not calculate fecundity, intrinsic value or reproductive value. I did not exclude it from survivorship analysis. In statistical comparisons of D. mendotae-dentifera at 20°H, one population of D. mendotae was excluded as an outlier; its

survivorship and fecundity were much worse than all other populations of the species complex. Although it is possible that this is a true response this environment, it is more likely a result of an undetected problem at the time the life table commenced. Because inclusion of this population renders some otherwise statistically different comparisons insignificant, I have included this population in all graphs so the reader can see its influence.

Explicit comparisons of life history plasticity within- and between-species complexes were performed on the population growth rate (r), and the persistence of fitness into late life. "Fitness persistence" was defined as the age when only 25% of intrinsic value remained (i_x25), an age sufficiently late in life to reflect most of the divergence among populations, yet minimally affected by any tails of very old ages. Response of r and i_x25 were tested in an ANOVA model that treated food, temperature and species complex as fixed effects, and taxon nested within species complex as a random effect with GLM in SAS v. 6.09. However, the test of an effect of taxon nested within complex was done with the Sheffé model, rather than the SAS model because I am interested in estimating genetic variances due to different taxonomic levels (Fry 1992). When significant genetic variation was indicated, the proportion of variation due to genetic differences within- versus between- species complexes was estimated via maximum likelihood (SAS v. 6.09, proc VARCOMP).

Hybrid populations were not included in tests comparing species' survival, reproductive value or intrinsic value within a given species complex because neither their mortality risks nor their genetics are understood sufficiently to predict their senescence relative to their parents. Hybrid populations were, however, included in comparisons between species complexes and in analyses of plastic responses to environmental variation as a population representative of their complex.

RESULTS

Differences between D. pulex and D. pulicaria life histories were moderately robust across environments. At 20°, D. pulex mortality rates accelerated substantially earlier and faster than in D. pulicaria. These effects were either minor or statistically insignificant at 26°. (Figure 1, Table 1). Age-specific fitness decreased faster in D. pulex than in D. pulicaria in all but the most stressful environment (26°L), regardless of whether age-specific contribution to fitness was measured with respect to R_0 (intrinsic value; Table 2, Figure 2) or r (reproductive value; Table 2, Figure 3). In no environment was there evidence that D. pulicaria senesced faster that D. pulex. Temperature mainly affected mortality schedules, whereas food level mainly affected fecundity. Overall, D. pulicaria exhibited a much more plastic mortality schedule than D. pulex (Figure 1), causing strong differences between species in senescence at 20°, while only a slight or no difference at

26°. The species achieved similar maximal reproductive values at 20° (Figure 3) with a similar effect of food level. However, at 26° D. pulex reproductive value was higher and more sensitive to food level, reflecting its greater early fecundity.

Differences between D. mendotae and D. dentifera were less pronounced and more variable than those between D. pulex and D. pulicaria. Comparisons of adult mortality are complicated by substantial juvenile mortality in low food environments, particularly at 26°. However, there was evidence that adult mortality accelerates later and more slowly at 26°H and 20°L in D. dentifera than in D. mendotae, whereas the reverse was true at 20°H (Table 1, Figure 4). Intrinsic value followed a pattern similar to mortality with loss occurring more slowly in D. dentifera than D. mendotae at 26°H and 20°L, but more rapidly at 20°H (Figure 5, Table 2). The reversed pattern at 20°H is dependent on exclusion of the apparent outlier; when it is included, no differences between these species are statistically significant at 20°H. Survivorship, and consequently, intrinsic value of D. mendotae-dentifera were most strongly influenced by temperature, with differences between species evident in three environments (Figure 5). For D. dentifera, reproductive value varied only mildly with age in all environments, causing a slow loss of age-specific fitness with respect to *r* (Figure 6). Sharper declines were evident in D. mendotae (Table 2), driven by higher fecundity peaks

(26°H, 26°L, 20°H) or earlier mortality (20°L), however confidence is low in the differences at low food.

ANOVA showed that temperature and food level affected life histories in markedly different ways. Food level strongly influenced *r* in all species, but had little impact on the index of fitness persistence ($i_x 25$); temperature had the reverse effects (Figure 7). Temperature also influenced the degree of differentiation in fitness persistence among the populations, with greater differentiation evident at the benign temperature. These patterns were strikingly similar in both species complexes, so they were analyzed jointly, pooling the effects of environment across taxonomy.

Food level was the only factor that had a substantial and significant influence on *r* ($p < 0.0001$, $F = 249.2$, $df = 1, 4.29$; Figure 7). Temperature had a slight effect on *r* ($p = 0.0178$, $F = 13.7$, $df = 1, 4.36$; Figure 7), but neither species complex ($p = 0.2095$, $F = 2.19$, $df = 1, 4.16$) nor taxon nested within species complex ($p = 0.4159$, $F = 1.79$, $df = 4, 1.68$) had any significant effect. There were no significant interactions among any factors. Driven by food, the total model explained >80% of the variation in *r*. The lack of any genetic role in explaining *r* variation indicates that comparisons of taxa in other aspects of life history are not confounded by differences in fitness with respect to the broad set of environments chosen.

Fitness persistence (i_x25), in contrast to r, was primarily influenced by temperature rather than food level, although food effects were significant (Table 3). Genetic variation was not apparent between complexes but was strong among taxa within complexes (Table 3). There was also a significant interaction of temperature with taxa within complex. This was caused by a larger portion of variation in fitness persistence being due to genetic variation within complexes at 20° (54.4% of total variation) than under thermal stress (29.1%). At neither temperature was any of the variation in fitness persistence due to genetic differences between the two species complexes. No other interactions were significant, although a marginally significant food by temperature interaction (Table 3) may be contributing to the environmental effects on fitness persistence.

DISCUSSION

The expected pattern of accelerated senescence under higher temperature and food level was evident in all four species of Daphnia. This is consistent with prior studies of these environmental variables on Daphnia longevity (MacArthur and Baillie 1929; Ingle 1933; Ingle et al. 1937; Korpelainen 1986). By studying them jointly, we see that the plastic response of senescence to food was curtailed under thermal stress. The response of senescence had little relationship to the response of population growth rate, r (Figure 7). Across species, r was similar and more responsive to food than

temperature. Despite these broad patterns, clear differences between species emerge from taxonomic comparisons made under different conditions.

Differences were not strong in comparisons between the two species complexes, the highest taxonomic level studied. None of the genetic variation in senescence, as indicated by fitness persistence ($i_x 25$), could be attributed to differences between the complexes. However, there was substantial genetic variation of senescence pattern among taxa within complex. Although this was true for both complexes, it is apparent that D. pulex-pulicaria harbors a wider range (Figures 2, 5). Because D. mendotae-dentifera has a smaller range of senescence variation, and its range occurs roughly midway between D. pulex and D. pulicaria, no inter-complex difference is apparent when their mean senescence is compared. The rank ordering of slowest senescence in D. pulicaria, moderate senescence in D. mendotae-dentifera and fast senescence in D. pulex suggests that senescence rate does not correspond to ancestry at the species level. Instead, this ranking roughly corresponds with known ecological variation in the mortality risk these species face, with D. pulicaria inhabiting a stable, low-predation environment, D. mendotae-dentifera facing substantial vertebrate and invertebrate predation, and D. pulex facing catastrophic mortality a few months after their environment becomes habitable.

There was also striking evidence of genetic variation in phenotypic plasticity among taxa within complexes, but no obvious difference between complexes (Table 3). Specifically, the effect of temperature on senescence patterns was strong in both complexes while the effect of food was modest. Temperature also affected the magnitude of genetic variation within species complexes, which accounts for more than half of the variation at 20°, but less than a third at 26° (Figure 7). This means that the phenotypic variation of senescence on which selection could act is greatest under benign conditions. Conversely, it suggests that evolved differences in senescence are reduced under stressful conditions. Though the pattern was the same across species, the response to temperature was most pronounced in D. pulex-pulicaria. Thus, there are two broad associations of genetic differences in senescence and the plasticity of senescence. First, variation in both the degree and plasticity of senescence is pronounced at the taxa-within-complex level, but is not found between complexes. Second, genetic variation of both senescence and the plasticity of senescence is greater within D. pulex-pulicaria than within D. mendotae-dentifera.

Senescence in D. pulex-pulicaria

The comparisons of life history between D. pulex and D. pulicaria suggest that in the natural environment of ponds and lakes, D. pulicaria can extend lifespan and reproduction to greater ages than D. pulex. Under the

benign temperature (20°) D. pulex senesced faster than D. pulicaria, regardless of food level or how age-specific fitness was measured. However, at the stressful temperature (26°), senescence was overall accelerated relative to the benign temperature and interspecific differences were reduced. In the field, the temperature animals experience will often be lower than those studied here. It is unlikely that at lower temperatures, D. pulex would exhibit such extraordinary survivorship plasticity to generate a slower senescence rate than D. pulicaria. It is similarly unlikely that lower temperatures would produce substantially faster senescence in D. pulicaria, given that this would be contrary to much that is known about senescence in ectotherms (Comfort 1979; Rose 1991). It seems safe to conclude that, despite some substantial genetic-environment interactions, the species from a temporary habitat senesces faster than the species from a permanent habitat, as predicted by the evolutionary theory of senescence.

The comparison of D. pulex with D. pulicaria at 26°H demonstrates that qualitatively different results can be obtained with intrinsic value and reproductive value, two different methods of combining mortality and fecundity to measure senescence. At 26°H, the intrinsic value of D. pulex appeared to decline more slowly than D. pulicaria (Figure 2); though slight, the difference in rates was significant (Table 2). However, reproductive value declined more rapidly in D. pulex, as a result of declining from a higher maximum reproductive value over approximately the same lifespan as D.

pulicaria (Figure 3); this difference in rates was also significant (Table 2). It is therefore especially important that a clear specification of the biological phenomenon of interest is made to draw accurate conclusions about senescence evolution. The interspecific differences in intrinsic value appear to be due to a higher level of plasticity in D. pulicaria to extend fitness into late life, whereas interspecific differences in reproductive value appear to be due to a higher level of plasticity in D. pulex to increase fecundity early in life.

Senescence in D. mendotae-dentifera

The comparison of life history in D. mendotae-dentifera is quite different than in D. pulex-pulicaria. This is expected, because the differences in ecological niches between D. mendotae and D. dentifera are not as large. Only under ideal conditions, abundant food and benign temperature, were the survivorships and fecundities of the two species strongly different. Even so, differences in mortality rate acceleration (timing and magnitude) were generally significant, and differences in age-specific fitness declines were significant when resources were abundant. Small but significant differences between the parent species were a result of the low differentiation among replicate populations within species.

Interpretation of the mortality patterns in D. mendotae-dentifera is hampered under scarce resources due to poor juvenile survivorship in both

species. In part, this may be due to plasticity of maturation time, which lengthened when food was low. This response was not evident in D. pulex-pulicaria, and implies that resources play different roles in juvenile success of the two complexes. Adult mortality patterns were clearer with high food because juvenile mortality was low. In general, the species showed similar levels of survival plasticity, except that a greater response to temperature was seen in D. mendotae at high food than in D. dentifera (Figure 4).

Considering all environments, the data suggest that the rate and timing of senescence have been decoupled in D. mendotae-dentifera. Because most work on the evolution of senescence has addressed only the rate of senescence, it is unclear how common decoupling of rate and timing is. D. mendotae had faster mortality rate accelerations, but the timing of mortality rate acceleration was later than in D. dentifera (Figure 4, Table 1). Declines of age-specific fitness as measured by reproductive value were also significantly different between D. mendotae and D. dentifera with high food. Because D. dentifera never achieved fecundity as high as those of D. mendotae, reproductive value in D. dentifera was always low, and consequently declined slowly. However, it was D. mendotae whose reproductive value typically peaked later (Figure 6). Because faster and earlier senescence do not appear to co-occur in this complex, this indicates that these are distinct aspects of senescence.

Patterns of intrinsic value decline were significantly different between the species at 20°, but the differences were opposite at the two food levels. The beginning of intrinsic value decline was similar between species, but the relative rates *reversed* depending on resource abundance (Figure 5). This reversal appears to be caused by a greater tendency for D. mendotae to respond to food by changing its investment in late life survival (Figure 4). The greater plasticity of D. mendotae is also evident in reproductive value (Figure 6), likely resulting from a greater responsiveness of fecundity. Although the low degree of within-species variation allowed detection of significant differences at 26°H, the small degree of difference may be of little import.

Environmental effects on senescence

Examining the relationship between r, a fitness measure that is highly sensitive to early-life events and insensitive to late-life, and an organism's ability to continue contributing to fitness as it ages is one approach to viewing the general structure of a life history. Food level had a strong positive effect on r through its effect on fecundity. However, it had a much smaller effect on fitness persistence ($i_x 25$). The reverse is true of temperature (Figure 7). Benign temperature led to extended fitness persistence relative to stressful temperature, but it had only a minor effect on r. Explanations of the genetic mechanisms causing the evolution of senescence assume, for simplicity, that the environment has no effect on the expression of deleterious mutations or

pleiotropies (Williams 1957; Hamilton 1966, Charlesworth 1980). However, it is clear that the environment does have an effect, in these data and in reports from other organisms (Comfort 1979; Finch 1990; Rose 1991).

Temperature also has an interesting impact on the expression of among-population genetic variation in *r* versus fitness persistence. At a stressful temperature, variation of fitness persistence was low relative to a benign temperature, suggesting that the impact selection could have is reduced under temperature stress. However, the stressful temperature also revealed stronger variation in *r* than the benign temperature, suggesting that evolutionary change will be rapid. The benign temperature allows expression of differential fitness persistence, while a high resource level elevates population growth rate, so even small variation can be selected on quickly. Thus selection should be most efficient in the most benign environment, and the life history relatively optimized for such an environment.

Collectively, the data reported here suggest that evolutionary differentiation of senescence in Daphnia has been recent and may be actively evolving. Differences in senescence were minimal across the deepest evolutionary split, but were strong within species complexes that are incompletely diverged. Genetic variation in senescence is apparently distributed at finer levels of phylogenetic relationship than subgenus. In general, differentiation of senescence patterns among taxa is more related to

ecological differences among habitats than to the span of time since evolutionary divergence. The pond-lake contrast of D. pulex-pulicaria is ecologically much greater than the lake-lake contrast of D. mendotae-dentifera, and we find a correspondingly larger contrast in overall senescence and plasticity of life history in D. pulex-pulicaria. This pattern of strong variation at the tips of phylogeny suggests that senescence is a trait that is actively evolving in the wild.

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Table 3-1. Differences in mortality rate acceleration between nominal species of Daphnia. PH refers to the proportional hazards model, testing whether the rate of mortality senescence differs. AFT refers to the accelerated failure-times model, testing whether the timing of senescence differs. For all tests df = 1.

	PH (rate)		AFT (timing)	
	p	χ^2	p	χ^2
D. pulex-pulicaria				
26° High Food	0.9039	0.015	0.8264	0.04
26° Low Food	0.0190	5.5	0.0966	2.8
20° High Food	< 0.0001	57.6	< 0.0001	566.2
20° Low Food	< 0.0001	82.3	< 0.0001	548.4
D. mendotae-dentifera				
26° High Food	0.0002	14.2	< 0.0001	24.1
26° Low Food	0.5813	0.30	< 0.0001	40,115.1
20° High Food	< 0.0001	23.4	< 0.0001	2896.9
20° Low Food	0.0076	7.1	0.0076	7.1

Table 3-2. Repeated measures ANOVA of intrinsic value and reproductive value in Daphnia. Differences in rates of senescence are indicated by significant time x species interactions, so only the interaction effect is listed.

	Intrinsic Value			Reproductive value		
	p	F	df	p	F	df
D. pulex-pulicaria						
26° H	< 0.0001	4.11	1, 18	< 0.0001	16.23	1, 17
26° L	0.1638	1.40	1, 19	0.0386	0.33	1, 18
20° H	< 0.0001	23.24	1, 46	< 0.0001	8.81	1, 45
20° L	< 0.0001	3.99	1, 76	0.0308	3.74	1, 75
D. mendotae-dentifera						
26° H	< 0.0001	3.72	1, 25	< 0.0001	3.98	1, 24
26° L	0.2689	1.21	1, 19	0.6443	0.47	1, 18
20° H	< 0.0001	23.76	1, 38	< 0.0001	24.44	1, 37
20° L	< 0.0001	6.38	1, 43	0.2898	6.28	1, 42

Table 3-3. Tests of genetic variation in the plasticity of the age when only 25% of intrinsic value remains (i_x25); for the full model $r^2 = 0.91$.

Source	df	F	p
Species complex	1, 4.0	1.03	0.37
Temperature	1, 4.0	22.32	0.0091**
Food level	1, 4.1	10.00	0.0332*
Taxon (complex)	4, 39	12.29	<0.0001***
Complex x temperature	1, 4.0	1.70	0.267
Complex x food level	1, 4.1	0.89	0.40
Complex x food x temperature	1, 4.2	1.43	0.29
Temperature x food level	1, 4.2	6.28	0.0634
Taxon (complex) x temperature	4,4	12.8	0.0149*
Taxon (complex) x food level	4,4	2.61	0.19
Taxon (complex) x food x temp	4, 39	0.79	0.54

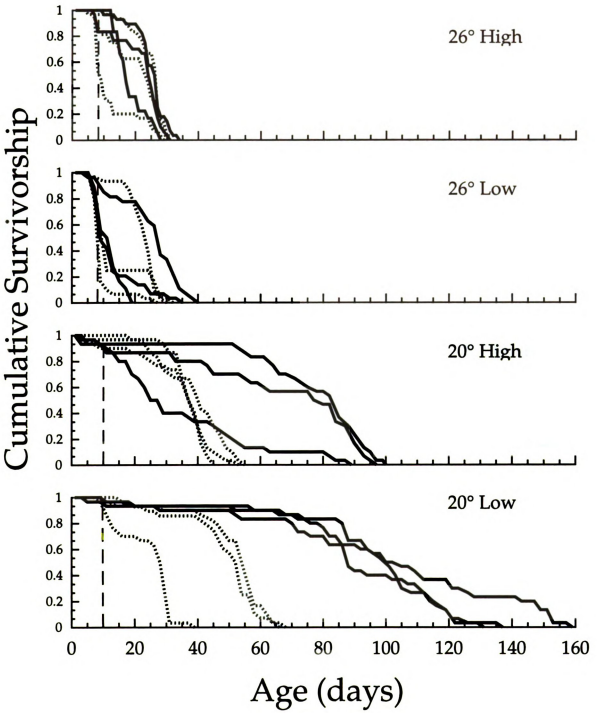

Figure 3-1. Survivorship of D. pulex-pulicaria in four environments. Solid lines are replicate populations of D. pulicaria, dotted lines are D. pulex. Dashed vertical lines indicate age at maturity.

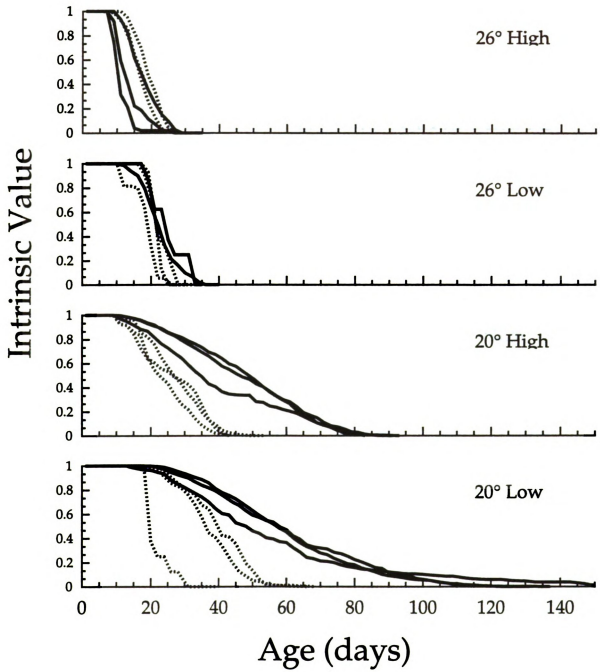

Figure 2. Intrinsic value of D. pulex-pulicaria. Solid lines show replicate populations of D. pulicaria, dotted lines are D. pulex. At 26°, one population of D. pulex is virtually hidden behind another.

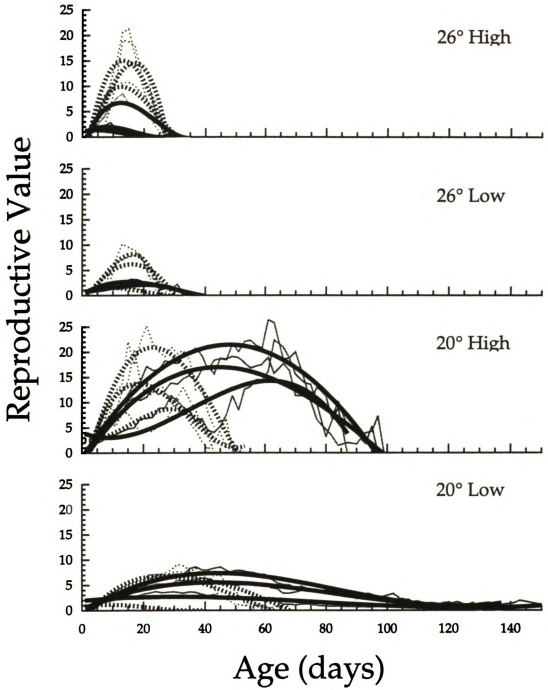

Figure 3. Reproductive value of D. pulex-pulicaria. Solid lines show replicate populations of D. pulicaria, dotted lines are D. pulex. Black curves are cubic polynomials fit to the data via least-squares regression.

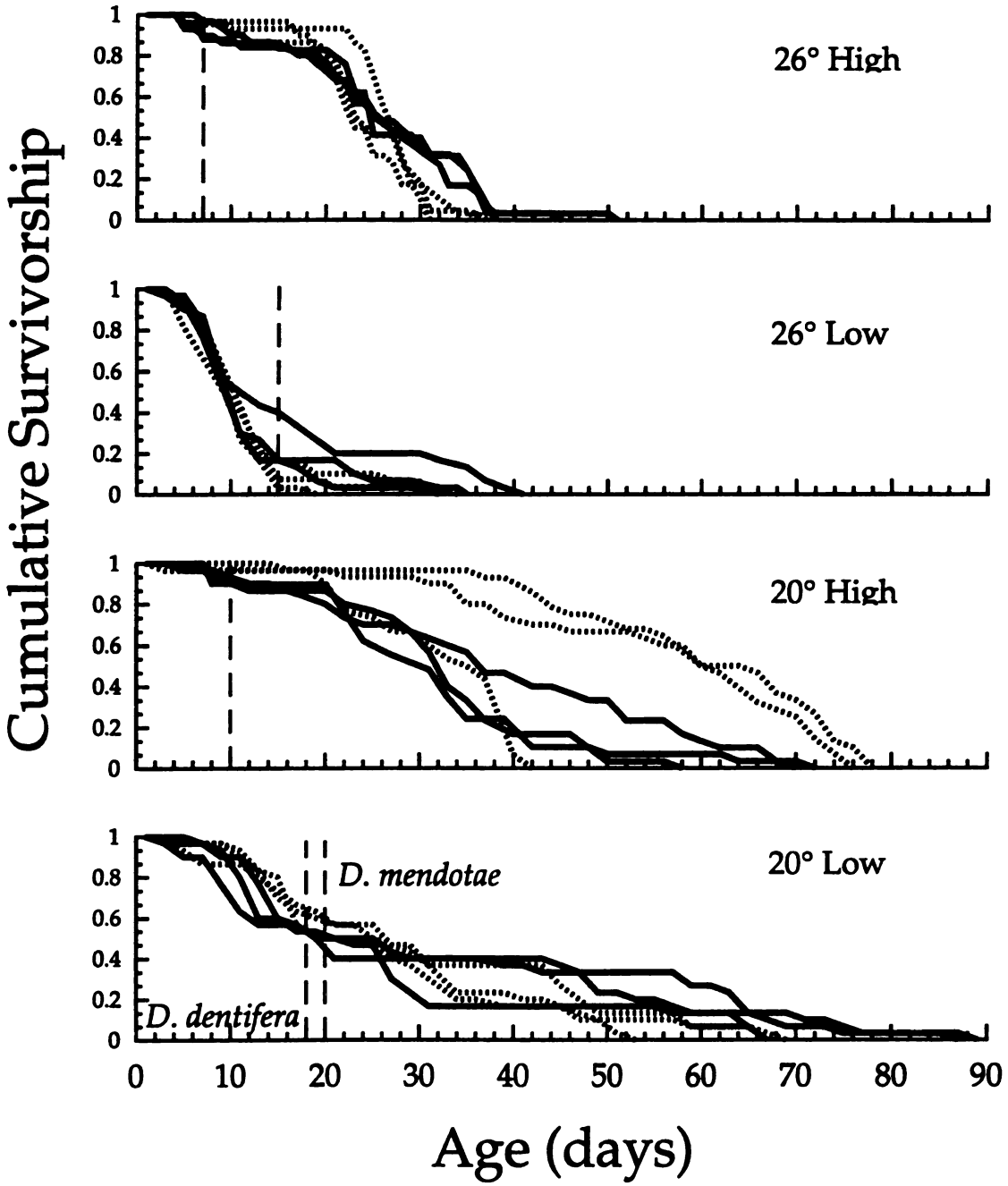

Figure 4. Survivorship of D. mendotae-dentifera in four environments. Solid lines are replicate populations of D. dentifera, dotted lines are D. mendotae. Dashed vertical lines indicate age at maturity. The extreme population of D. mendotae is excluded from statistical comparison (see text for explanation)

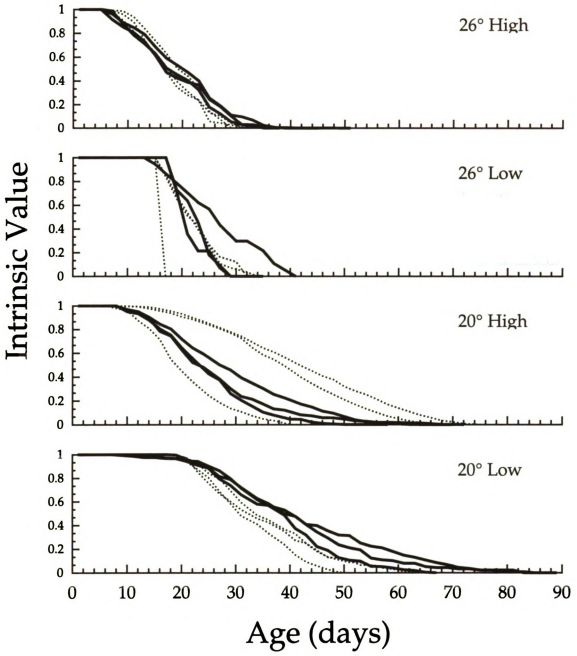

Figure 3-5. Intrinsic value of D. mendotae-dentifera. Solid lines show replicate populations of D. dentifera, dotted lines are D. mendotae. The extreme population of D. mendotae is excluded from statistical comparison (see text for explanation)

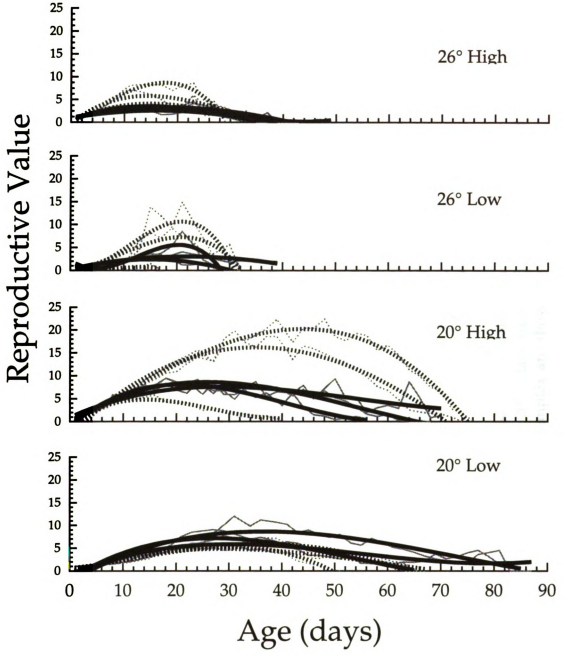

Figure 3-6. Reproductive value of D. mendotae-dentifera. Solid lines show replicate populations of D. dentifera, dotted lines are D. mendotae. Black curves are cubic polynomials fit to the data via least-squares regression. The extreme population of D. mendotae is excluded from statistical comparison (see text for explanation)

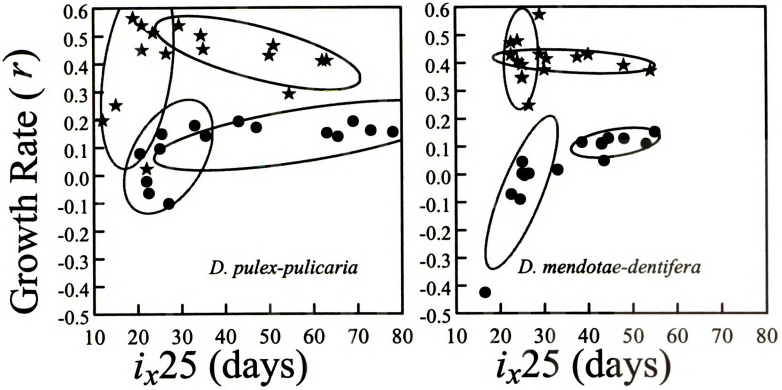

Figure 3-7. Estimated population growth rate and persistence of fitness into late life in four laboratory environments. Grey symbols are 26°, black symbols are 20°. Stars are high food, circles are low food. For each environment, eight populations of each species complex are shown, including two hybrid populations. Standard error probability ellipses are drawn around data for each environment.

CHAPTER FOUR: MORTALITY RISK AND THE ECOLOGICAL DISTRIBUTION OF SENESCENCE VARIATION

Introduction

Ecology is classically described as the study of the distribution and abundance of organisms. Ecologists have long argued that how organisms respond to their environment is critical to understanding their distribution and abundance. Ecology is fundamentally joined with evolutionary biology in a feedback loop because selection is determined by ecological interactions and the traits through which an organism interacts with its environment are determined by the evolutionary forces its ancestors experienced. This basic link is at the root of a view that exploring the distribution of traits in the environment is an illuminating approach to understanding why particular distribution patterns of organisms exist (Schoener 1986; Chapin, et al. 1997; Tessier, et al. in press). In this approach, researchers ask if environmental heterogeneity determines the distribution of traits across nature, and if so, how?

Life history encompasses survival and reproduction, the ultimate traits through which all other traits are filtered by natural selection. Because life history is a summary outcome of all of an organism's interactions, it can not directly reveal how an organism interacts with its environment. Instead it reflects the net effect of positive and negative interactions. Futhermore, life history is constrained by evolutionary history and tradeoffs, preventing an organism from having a life history that is ideally responsive to ecological

variation. No one life history can be optimal in all situations. An enormous diversity of life histories is distributed throughout nature and ecological factors determine whether a particular life history will succeed or fail through natural selection. Patterns of ecological heterogeneity generate variation in the traits favored by selection and therefore influence the patterns in which life histories are distributed.

Senescence is not traditionally approached as a life history trait crucial to understanding the relationship between the environment and the distribution of organisms. Conversely, most scientists seeking to understand senescence have not sought ecological explanations for why it is highly variable among taxa. However, senescence is potentially useful for understanding how organisms perform in their environments because as a component of integrated life histories it may affect overall constraints on life history optimization. It is also possible that senescence has widespread, if subtle, effects on ecological interactions, by lowering an individual's capabilities.

The basic conundrum of senescence is that it is a deleterious, nearly ubiquitous trait, and yet natural selection fails to eliminate it. Throughout this paper I treat senescence as the degradation of an organism's state (i.e., the ability to function) that accompanies advancing age. This means that senescence is a decline in age-specific fitness from the physiological perspective, and consequently, the Darwinian perspective as well. In life history statistics, senescence appears as age-specific declines in fecundity and the conditional probability of survival.

Why has natural selection failed to eliminate this trait that is directly deleterious to Darwinian fitness? Or, in a simplified view, why doesn't everything live longer than it does? The obvious answer, that there are costs to prolonging physiological fitness, is unsatisfactory because cost alone can not explain why there is so much variation in senescence. The evolutionary theory of senescence offers an explanation because it predicts a causal relationship between mortality risk and senescence (Williams 1957). The mortality risks organisms face are highly variable in space, time and scale and therefore lead to variation of senscence.

The evolutionary theory of senescence is based on the notion that the force of natural selection declines as organisms age (Medawar 1952). This decline occurs because as organisms age, an increasing amount of their reproductive potential is in their past. This occurs even in an imaginary non-senescing population that is exposed to extrinsic mortality risks. Traits expressed at specific ages can only influence the realization of future reproductive potential. As time passes and future reproductive potential decreases, logically the force of natural selection will also decrease because genes causing deleterious traits at late ages will have been passed on to the next generation when the individual was younger.

The theory leads to a direct link between the extrinsic mortality risk (i.e., the risk of death due to causes other than senescence) organisms face and the degree of senescence they will evolve towards (Williams 1957). As extrinsic

mortality risk varies, so does the potential benefit of investing in maintaining a body so that fitness persists into later life. In an environment with a high risk of extrinsic mortality, there is little utility in late-life investment because organisms will likely die before a payoff can be realized. This argument assumes that there are indeed costs to investment in late-life, otherwise even the slightest chance that an individual may continue surviving is worth betting on. The basic prediction is that there will be a negative relationship between extrinsic mortality risk and investment in late-life performance.

A handful of studies have explored the mortality risk hypothesis of the evolutionary theory of senescence, generally using coarse descriptors of mortality risk in conjunction with estimates of mean lifespan. The ability to fly, a trait that presumably improves predator avoidance, is associated with longer maximum lifespan in mammals (Prothero and Jurgens 1987) and birds (Holmes and Austad 1995). A comparable rationale was used by Keller & Genoud (1997), who attributed the relatively long lifespans of eusocial queen ants to their protection from extrinsic mortality by workers.

Three studies are noteworthy because they sought to measure senescence based directly on changes of age-specific life history. Tatar, et al. (1997) predicted that potential lifespan of grasshoppers would be related to season duration and quanified age-specific intrinsic mortality rates in male grasshoppers from populations at different elevations. They showed that, when raised in a common environment, low elevation grasshoppers (i.e., long season) live longer and have slower increases in age-specific intrinsic mortality than those from high

elevation. In a similar vein, Dudycha and Tessier (1999) demonstrated a relationship between habitat permanence and senescence of *Daphnia*, with populations in temporary ponds senescing more rapidly than those from permanent lakes. Ricklefs (1998) applied a three-parameter Weibull model to mortality schedules of birds and mammals to estimate initial adult mortality, assumed to be representative of the extrinsic mortality risk, and showed this is related to the rate at which mortality increased later in life. None of these studies actually measured the mortality risk animals face in the wild.

Objective of this study

Here I report on a study designed to test the hypothesized relationship between extrinsic mortality risk and senescence by directly measuring the mortality risk natural populations experience and quantifying genetic variation of senescence among those populations. Dudycha and Tessier (1999) documented substantial genetic variation of senescence in the *Daphnia pulex-pulicaria* species complex of freshwater microcrustaceans. They showed that the genetic variation roughly corresponded to the catastrophic mortality risk that populations in temporary ponds face or the low mortality risk known from local permanent lakes (Leibold and Tessier 1998). I expand upon this work by measuring population dynamics, birth rates and death rates of *D. pulex-pulicaria* in permanent and temporary waters. I measured these properties throughout a year because there is potentially much seasonal variation in mortality risk. Additionally, I constructed a phylogenetic tree from mitochondrial DNA to

evaluate whether selection was enforcing a match between populations' life history characteristics and their ecological conditions.

The model system

Daphnia are an ideal model system for the study of evolutionary ecology because their natural dynamics are simple to follow, and genetic variation of traits can be easily quantified. *D. pulex* and *D. pulicaria* are common microcrustaceans, whose status as independent species is uncertain because interspecific hybridization is common. A hierarchical phylogeny based on DNA failed to resolve them as discrete species (Lehman, et al. 1995). Others have found only weak support for their separation (Crease, et al. 1996; Colbourne, et al. 1998). Systematics work historically regarded them as one species (Brooks 1957) and they are generally indistinguishable by morphology (Hebert 1995). However, their nominal labels have remained useful to ecologists and they are typically applied on the basis of a diagnostic allozyme (LDH) or habitat (Hebert 1995, Deng 1997). I will continue to use both species names as a shorthand for the ecological conditions populations experience, despite new evidence that the specific populations studied here also fail to resolve into two distinct genetic species (see Local Phylogeny, below).

Daphnia pulex is typically found in temporary ponds, while *D. pulicaria* is found in deep lakes (Hebert 1995). Variation in pond size, local hydrology and annual weather patterns create variation in the length of time ponds are habitable by *D. pulex*, but all of my study ponds repeatedly dry by summer.

Hence, maximum lifespan of *D. pulex* is constrained to a few months by the temporary nature of their habitat. In contrast, *D. pulicaria* lives in a permanent and environmentally more stable habitat, and can persist year-round in deep lakes (Geedey, et al. 1996; Geedey 1997). In the absence of any strong abiotic constraints, resources and predators regulate *D. pulicaria* population dynamics. Planktivorous fish are the most important predator, but *D. pulicaria* can effectively avoid them in deep water (Tessier and Welser 1991). Populations of *D. pulicaria* display low birth and death rates even at the time of peak planktivory (Leibold and Tessier 1998).

Genetic variation of senescence

Trait measurement.— I quantified genetic variation of senescence by constructing mortality and fecundity schedules on replicate populations of *D. pulex* and *D. pulicaria*. To combine mortality and fecundity into a single measure of age-specific fitness, I calculated the ratio of current and expected future fecundity to the expected lifetime fecundity (Dudycha and Tessier 1999). This measure, hereafter called intrinsic value, gives the proportionate age-specific contribution to R_0. Intrinsic value is a useful measure of senescence because it is uncomplicated by age-dependent tradeoffs between mortality and fecundity and it reasonably represents the physiological state of an average individual (Dudycha and Tessier 1999).

In this report, I augment the data from six populations reported in Dudycha and Tessier (1999) with life history information on an additional three populations of *D. pulex* and four of *D. pulicaria*. Most populations were used in both the estimation of life history traits and field demography measurements; however, one *D. pulicaria* population (Deep Lake) and one of *D. pulex* (Mormon Creek Pond) were logistically difficult to sample and omitted from demographic measurements. Clones from one *D. pulex* population (Woodfrog) were not available when life tables were constructed.

Populations were chosen to represent the extremes of the habitat permanence gradient. This did not affect the choice of *D. pulex* populations, but *D. pulicaria* populations were chosen based on data indicating they were perennial populations (Geedey 1997). All populations are in SW Michigan, except one of *D. pulex*, which is in Michigan's Upper Peninsula. *Daphnia* will reproduce in the lab by ameiotic parthenogenesis, allowing the capture of natural genetic variation by randomly sampling a population and establishing clonal lines. I isolated five randomly chosen clones from each population. Five clones will not completely represent the variation present within a population, but will reduce the chance of being misled by a single clone. Lineages were acclimated to lab conditions (20-22°, satiating food) for ≥ 3 generations prior to life history trait measurement (Tessier and Consolatti 1989, 1991). Routine monitoring and microscopic examination (at 460x) verified that lineages were free of parasites.

Mothers of experimental animals were raised from birth for several weeks with satiating food at low density (1/50 mL) to minimize variation due to

maternal effects. Life tables were run in two blocks (including 6 or 7 populations, respectively), starting each with neonates (~12 hr old) from the mothers' third or later clutches. For each population, 3 or 4 cohorts consisting of 2 neonates from each of the 5 clones were set up, for a total of 30 or 40 individuals per population. Minor deviations from this ideal occurred in a few populations because some clones produced only a few female neonates on the starting day. The life tables were therfore constructed in a nested design, with cohorts (the lowest level of measurement) nested in population nested in species. Juvenile suvivorship was near perfect in all populations, with no bias between species. Animals were fed a satiating food level (20,000 cells/mL *Ankistrodesmus falcatus* daily), and raised in growth chambers at 20° on a 16:8 L:D cycle, and transferred to fresh, filtered lakewater every other day. As animals grew and died, water volume was adjusted so animals could not filter more than 50% of the water between feedings (Knoechel and Holtby 1986). Survivorship was recorded daily and fecundity every 2 d until all animals died. Females who produced diapausing eggs were temporarily excluded from estimates of m_x because information to convert investment in diapause into an equivalent investment in immediate reproduction is inadequate (Lynch 1989; Dudycha & Tessier 1999).

Data analysis.— Throughout the statistical analyses, I treated the data from Dudycha and Tessier (1999) and the new populations as two separate blocks. The experimental blocks were not measured concurrently, and it is possible that seasonal differences in water chemistry or laboratory differences in maternal quality affected the experimental animals' life histories. The first block

(30 individuals/population) included Warner, Pine, Lawrence (*D. pulicaria*), Wisdom, Otislake III, and Mormon Creek Pond (*D. pulex*). The additional populations in block two (40 individuals/population) were Hamilton, Three Lakes II, Lake XVI, Deep (*D. pulicaria*), POVI, Bittern, and Roughwood (*D. pulex*).

It is common practice in studies of senescence to focus on mortality despite the potential for tradeoffs with fecundity. Separating the analysis of mortality and fecundity provides insight into the pathways through which senescence affects evolutionary fitness. To determine if a degradation in survival ability was apparent, I pooled the data for each ecologically-defined species and fit it to a Weibull model. In a Weibull model, age-specific mortality (μ) at age x is defined $\mu_x = \lambda\gamma(\lambda x)^{\gamma-1}$ (Lee 1980). If $\gamma > 1$, the hazard function increases with age, indicating a declining ability to survive that is typically interpreted as senescence. λ scales the model to a baseline rate of mortality, but is not indicative of senescence.

I applied two types of regression model to the mortality data to test for interspecific differences in the degradation of survival ability. With an accelerated failure-time (AFT) model, I tested the *timing* of decreasing survival ability. (Kalbfleisch and Prentice 1980; Fox 1993). AFT models require one to specify an underlying distribution; I chose a gamma distribution because it is a flexible general distribution that permits, but does not force, the expectation of monotonically increasing age-specific mortality. I also ran tests using Weibull and exponential distributions, and found the results to be robust regardless of

distribution. With a proportional-hazards (PH) model, I tested for differences among taxa in the magnitude of mortality hazard increase, which can be interpreted as the *rate* of senescence. Juvenile mortality was excluded from these analyses.

I used univariate repeated-measures ANOVA to test for differences between species in the pattern of age-specific fecundity and in the shapes of their intrinsic value curves. A significant time × species interaction indicates differences in the rates of change within fecundity profiles. In r-m ANOVA, the degrees of freedom for interactions between time and the between-subjects factor (here, species) are drawn from both replication of the experimental unit (here, population) and the number of repeated measurements made. R-m ANOVA assumes that the data are circular, meaning that the variance of the differences in trait value between any two ages is homogeneous. Although the tests are moderately robust to violations of this assumption, F-statistics can be inflated for the within-subjects factor (time) and its interactions (time × species) (von Ende 1993). When the assumption of circularity was not met, I adjusted the F-test degrees of freedom by the Huynh-Feldt epsilon (von Ende 1993). All life history analyses were run in SAS v. 6.09 with Lifereg, PHreg or GLM.

Life history results.— Life history results confirmed the differences reported in Dudycha and Tessier (1999). Lifespan was substantially shorter in *D. pulex* than in *D. pulicaria* (Figure 1). Both species had increasing age-specific mortality hazards. The Weibull shape parameter was notably larger in *D. pulex* (γ

= 4.7; 95% CI = 4.3 - 5.3) than in *D. pulicaria* (γ = 2.3; 95% CI = 2.0 - 2.5), indicating that in *D. pulex* survival declines faster than in *D. pulicaria*. Both of these parameter values are somewhat lower than those reported for the analysis of only block one (Dudycha & Tessier 1999), reflecting the greater genetic variation in the pooled data. As in the earlier analysis, *D. pulex* had both an earlier period of mortality senescence (AFT model; $p < 0.0001$, $\chi^2 = 97.04$, df = 1) and a faster rate of mortality senescence (PH model; $p < 0.0001$, $\chi^2 = 49.02$, df = 1). Fecundity also differed strongly between the two species (Figure 2). Fecundity peaked earlier and declined faster in *D. pulex* than in *D. pulicaria* (time × species interaction; $F = 4.75$, $p < 0.0001$, df = 1, 48, $\varepsilon = 0.54$), but did not differ between blocks ($p = 0.25$, $F = 1.49$, df = 1,9). Intrinsic value also declined faster in *D. pulex* than in *D. pulicaria* (time × species interaction; $F = 40.7$, $p < 0.0001$, df = 1, 48, ε = 0.10; Figure 3).

Field Demography

Methods.— In order to estimate extrinsic death rates, demographic parameters were measured in six replicate populations each of *Daphnia pulicaria* and *D. pulex* from October 1997 through September 1998. The general procedure was to measure change in population density to estimate instantaneous population growth rates (r) and clutch size, egg age distribution and temperature to estimate instantaneous birth rates (b), similar to Tessier, et al. (1992). Egg age distribution was determined based on developmental stages described in

Threlkeld (1979) and pictured in Esslová (1959). By incorporating information about the egg age distribution, many assumptions in calculating birth rates from clutch size and temperature are eliminated and a more accurate estimate of b can be obtained (Bottrell, et al. 1976; Threlkeld 1979; Rigler and Downing 1984). Once r and b have been estimated, the instataneous death rate (d) is taken from the equation $r = b + d$. Because I used the egg age distribution in birth rate calculations, estimates of d produced this way are primarily subject to sampling error through measurement of r (DeMott 1980). For this reason I adopted sampling protocols for population density that are spatially integrative and highly repeatable, minimizing sampling variation in r.

Different sampling protocols for lakes and ponds were dictated by habitat differences. In the six lakes, samples were taken with vertical hauls of an 80μm mesh, 11.5 cm diameter Birge plankton net. On each sampling date, three replicate samples were taken. Each sample consisted of three vertical hauls made at different locations (> 10m apart) in the deepest area of the lake and then pooled. No attempt was made to precisely duplicate locations on different dates other than returning to the deepest area of the lake. Lakes were sampled to obtain population densities throughout the year, and estimates of birth rate during focal periods (late October, late March, late May and early September) representative of the major changes in *D. pulicaria* dynamics (Hu and Tessier 1995; Threlkeld 1979). For each focal period, samples were taken on three dates separated by the egg development time indicated by the water's average temperature. Birth rate was also estimated in mid December (under ice-cover) in four populations.

In the six ponds, three replicate spatially pooled samples were taken with a 3-L pitcher. For each sample, I plunged the pitcher into the water at 10 - 20 locations, moving through the pond without backtracking into disturbed areas. At each location, I collected three pitcherfuls of water, passing each through a separate 80μm mesh screen. At successive locations, I varied both the depth from which the water was taken and the order in which the screens were used. This procedure resulted in three samples composed of 30 - 60 L of water taken from the same locations, but minimized the effects of spatial structure in *Daphnia* distribution or behavioral response to disturbance. In some instances, water levels were too low to take three complete samples and fewer or smaller samples were taken. Ponds were monitored for animals starting when they first had water in them, which varied from November to late February. Once *D. pulex* appeared, samples were taken continuously, spaced by the egg development time, until ponds dried.

Samples were either preserved with cold sugar-formalin (Prepas 1978) immediately or placed on ice for preservation in the lab. On dates when birth rate was estimated, egg data on at least 100 randomly chosen adult females (if available) from one sample was recorded within 36 hours of preservation. Numbers of adult females, juvenile females and males were counted later. Usually a 5 - 10% subsample was counted, but if animals were rare, the entire sample was counted.

There can be substantial vertical layering to the spatial distribution of *D. pulicaria* in lakes, therefore it is inappropriate to report densities based on water

volume. Here, I report densities based on the surface area sampled (312 cm^2), assuming that horizontal structure is low and its effect is minimized by the sampling design. In contrast, I report densities in ponds based on volume. Although this is problematic for absolute comparisons of the population size, I am primarily interested in the dynamics of population growth.

Results.— Populations were much more dynamic in temporary ponds than in permanent lakes (Figure 4). *D. pulex* populations expanded and contracted over four orders of magnitude in less than five months, whereas some *D. pulicaria* populations fluctuated at most by three orders of magnitude over a year. The stability of *D. pulicaria* produces population growth rates that are generally near zero. *D. pulex*, in contrast, shows extreme growth rates, flipping from +0.3 to –0.4 per day in the span of a month (Figure 5). Birth rates in *D. pulicaria* were relatively low, with spring and fall peaks remaining below 0.5 neonates per adult female per day in most cases. In *D. pulex*, most populations had a birth rate peak in excess of 2.5 neonates per female per day (Figure 6).

The differences in population growth and birth rates between the habitats resulted in striking differences in death rates. Death rate in *D. pulicaria* was both lower and more constant than in *D. pulex* (Figure 7). In a few populations, negative death rates were observed. In zooplankton, this is not surprising because individuals hatched out of the sedimentary egg bank can contribute to poulation gowth. Such individuals cause underestimation of death rates, and negative death rates have been used to estimate the minimum hatching rate of zooplankton from the egg bank. The negative death rate observed in *D. pulicaria*

in late September and in *D. pulex* in late March occur at times when hatching is expected to be highest for these species.

In the *D. pulicaria* populations I study, I assume that the hatching rate is negligible relative to overall population dynamics. This assumption is reasonable because these populations have a perennial phenology, and are therefore predicted to invest little in diapause. The prediction has not been well-tested, but I rarely observed *D. pulicaria* females producing diapause eggs, and on those occasions it included only a small portion of the adult females. *D. pulex*, in contrast, must invest in diapause to persist through the dry period. In temporary ponds, genotypes that hatch late suffer a demographic cost relative to genotypes that hatch early, leading to a narrow hatching window. I assumed that hatching was essentially complete by the time population densities were sufficiently large to estimate birth rate, and should therefore minimally affect my death rate estimates. However, failure of this assumption means that *D. pulex* death rates, and consequently the difference between species, was underestimated.

Relationship between Demography and Life History

As a test of whether senescence is a function of extrinsic mortality, I examined the relationship between mean per capita death rate and an index of the fitness persistence into late life (l_x25, the age when only 25% of intrinsic value remains). I summarized the mortality measurements by calculating weighed mean death rates over the year in each population, with each death rate estimate for a population weighted by population density at the time of measurement.

There was a strong negative relationship between mortality risk and the index of fitness persistence (Figure 8). Linear regression showed that the relationship is significantly negative ($p = 0.0012$, $F = 21.79$, $df = 1,9$), but a power relationship had a better fit to the data (linear $R^2 = 0.68$, power $R^2 = 0.95$).

Local Phylogeny

DNA sequencing—In order to better understand the relationships among the replicate populations, I estimated their phylogenetic relationships based on a mitochondrial DNA sequence. I generally followed the protocol described in Lehman, et al. (1995). When possible, I used up to three clones per population to better evaluate whether migration was homogenizing the metapopulation. DNA was extracted by autoclaving fresh or frozen individuals in 250 ul 10% chelating resin for 15 min (Walsh et al. 1991). The supernatant from these extractions was used in the polymerase chain reaction (PCR) to amplify the rapidly-evolving control region. I used the primers specified in Lehman, et al. (1995) in a nested PCR to produce DNA fragments roughly 730 bases long. PCR reactions were performed with 10 pmol of each primer, 0.2 units of Taq DNA polymerase and 10 ul extraction supernatant or 2 ul PCR product in 50 ul reaction volumes. External primers were thermocycled for 92° 10 min + (92° 1 min, 50° 1 min, 72° 1 min) X 40 + 72° 10 min. Internal primers were amplified with a similar program, but denatured at 94° and annealed at 53°. The product of the internal primers was quantitated on a 1% agarose gel with Boehringer-Mannheim molecular weight marker XIV. This product was then purified with a Gene Clean® kit (Bio

101) and sequenced at Michigan State University's automated squencing facility. Sequencing reactions normally included >100 ng purified PCR product and 20 pmol primer. The sequences were then base-checked and aligned by eye for analysis.

Gene tree construction.— I constructed a tree based on 551 bp of sequence starting at the 5′ end of the amplified fragment using PAUP* 4.0b2a (Swofford 1998). My goal was to evaluate whether ancestry was entirely confounded with habitat differences, thus I am interested in whether the ecologically-defined species are strictly monophyletic. I do not argue that I can recover the true phylogenetic relationships among the study populations. Rather, I seek to understand whether there is evidence that selection repeatedly causes only certain genotypes to be successful in the different habitats. This may occur through repeated evolutionary divergence, clonal selection, migration between habitats with subsequent introgression, or long-distance dispersal into the local area.

I constructed trees using parsimony, phenetic and maximum likelihood approaches. For the parsimony approach, I performed heuristic searches on 1000 bootstrapped pseudoreplicates of the data and created a 50% majority-rule consensus tree, using *D. melanica* as an outgroup (Colbourne and Hebert 1996; unpublished sequence provided by N. Lehman). I treated gaps as a fifth character state, which produced 45 equally weighted parsimony-informative characters. In each pseudoreplicate, tree bisection-reconnection branch swapping with steepest descent in effect was performed on 25 starting trees generated by

random stepwise addition. Character states were optimized via accelerated transformation. This produced a tree with treelength = 117, CI = 0.73 and RI = 0.90 (Figure 9). Phenetic analysis was performed by neighbor-joining (NJ) and the unweighted pair-group method (UPGMA) under the same parameters as parsimony except that gaps were treated as missing data. 50% majority-rule consensus trees of 1000 bootstrap pseudoreplicates from NJ and UPGMA were topologically similar to Figure 9. Maximum likelihood (ML) analysis used the empirical nucleotide frequencies, assumed all sites to evolve at the same rate and did not enforce a clock. For the ML, I used only 10 replicate addition sequences. This produced three trees of maximum likelihood, whose consensus is identical to Figure 9.

All methods strongly supported a division of the populations into two clades, one containing only *D. pulicaria* genotypes and one including a mixture of *D. pulex* and *D. pulicaria* (Figure 9). This rejects the possibility that ancestry and habitat perfectly covary. Furthermore, multiple clones from single populations never had identical sequences, but generally grouped together. This indicates that populations are not simply representatives of one panmictic population. However, some notable exceptions to this (Three Lakes II and Lawrence Lake clones are found in both major clades) suggests migration is occurring.

Discussion

The data reported here clearly support the hypothesis that investment in late life performance is negatively related to extrinsic mortality risk. *D. pulex*

inhabits temporary ponds, resulting in death rates that are both high and seasonally variable. *D. pulicaria*, in contrast, inhabits permanent lakes that result in a much more stable population dynamics and low death rates. Logically, if maintaining physiological fitness incurs costs that are equal between these taxa, *D. pulex* should invest relatively little in maintenance compared to *D. pulicaria* because its expected returns are lower. Using demographic life history traits to integrate physiological traits, the data shows that *D. pulex* does invest less in sustaining fitness in late life. This is evident in both intrinsic survival and fecundity, and also in a combined measure of age-specific fitness.

Although the major differences occur between nominal species associated with strikingly different habitats, there is good evidence that that nominal taxonomy does not adquately reflect the genetic history of this species complex. Thus, the labels applied to *D. pulex* and *D. pulicaria* are more appropriately thought of as defining an ecological rather than a genetic difference. This means that the relationship is not simply one of two species that happen to differ in both their habitat occupied and life history. Populations of *D. pulex-pulicaria* genetically vary in their senescence characteristics and rapidly senescing populations occupy habitats with high extrinsic mortality risk. This suggests that the distribution of senescence variation is directly influenced by ecological variation.

One apparent discrepancy is that life expectancies in the field are notably shorter than median lifespans in the lab. In *D. pulex*, instantaneous mortality rates average ~0.25 per day, yielding a life expectancy of about 4 days. However,

their median lab lifespan is 20-40 days. A similar pattern is true of *D. pulicaria*, with a typical instantaneous mortality rate of ~0.1 per day, a life expectancy of 10 days, but a median lab lifespan of 30-90 days. Environmental differences between the field and the lab are unlikely to have caused this discrepancy through phenotypic plasticity. Temperature and resource level are the environmental factors likely to have the strongest influence on senescence phenotype. Temperature has been shown to be more important in determining lifespan than resources for *D. pulex-pulicaria* (Dudycha, in review) and conducting the laboratory measurements of lifespan at average field temperatures (i.e., colder) would simply enlarge the difference between field life expectancy and lab median lifespan. This difference suggests that death due to intrinsic senescent deterioration, i.e., dying of old age, is not a major factor in the wild. Instead, mortality in the wild is primarily caused by extrinsic factors such as predation, starvation, disease or anoxia. Hence, the relationship between death rates in the field and senescence is not caused by senescence determining the field death rates.

Juvenile mortality is present in both habitat types (Dudycha, unpubl. data) but was essentially non-existent in the laboratory. This could partially account for the differences in field life expectancy and median lifespan. Indeed, field life expectancy barely appoaches adulthood in *D. pulicaria*, and is well within juvenile range for *D. pulex*.

Juvenile mortality is not just important for comparing life expectancy and median lifespan; it potentially alters the predicted effect of selection on

senescence. As presented, the current analysis does not decompose mortality risk into juvenile and adult mortality risk. This can be important for the evolution of senescence because the predicted relationship between mortality risk and senescence holds true only if juvenile mortality is nil, is constant across compared groups, or is positively related to adult mortality risk. The reason for these restrictions is that juvenile mortality risk, by increasing the evoluitonary value of individuals who have already survived to maturity, will drive selection for increased preservation of adulthood (Abrams 1991) Consequently, a limitation of this report is that juvenile mortality, generally associated with invertebrate predation or starvation, is likely to be important for both *D. pulex* and *D. pulicaria*. It will be important to confirm that the age-structure of mortality risk does not reverse predictions for *D. pulex-pulicaria* senescence. Indications are that adult mortality is higher in *D. pulex* than *D. pulicaria*, because few juveniles are present during much of *D. pulex* phenology, but it is not clear how juvenile mortality differs.

Few sources of extrinsic mortality are likely to have equal effects on juveniles and adults. The importance of the balance between juvenile and adult mortality risk, and of variation in the balance among populations, suggests that an understanding of what mechanisms cause *Daphnia* mortality along the pond-lake gradient will be necessary to understand senescence evolution fully. Predation, starvation, disease and abiotic conditions are likely to be the main factors, but can operate differently in ponds and lakes. No information is available on the comparative risk of disease-related death either among habitats or across ages, so it will not be discussed further.

Predation can be roughly divided into that by vertebrates (planktivorous fish, newts) and invertebrates. Vertebrates are known to preferentially prey on large (=old, *Daphnia* have indeterminant growth) individuals and can be thought to select for accelerated senescence. However, local populations of *D. pulicaria* are known to avoid fish behaviorally and seasonally, minimizing their risk exposure. During my demographic monitoring, I observed only one newt in one pond, although I observed them to be locally abundant in shallow permanent waters. In lakes, the major invertebrate predator on *D. pulicaria* is the predatory midge *Chaoborus*, which is known to preferentially prey on small individuals. Chaoborus also avoids fish predation, therefore it spatially co-occurs with *D. pulicaria* and can strongly influence *D. pulicaria* dynamics. Temporary ponds harbor a wide range of invertebrate predators, mostly insects, that are known to prey on *Daphnia*. However, it is unclear which, if any, exert important predation pressure on *D. pulex*. In sum, it is unclear whether the age-structure of predation will act to reinforce or countermand selection on senescence based on total mortality rates.

Starvation is probably more important to juvenile mortality than to adult mortality. In the lakes I studied, it has been shown that resources peak in the spring and fall (similar to the birth rate peaks, Figure 5) but that during most of the warm months resources are low due to competition from other zooplankton. Winter resource availability is poorly known, but will be low if snow cover prevents light from penetrating the ice. In contrast, ponds have a high resource availability until after *D. pulex* populations have peaked. At that time, resources

may decline to levels where starvation is possible, but *D. pulex* populations are overwhelmingly adult. The among-habitat pattern of starvation potential, combined with an expectation that juveniles are more susceptible, indicates that age-dependent starvation will act to reinforce selection on senescence based on total mortality rates.

Dessication and anoxia are likely to be the most important abiotic causes of mortality. Dessication is only relevant in the ponds and is indiscriminant with respect to age. At the time ponds dry, there are few juveniles in the population because reproduction has switched to producing diapausing eggs. Therefore, the actual influence of dessication is primarily on adult death, but this influence may be small overall because populations decline prior to drying. Anoxia could play an important role in both habitat types. In lakes, oxygen is depleted in bottom waters between spring and fall due to decomposition combined with a lack of mixing with surface waters. Because *D. pulicaria* use the bottom waters as a vertebrate predation refuge in the daytime, their safe habitat constricts seasonally. I expect that suffocation is a more significant risk for juveniles, because hemoglobin production is costly and adults can aquire more resources per unit time. However, juveniles may not be exposed to this risk, since they have a relatively low risk of fish predation and may choose to remain continuously in the upper waters (where higher resources and temperature result in faster development). In ponds, anoxia is temporally structured, but not spatially structured. Oxygen levels in ponds can have strong diel fluctuations due to daily cycles of photosynthesis, respiration and decomposition combined with a seasonal progression towards anoxia as the canopy closes and insolation

is reduced. These patterns make it unclear whether anoxia-related death will reinforce or counteract selection on senescence based on total mortality rates.

One surprising aspect of the mortality dynamics is that most *D. pulex* populations experience a decline in mortality risk towards the end of the season well before the ponds dry. This contrasts with the expectation that pond-drying causes mortality en masse at the end of the season. Two other major demographic shifts are occurring substantially before pond-drying: total density typically declines, and females shift reproduction to diapausing eggs. The shift in reproduction produces a large reduction of birth rates. Consequently, juvenile density falls. A reduction of juveniles would reduce total mortality risk if that mortality disproportionately fell on juveniles. Adult death rates could also be dropping if the proportion of adults that have grown large enough to enter a size refuge from invertebrate predation is increasing.

The potential for a relationship between longevity (duration of reproductive lifespan) and diapause exists from both ecological and evolutionary considerations and deserves focused attention. In *Daphnia*, diapause and longevity are alternative mechanisms of dispersing one's offspring through time. These are fundamentally similar approaches to temporal dispersal, but their usefulness is likely to depend on the scale, degree and predictability of environmental variation. It would be interesting to examine the relative importance of these dispersal mechanisms along a gradient of habitat quality variation that ranges from near-term extreme fluctuation to long-term moderate changes. Diapause and longevity may trade off in *Daphnia*, because diapause is

not the proximate mechanism producing long life. Such a tradeoff is akin to tradeoffs between reproduction and survival, but alters the picture because it introduces a second mode of reproduction. Life history evolution may therefore be balancing three fundamental fitness components: survival, immediate reproduction and diapause investment. Experimental approaches to life history optimization under this scenario may require theoretical development of multidimensional tradeoffs, rather than examining a collection of pairwise tradeoffs.

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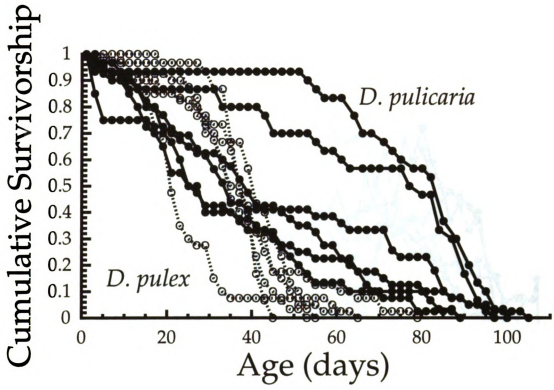

Figure 4-1. Survivorship of 12 populations of *D. pulex-pulicaria*. Open symbols and dotted lines are populations from temporary ponds, closed symbols and solid lines are populations from permanent lakes.

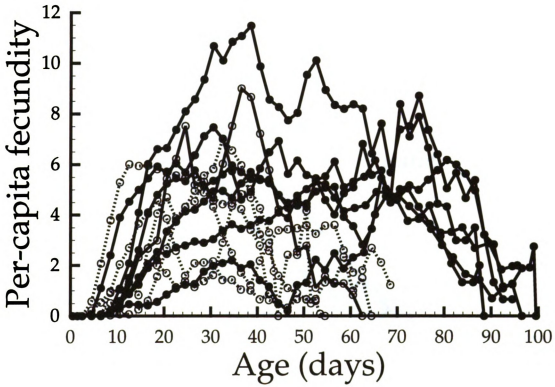

Figure 4-2. Fecundity of 12 populations of *D. pulex-pulicaria*. Symbols are as in figure 1. Fecundity measurements are per 2-day period, smoothed over three periods.

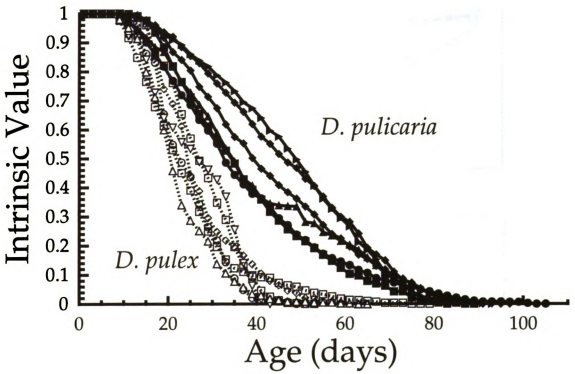

Figure 4-3. Intrinsic value of 12 populations of *D. pulex-pulicaria*. Symbols are as in figure 1. Intrinsic value is the sum of current + expected future fecundity scaled by the expected lifetime fecundity.

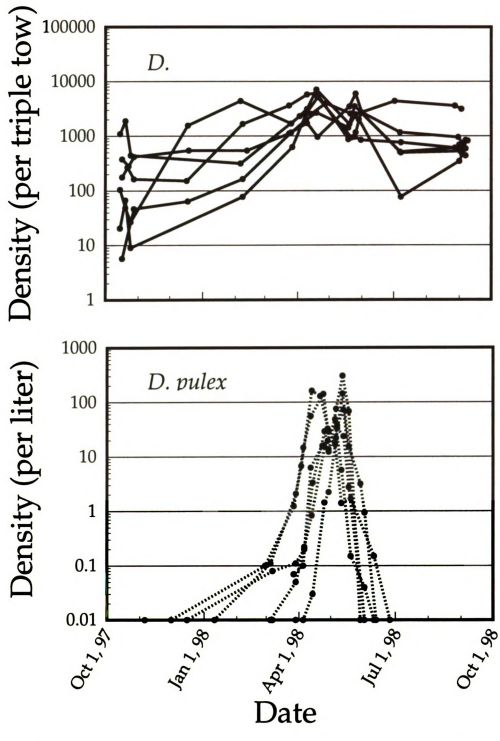

Figure 4-4. Population density of *D. pulex-pulicaria* over one year. Top: density of six *D. pulicaria* populations (permanent lakes) measured per unit surface area. Bottom: density of six *D. pulex* populations (temporary ponds) measured per unit volume. The scale of absolute numbers differs between panels, but the scale of *change* in density is identical. Symbols are as in figure 1.

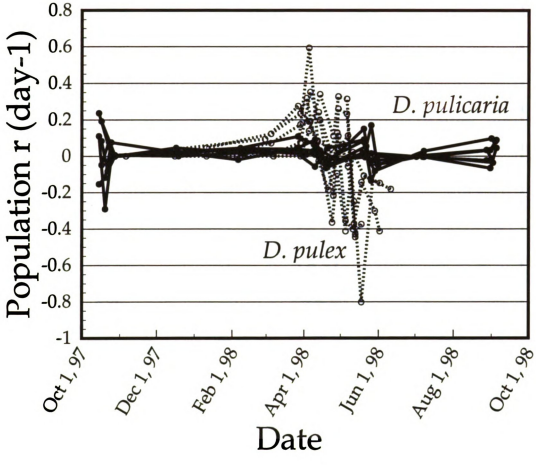

Figure 4-5. Comparison of population growth rates in *D. pulex-pulicaria* over one year. Symbols are as in figure 1.

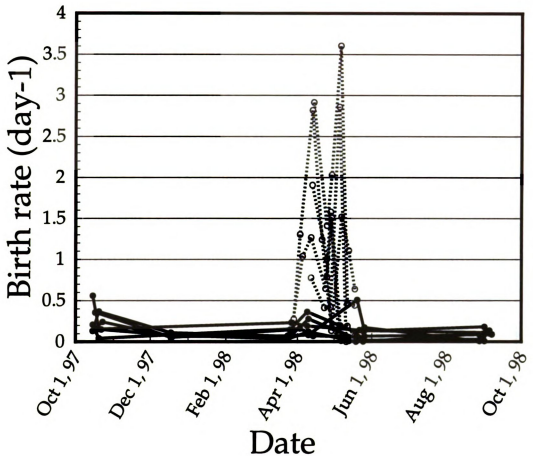

Figure 4-6. Instantaneous birth rates in *D. pulex-pulicaria* over one year. Symbols are as in figure 5.

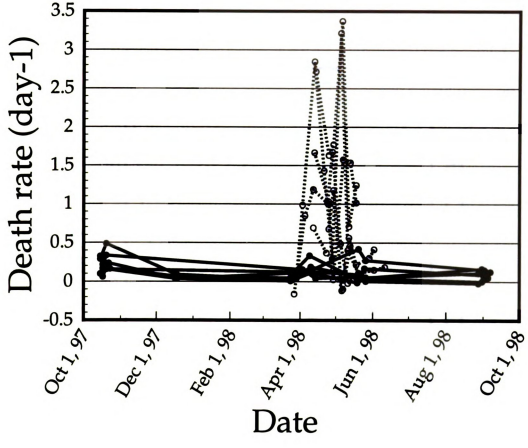

Figure 4-7. Instantaneous death rates in *D. pulex-pulicaria* over one year. Symbols are as in figure 5.

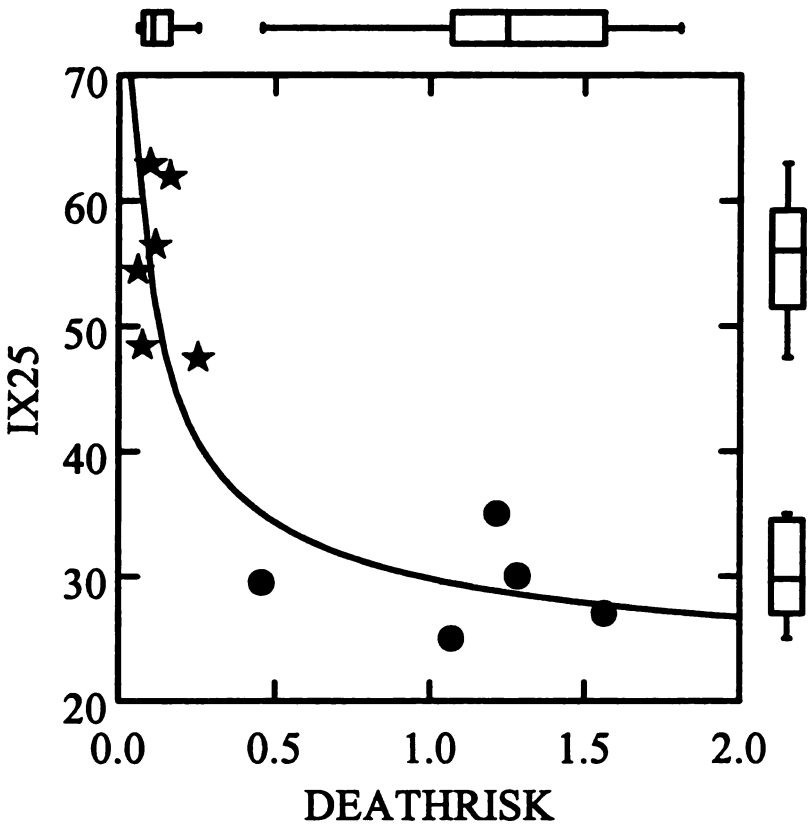

Figure 4-8. Relationship between ecological mortality risk and senescence. The x-axis is the average instantaneous death rate over one year, weighted by population density. The y-axis is the age when 25% of intrinsic value remains, an index fitness persistence into late life. Boxplots show the data range for *D. pulex* (temporary ponds; circles) or *D. pulicaria* (permanent lakes; stars).

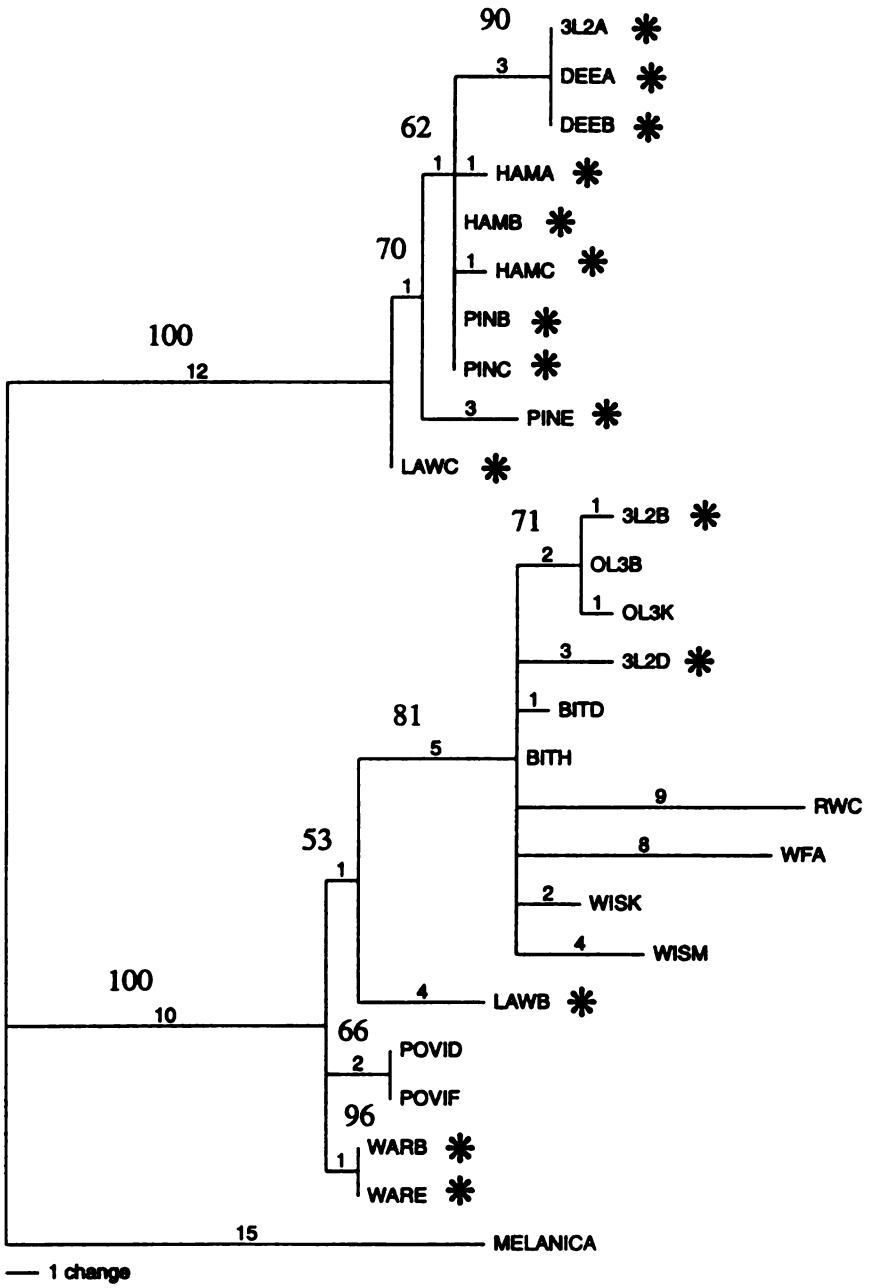

Figure 4-9. Gene tree of 25 clones of *D. pulex-pulicaria*. Clones are identified by source population and a letter identifying unique isolates. This tree (length 117; CI = 0.73; RI = 0.90) is a 50% majority rule consensus of 1000 bootstrap pseudoreplicates searched heuristically for the most parsimonious trees. Large numbers are bootstrap percentages, small numbers are branch lengths. Clone labels with an asterisk are nominally classified as *D. pulicaria*, all others are *D. pulex*. See text for details.