



2011-07-08

Changes in Life History within an Individual's Lifetime

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Changes in Life History within an Individual's Lifetime

Eric J. Billman

A dissertation submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

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August 2011

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ABSTRACT

Changes in Life History within an Individual's Lifetime

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A central goal of life history theory is to understand the selective factors that generate the diversity of reproductive patterns observed in nature. Within lifetime changes in reproductive investment will determine an organism's fitness; however, this area of life history theory has received less attention than comparisons among population that characterize life history traits as a single population mean. Reproductive allocation can be affected by multiple cues; the integration of these cues across an organism's lifetime generates the diversity in life history strategies observed in nature. Life history studies should examine the interacting effects of multiple cues on life history strategies to generate better predictions and generalizations of age-related changes in reproductive investment. An individual's life history strategy is inherently multivariate consisting of a coordinated suite of life history traits that, when combined across the organism's lifetime, determines its fitness. Life history strategies can therefore be described as a trajectory through multivariate space defined by life history traits. Here I describe life history trajectory analysis, a multivariate analytical approach for quantifying and comparing phenotypic change in life history strategies; this methodology is adapted from an analytical framework originally described for studies of morphological evolution. Life history trajectories have attributes (magnitude, direction, and shape) that can be quantified and statistically compared among taxa to determine if life history patterns are predictable. Using the life history trajectory analysis, I demonstrate the effect of prior experience on reproductive allocation in the burying beetle *Nicrophorus orbicollis*. The effect of prior experience resulted in a terminal investment or accentuated response to age-based cues, or resulted in a conservative investment strategy or reproductive restraint. In the livebearing fish *Gambusia affinis*, females adjust the level of reproductive investment to current reproduction based on age- or environment-based cues. Age-0 females decreased the level of reproductive investment to current reproduction in late summer prior to the onset of fall and winter months. Old females, on the other hand, increased the level of reproductive investment as the summer progressed. The reproductive restraint and terminal investment patterns exhibited by age-0 and age-1 females, respectively, were consistent with the predictions from the cost of reproduction hypothesis. These studies demonstrate how the life history trajectory analysis provides an analytical tool to test predictions of life history theory. Additionally, I provide evidence that organisms use multiple cues to determine the level of reproductive investment and that the strength of the effect of each cue will depend on the age of an individual.

Keywords: life history evolution, life history trajectory analysis, cost of reproduction, trade-offs, terminal investment, Poeciliidae, burying beetle

ACKNOWLEDGEMENTS

I would like to thank my family for the love and support provided during the past five years. My wife always motivates me to do better and to work hard in all my endeavors. All my success I therefore owe to her. My children bring me great joy and similarly inspire me to be an excellent role model and teacher to them. I love their fascination for life in its many forms. I owe my gratitude to Mark Belk for his guidance through my research, as well as my committee: Jerry Johnson, Steve Peck, Russ Rader, and Bruce Schaalje. I am grateful to Curtis Creighton who introduced me to the fascinating world of burying beetles and helped me with my research with these amazing critters. I have learned much from my fellow graduate students including Josh Rasmussen, John Aedo, Josh Kreitzer, Jared Lee, Steve Merkley, and countless others. Jeff Wesner provided much encouragement and insight as I prepared this dissertation. Much of the research benefited from the labors of countless undergraduates, most significantly Peter Meyers. Last of all, I would like to thank the members of the department, especially the secretaries who helped me keep on track to finish this degree.

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Chapter 1: Evolution of Life History Strategies: the Balance of Costs and Benefits of Reproduction

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Abstract

A central goal of life history theory is to understand the selective factors that generate the diversity of reproductive patterns observed in nature. Within lifetime changes in reproduction will determine an organism's fitness; however, this area of life history theory has received less attention than comparisons among populations that characterize life history traits as a single population mean. In this review, we describe general patterns of reproductive allocation that have been documented in a variety of organisms. Next, we discuss the cost of reproduction paradigm and how it predicts the level of reproductive investment for the current reproductive bout. This paradigm has led to the cost of reproduction hypothesis which predicts an increasing level of reproductive investment as individuals increase in age. However, variation in environmental conditions and in the condition or state of individuals can cause observed reproductive patterns to deviate from these predictions. We are particularly interested in how the interaction of age with extrinsic and intrinsic cues generates variation in life history strategies among populations and species. Therefore, we provide direction for future life history studies to establish better understanding of the causes and consequences to patterns of reproductive allocation. Life history studies should examine the interacting effects of multiple cues on life history strategies to generate better predictions and generalizations of age-related changes in reproductive investment.

Keywords: life history evolution, cost of reproduction, terminal investment, reproductive restraint, reproductive constraint, state-dependent

Introduction

A fundamental goal of the study of life history evolution is to determine the forms of divergent selection that have produced the diversity of reproductive patterns observed in nature. Life history tradeoffs have been studied extensively, producing general predictions of life history strategies expected due to particular selective regimes (Roff 2002). Many of these studies examine life history tradeoffs among populations or species by representing each life history trait separately as a single mean for a population (Stearns 1976). However, of equal interest is an understanding of how an individual's life history strategy, or pattern of reproduction across an individual's lifetime, contributes to its evolutionary fitness (Williams 1966; Gadgil and Bossert 1970; Schaffer 1974; Stearns 1992; Roff 2002). While the number of studies has increased over the past decade, life history strategies characterized across an individual's lifetime have received less attention than studies among populations or species that characterize life history as a single mean. Additionally, studies of changes in lifetime reproductive patterns have not provided overwhelming support for a single model or hypothesis, but instead have provided evidence for multiple models that predict life history changes over an individual's lifetime. As a result, the study of life history strategies currently lacks predictability and generality of patterns among taxa.

Here we provide a review of within lifetime changes in reproductive patterns and provide future directions for studies of life history evolution. First, we describe within lifetime variation in life history traits observed among organisms to identify specific patterns. Next we review the cost of reproduction paradigm and the cost of reproduction hypothesis that generate predictions for changes in life history tradeoffs over a lifetime. While these age-based

predictions provide a general framework for assessing life history strategies, organisms face several selective factors over their lifetime. It is the interaction of these extrinsic and intrinsic factors with age that generate variation in life history strategies within and among populations. As such, we provide directions for future research to establish testable patterns of reproduction over a lifetime, to determine how to integrate multiple selective factors into life history models, and to establish the generality and predictability of within lifetime change in reproductive patterns across taxa.

Age-related variation in life history

Organisms exhibit a range of lifetime reproductive allocation patterns including 1) increased reproductive allocation with age, 2) decreased reproductive allocation with age, 3) no change in reproductive allocation with age, and 4) variable reproductive allocation with age.

The pattern of increased reproductive allocation with age has been observed in a variety of taxonomic groups including insects (Dixon et al. 1993; Creighton et al. 2009; Cotter et al. 2011), fishes (Poizat et al. 1999; Poizat et al. 2002; Baker et al. 2008; Belk and Tuckfield 2010), birds (Pärt et al. 1992; Velando et al. 2006), and mammals (Clark et al. 2002; Isaac and Johnson 2005; Mysterud et al. 2005; Broussard et al. 2006; Descamps et al. 2007; Galimberti et al. 2007). Increased reproductive allocation can also be seen in a variety of life history traits. Evidence from life history studies has demonstrated an increase with age in both offspring size (Dixon et al. 1993; Lock et al 2007) and offspring number (Sade 2004; Blas et al. 2009; Wilson et al. 2009). Increased reproductive allocation patterns are also manifest in sex allocation in hermaphroditic organisms (Ohbayashi-Hodoki et al. 2004; Ohbayashi-Hodoki and Shimada 2005; Aira et al 2007; Pavel and Bures 2008) and in mammal species (Sade 2004; Bercovitch et

al. 2009), both of which have a higher probability of producing the costly sex with increased age. Some organisms demonstrate an increase in reproductive allocation with age by suffering greater decreases in predator avoidance (Korpimaki et al. 1994; Ghalambour and Martin 2000, 2001; Pflanz 2002; Ackerman et al. 2006; Belk and Tuckfield 2010) or decreases in physiological processes (e.g. allocation of energy to sexual signals as opposed to antioxidant defenses; Cote et al. 2010). Finally, life history studies have demonstrated increased reproductive allocation in organisms that provide parental care, manifest as an increase in the amount (quality and duration) of parental care provided (Pugesek 1981; Pugesek and Diem 1983; Clutton-Brock 1984; Berteaux and Boutin 2000).

Another common pattern of age-related reproductive allocation is a decrease in reproductive allocation with age. These decreases that occur in old age are generally attributed to a decline in physiological performance due to damage accumulation across an individual's lifetime that results in an increase in the probability of mortality and a decrease in reproduction (e.g. senescence; Kirkwood and Rose 1991; Monaghan et al. 2008; Ricklefs 2008). A decline in reproductive allocation with increasing age has been reported in a variety of long-lived birds (Weimerskirch 1992; Reed et al. 2008; Berman et al. 2009; Beamonte-Barrientos et al. 2010; Lecomte et al. 2010; Rebke et al. 2010) and mammals (Ericsson et al. 2001; Beauplet et al. 2006; Bowen et al. 2006; Baker and Thompson 2007; Festa-Bianchet and King 2007; Harting et al. 2007). However, similar patterns have also been reported in other taxonomic groups, including insects (Creighton et al. 2009; Cotter et al. 2011) and fish (Reznick and Elder 2001). Evidence for decreased reproductive allocation have been observed in offspring number (Festa-Bianchet and King 2007; Low et al. 2007), although decreasing patterns have also been reported for parental care (Ericsson et al. 2001).

A far less prevalent age-related pattern of reproductive allocation is that of no change in reproductive allocation with age. To our knowledge, empirical evidence of this pattern has only been reported in a single organism, tsetse flies *Glossina morsitans morsitans* (Diptera: Glossinidae), a short-lived viviparous insect (Langley et al. 1998). Over their lifetime, tsetse flies did not change the level of energetic investment into reproduction, and the size of offspring was similarly constant with age. Fecundity for this species was determined as the number of offspring produced per time interval; fecundity remained constant across the flies' lifetime although this rate of reproduction may decrease at the end of life (Langley et al. 1998).

Finally, the level of reproductive allocation may vary across an organism's lifetime, a pattern attributable to variation in environmental conditions. Resource allocation is dependent on the amount of energy available for an individual to invest into reproduction and other competing somatic processes. Therefore, variation in environmental conditions that affect the availability of resources can affect an individual's level of reproductive allocation independent of age (Heimpel and Rosenheim 1995; Heimpel and Collier 1996; Festa-Bianchet and Jorgenson 1998; Tveraa et al. 2003; Lake et al. 2008). Offspring number has been demonstrated to be positively correlated with environmental conditions; organisms will decrease offspring number during periods of low resources, the most extreme examples being termination of pregnancy (Trexler and DeAngelis 2003; Lake et al. 2008). For some bird species, high resource availability increases reproductive allocation in the current reproductive period manifest either in larger clutch sizes and/or more broods within the reproductive season (Martin 1987; Grant et al. 2000; Christman 2002; Preston and Rotenberry 2006). For species that have one sex with more variable reproductive success (e.g. males in polygynous species), females produce more of the

sex with variable reproductive success (i.e. variation in sex allocation) during periods of high resource availability (Trivers and Willard 1973; Cameron 2004).

Organisms both within and across taxonomic groups exhibit a wide-range of patterns of reproductive allocation. However, the ability to make inferences about the generality of these patterns and predictability given selective regimes is lacking due to poor taxonomic coverage and due to the lack of comparative measures of reproductive allocation. One challenge of these life history studies is the difficulty and the cost in both time and resources to acquire sufficient data of individual patterns of life history in longitudinal studies, particularly for long lived organisms (Nussey et al. 2008). Additionally, for most organisms it is difficult to determine the proportion of energy available that is allocated to reproduction (Clutton-Brock 1984; Langley et al. 1998); therefore, other measures are quantified (e.g. change in female body mass) as surrogates to energetic investment, or researchers simply examine patterns of reproductive success (e.g. number of offspring) without examining energetic investments. This demonstrates a need for both an increase in longitudinal studies as well as a standardization of appropriate measures of reproductive allocation to facilitate broader taxonomic comparisons of life history strategies.

Cost of reproduction and life history strategies

The fitness of an organism will be determined by its life history strategy, or the balance of life history tradeoffs incorporated over its lifetime. In each reproductive bout, an individual must decide the level of reproductive allocation or investment that will contribute to its maximum attainable fitness. We define reproductive allocation (reproductive allocation and reproductive investment are used interchangeably) as the level of energetic investment devoted

for a reproductive bout and how that energy is divided into competing demands including but not limited to offspring size, offspring number, sex allocation, quality and duration of parental care, risk adverse behaviors, etc. (Williams 1966; Roff 2002). While increased allocation of energy to the current reproductive bout will necessarily provide benefits to an individual's fitness, it will also come at a cost to future reproductive opportunities (i.e. the cost of reproduction) either by decreasing the probability of survival or by decreasing future reproductive capacity (Williams 1966). Therefore, an individual can maximize its evolutionary fitness by balancing the costs and benefits of reproduction across its lifetime. Within a population, allocation strategies that offer a positive fitness benefit from the normal expenditure will experience positive selection and vice versus.

One question the cost of reproduction idea presents is how does an individual assess future reproductive potential? The prevailing idea following Williams' synthesis (1966) was that age was the primary cue indicating future reproductive potential. An individual of a given age has an expected amount of future reproductive success or reproductive value (Fisher 1930; Williams 1966). The cost of reproduction hypothesis predicts that the level of reproductive allocation to current reproduction will be inversely correlated with an individual's reproductive value, or expected future fecundity (Fisher 1930; Williams 1966; Clutton-Brock 1984). A life history strategy that follows the predictions of the reproductive value hypothesis will be characterized by an increasing pattern of reproductive allocation with age, or reproductive restraint early in life and terminal investment (Clutton-Brock 1984) late in life. Early in life, an individual with a high reproductive value should only be willing to incur a small cost of reproduction resulting in an individual allocating a smaller amount to reproduction (i.e. reproductive restraint) and more energy to growth and soma to ensure future reproductive

opportunities. As reproductive value decreases, the level of reproductive investment to current reproduction should increase, culminating in the highest level of allocation and subsequent cost of reproduction at or near the end of an individual's reproductive life (i.e. terminal investment; Clutton-Brock 1984). This strategy allows an individual to maximize reproductive output at the end of its life when future reproductive opportunities are unlikely or absent.

The cost of reproduction hypothesis also assumes that individuals in the population have a constant supply of resources (i.e. no variation in energy available) and individuals of the same age have the same condition or state. Therefore, deviations from expected reproductive patterns given predictions from the cost of reproduction hypothesis can result from environmental conditions that cause stochasticity in resource availability and within cohort variation in state. These deviations in reproductive phenotypes represent genotype*environment interactions.

Reproductive investment is directly tied to resource acquisition; variation in environmental conditions, whether spatially or temporally, will affect the availability of resources and consequently the energy available for an individual to invest into reproduction (Reznick et al. 2000; Millon et al. 2010). Therefore, environment-based cues can affect how individuals assess the value of current versus future reproduction, and consequently the individual's pattern of reproductive investment across its lifetime. When resource availability is high, all individuals should have sufficient energy to invest into reproduction, and in fact investment may be higher than predicted given an individual's age. Poor environmental conditions, however, can have contrasting results depending on an individual's reproductive value. Organisms with a high reproductive value should allocate to future reproduction by reducing reproductive investment and costs incurred on current reproduction (Tolonen and Korpimaki 1994; Festa-Bianchet and Jorgenson 1998; Lake et al. 2008). Alternatively,

individuals with a low reproductive value should increase or maintain current investment despite the increased costs of reproduction that will be incurred (Hamer and Furness 1991; Descamps et al. 2009). The environment-based cues previously experienced by an individual can determine how the individual assesses current versus future reproductive opportunities and therefore determine the individual's decisions on the extent of current reproductive investment (Hayward et al. 2009; Cotter et al. 2011).

Age-based life history theory assumes that reproduction and survival are functions of an organism's age and that same-aged individuals have the same state, or condition, thereby ignoring underlying physiological and environmental conditions that also affect reproduction and survival (McNamara and Houston 1996). There is a growing body of literature that demonstrates the importance of an individual's state on its lifetime reproductive success (Fisher 1999; Wendeln and Becker 1999; McElligott and Hayden 2000; Beauplet and Guinet 2007; McCleery et al. 2008; Lescroel et al. 2009; Lescroel et al. 2010). For example, Beauplet and Guinet (2007) reported that female fur seals in the best condition (33% of females as determined by body size) accounted for 71% of the viable offspring in the next generation. While individuals in better condition have greater lifetime reproductive success (McElligott and Hayden 2000; McCleery et al. 2008), individuals with a reduced state (e.g. due to parasitic infections) may have less energy available for reproductive investment, thus suffering a reduction in lifetime reproductive success (Gustafsson et al. 1994; Kolluru et al. 2009). Characterizing individual quality can be challenging and dependent on both the study species and the research questions (Moyes et al. 2009; Wilson and Nussey 2010).

State-based cues represent a means by which an individual can assess its future reproductive potential. This has led to an increasing number of studies that incorporate an

immune or condition (e.g. clipping feathers; Ardia and Clotfelter 2007) challenge to simulate a decrease in reproductive value to determine its effect on reproductive strategies.

Overwhelmingly these studies have demonstrated that organisms use state-based cues to determine reproductive value, and will exhibit an increase in reproductive investment (i.e. terminal investment) when state-based cues indicate a reduction in reproductive value (Bonneaud et al. 2004; Javois and Tammaru 2004; Hanssen 2006; Velando et al. 2006; Blanckenhorn et al. 2008; Cotter et al. 2011).

Reproductive restraint late in life

The cost of reproduction hypothesis predicts that old individuals should exhibit terminal investment or increased allocation to current reproduction (Clutton-Brock 1984). However, the paradigm of cost of reproduction (i.e. the costs-benefits concept of reproduction) can also generate the opposite pattern, or reproductive restraint late in life. Terminal investment is suggested to be the optimal strategy when an organism has a fixed maximum lifespan; an organism should increase its reproductive effort when approaching the end of its life (Williams 1966; Gadgil and Bossert 1970). However, an organism's lifespan may be limited by the deterioration of its body over time rather than a fixed age. McNamara et al. (2009) developed a model incorporating the effect of somatic deterioration demonstrating that organisms with intrinsic causes of mortality benefit a fitness advantage by exhibiting reproductive restraint late in life. By reducing reproductive investment, these organisms slow deterioration and delay death, extending their lifespan to gain more time to reproduce. However, organisms that utilize reproductive restraint late in life will still respond to extrinsic variables that compromise survival prospects by dramatically increasing reproductive investment as predicted by the reproductive

value hypothesis (Bonneaud et al. 2004; Velando et al. 2006; Cotter et al. 2011). Organisms that have a long, slow life history may be more likely to exhibit reproductive restraint compared to organisms with a short, fast life history because death is determined by intrinsic rather than extrinsic factors (Monaghan et al. 2008; Hamel et al. 2010; but see Reznick et al. 2004).

Future research

Age related changes in reproductive investment represent shifts in life history strategy to allow individuals to maximize their evolutionary fitness. The cost of reproduction paradigm provides a framework to examine the balance of reproductive costs and benefits. Predictions from the cost of reproductive hypothesis represent a null hypothesis with which to compare life history strategies of populations and species. However, of greater interest are the genotype*environment interactions: how does age interact with extrinsic and intrinsic cues to determine the optimal life history strategy? These cues interact to generate the variation in life history strategies observed both within and among species. Additionally, the cues are likely to generate varying levels of response depending on the life history traits that are quantified and the age of the individual (Crocker et al. 2001; Alonzo and Heckman 2010). Thus, the challenge of life history studies is to determine which cues most likely influence reproductive investment, how these cues interact, how they affect the coordinated life history response, and if they generate consistent life history responses across taxa. Therefore, we provide the following suggestions for future research to help advance this field of study.

Descriptive studies of lifetime patterns

The study of life history evolution attempts to provide generality and predictability to the diversity of reproductive patterns observed among organisms. To achieve that goal, it is

necessary to continue descriptive studies to provide necessary data to establish general patterns (Nussey et al. 2008). Descriptive studies of life history have consisted of both cross-sectional and longitudinal studies. Cross-sectional studies describe the average age-related patterns of reproduction of individuals in a population; as such they are useful for generating hypotheses regarding the relative effect of different cues on the organism's life history strategy. However, life history strategy is a within individual trait that is dependent on the individual's state, prior reproductive experience, and its genotype*environment interaction (Reznick et al. 2000; Nussey et al. 2008). Longitudinal studies describe the average within-individual lifetime reproductive strategy allowing researchers to confirm patterns observed in cross-sectional studies (Nussey et al. 2008). Additionally, longitudinal studies provide a method to determine how individuals respond to multiple cues and the extent to which these changes in reproductive investment are consistent among individuals. Because of the difficulties of acquiring longitudinal data (Nussey et al. 2008), a combined approach using both cross-sectional and longitudinal data will provide the most robust results in observational life history studies. A challenge of both cross-sectional and longitudinal studies is proper measurement of life history traits associated with reproductive investment (Reznick 1985). Therefore, observational life history studies are useful and essential for establishing age-related patterns of reproduction from which experiments can be designed to determine the cues responsible for generating these patterns.

Experimental studies of life history

Experimental life history studies are necessary to empirically determine life history responses to individual cues as well as integrated responses to multiple cues, thus providing the mechanism for observed patterns from descriptive studies. Experimentation also allows researchers to quantify life history traits that are otherwise too difficult in descriptive studies of

wild animals. Individual cues have already been the focus of some experimental studies, such as immune challenge research that determines how a change in state affects reproductive allocation (Bonneaud et al. 2004; Javois and Tammaru 2004; Hanssen 2006; Velando et al. 2006; Blanckenhorn et al. 2008; Cotter et al. 2011). By utilizing multiple approaches or manipulations, researchers can assess the relative effect of multiple cues and how these cues interact across multiple ages.

Two recent studies, both utilizing burying beetle species (*Nicrophorus spp.*), exemplify how experimentation can separate the effects of multiple factors on age-related reproductive patterns. In a laboratory study, Creighton et al. (2009) demonstrated how costs incurred during reproduction resulted in senescent patterns of reproduction and survival. However, the authors also demonstrated that the female beetles exhibited patterns of terminal investment that would have otherwise been masked by reproductive senescence. Similarly, Cotter et al. (2011) utilized prior experience and immune challenge to demonstrate that burying beetles experience senescence and as a result will utilize reproductive restraint to minimize somatic deterioration. However, the beetles increase reproductive investment when activation of their immune system induces the individuals to believe they have a reduced probability of future survival, and therefore a lower reproductive value (Cotter et al. 2011). These studies demonstrate how multiple experimental approaches can determine the influence of multiple cues on age-related reproductive investment. Future experimental research should continue to examine how cues interact to produce observed patterns of reproductive investment. Burying beetles provide a good model system to examine the interaction of age-based and environment-based cues, for example to determine how prior experience determines how individuals assess future

reproductive opportunities and how environmental gradients (e.g. across latitudinal clines) affect age-based reproductive investment.

Comparisons across taxa

Through studies of phenotypic change, evolutionary biologists have sought to determine if the course of evolution is highly stochastic and unpredictable, highly deterministic and predictable, or somewhere between these extremes (Langerhans 2010). To determine the predictability of within lifetime change in life history, life history comparisons are necessary among species and higher taxa. Broad taxonomic comparisons present some challenges to life history comparisons due to variation in body size, longevity, and mode of reproduction (Grime 1977; Winemiller 1992; Winemiller and Rose 1992; Charnov 2002). However, standardization of life history traits (e.g. by body size or maximum age) could provide common measures that could be used to assess the effect of selective factors on phenotypic variation in life history strategies. Additionally, a recently proposed conceptual framework provides the ability to test the predictability of changes in phenotypic traits to determine or understand the causes and consequences of phenotypic evolution (Langerhans 2008; Langerhans 2010). Using this approach, the researcher develops a mechanistic model describing how the system is believed to function under selection from the primary selective factors. From this model, predictions are generated to which observed patterns can be compared (Langerhans 2010). Using this unified framework, researchers could determine the extent to which patterns of within lifetime change in life history due to various selective factors are predictable across distant taxa.

Analysis of multivariate life history strategies

The reproductive response to intrinsic or extrinsic cues will not only vary across age but can also vary depending on the life history trait that is measured (Crocker et al. 2001; Alonzo and Heckman 2010). Therefore, life history studies should examine multiple life history traits to determine the coordinated response to cues that affect patterns of reproductive investment across an organism's lifetime. Because life history strategy is a multivariate phenotype, it can be described across two or more ages as a multivariate trajectory. Life history trajectories could then be compared among populations or species using a recently described framework for comparing multivariate phenotypic change (Adams and Collyer 2007; Collyer and Adams 2007; Adams and Collyer 2009). Using this analytical framework, researchers could quantify and statistically compare attributes (amount and direction of phenotypic change) of life history strategies of two or more populations or species. Additionally, researchers could generate predicted trajectories based on cues that are anticipated to affect individuals within the study system and could compare observed to predicted trajectories. In this manner, trajectory analysis can elucidate the cues that influence reproductive investment, the ages at which their effect is the greatest, and the extent to which these patterns are consistent across selective regimes both within and among species. For example, livebearing fish have demonstrated consistent patterns of life history evolution across selective regimes. However, few studies have examined age-related patterns of reproductive investment (but see Reznick et al. 2004, Belk and Tuckfield et al. 2010). Livebearing fish lend themselves to broader phylogenetic comparisons (e.g. within the genus *Brachyrhaphis*) to compare age-related reproductive patterns among species and across selective regimes to determine the extent to which patterns are repeatable and the extent that the taxa are evolutionary constrained (Langerhans 2008, 2010).

Conclusion

Organisms exhibit a great variety of within lifetime changes in patterns of reproductive allocation. These patterns have been compared to theoretical models providing inconclusive support for models and their predictions. Studies of age-related patterns of reproduction have demonstrated that multiple cues simultaneously affect reproductive allocation, interacting to elicit observed responses. Future research must examine not only the effects of age but also the interaction of age with other cues on how individuals determine the extent of reproductive investment across their lifetime. The results of this research will provide the evidences necessary to modify current age-based life history hypotheses to generate a more robust theoretical model that incorporates multiple cues, providing better generality of evolutionary patterns (Langerhans 2010).

Acknowledgements

Funding was provided by the Department of Biology at Brigham Young University.

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Chapter 2: Life History Trajectory Analysis: A Multivariate Approach to the Analysis of within Lifetime Variation in Life History

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Abstract

A major goal of the study of life history evolution is to understand how an organism determines the optimal pattern of reproductive investment across its lifetime. An individual's life history strategy is inherently multivariate consisting of a coordinated suite of life history traits that, when combined across the organism's lifetime, determines its fitness. Life history strategies can therefore be described as a trajectory through multivariate space defined by life history traits. Additionally, ecological gradients can provide divergent selective pressures on organisms, and can create differences in the optimal pattern of reproductive allocation across an organism's lifetime. Therefore, there is a need in life history studies for an analytical framework to compare multivariate phenotypic change in life history strategies between populations to determine if patterns are consistent with predictions from age-based life history hypotheses. Here we describe life history trajectory analysis, a multivariate analytical approach for quantifying and comparing phenotypic change in life history strategies; this methodology is adapted from an analytical framework originally described for studies of morphological evolution. Life history trajectories have attributes (magnitude, direction, and shape) that can be quantified and statistically compared among taxa to determine if life history patterns are predictable. We use two examples to demonstrate the utility of life history trajectory analysis. Particularly, we demonstrate how trajectories can be constructed using levels characterized by individuals with the same age or a similar state. Also, we illustrate how this approach can be

used to compare life history strategies to predictions from life history theory. Life history trajectory analysis provides a method for statistically testing age- and state-based predictions of life history theory as well as identifying environmental, morphological, and physiological factors responsible for patterns of life history evolution.

Keywords: life history evolution, phenotypic plasticity, *Nicrophorus*, Poeciliidae, multivariate trajectory

Introduction

A central goal of life history evolution is understanding how changes in phenotypic life history traits across an organism's lifetime are affected by changes in survival probabilities due to ecological selection (Roff 2002). Phenotypic variation in life history strategies occurs because organisms utilize different strategies to balance investment of energy to competing demands to maximize fitness. Energy available to an organism must be allocated to competing demands in three main categories: soma (body maintenance, storage, etc.), growth, and reproduction. For both semelparous and iteroparous organisms, energy in early life stages is allocated primarily to soma and growth, but semelparous organisms will allocate all or nearly all energy to reproduction and associated activities during the single, terminal reproductive bout. An iteroparous organism must balance trade-offs in current versus future reproduction (Williams 1966). Therefore, the coordinated responses of life history to environmental factors at each reproductive bout (i.e. the interaction between age and environment) determine an individual's lifetime reproductive strategy and fitness.

The cost of reproduction hypothesis provides general predictions about how an iteroparous organism should allocate energy to reproduction during each reproductive bout based on the reproductive value, or future reproductive potential (product of future probability of survival and fecundity potential; Fisher 1930; Williams 1966; Roff 2002). These predictions are based on the premise that reproductive investment comes at a cost: allocation to current reproduction results in a cost to future reproductive opportunities (Reznick 1985). When the reproductive value of an individual is high, an organism should tolerate only relatively low costs of reproduction on the current reproductive bout to ensure future reproductive opportunities.

Conversely, an organism should be more willing to incur a high cost of reproduction on the current reproductive bout when its reproductive value is low. Thus, individuals should show restraint in reproduction early in life (restraint hypothesis; Curio 1983; Forslund and Pärt 1995) and terminal investment later in life (terminal investment hypothesis; Clutton-Brock 1984). Similarly, as the probability of survival changes across selective regimes, the coordinated life history response over an individual's lifetime should likewise change to maximize the individual's fitness.

Life history response to age will vary depending on how the environment affects the probability of survival and depending on which life history traits are quantified (Crocker et al. 2001; Alonzo and Heckman 2010). Therefore, comparisons of life history strategies among populations should examine the coordinated or multivariate nature of the life history response as well as the age by environment interaction. Many studies of age-related patterns of reproduction examine life history strategies by examining each trait separately (Stearns 1976; Roff 2002). However, life history traits represent competing demands such that the response of one life history trait is dependent on the responses of other traits; thus, life history traits considered collectively represent a multivariate life history strategy. Studies of evolutionary ecology compare patterns of phenotypic variation to elucidate the general predictability of evolutionary change or the genotype by environment interaction (Langerhans 2010). In life history evolution, we are interested in the interaction between age and environment, or how age interacts with environment to generate variation in life history strategy. Thus, there is a need in age-based life history studies for an analytical framework that can compare the coordinated multivariate responses among populations. Additionally, this framework would employ a technique to

interpret significant age by environment interactions to determine if patterns of life history variation are consistent with life history hypotheses (Adams and Collyer 2007).

An organism's lifetime life history strategy can be described by a phenotypic change vector (Collyer and Adams 2007; Adams and Collyer 2009): a trajectory across a set of ages in multivariate phenotypic space (life history traits) representing the balance of tradeoffs at each reproductive bout that is utilized by an individual. Comparisons of life history trajectories will provide an assessment of the patterns of phenotypic change (amount and direction) among populations or species and will indicate how fitness is maximized by each population or species given environmental, physiological, morphological, or other constraints. Such comparisons could provide greater understanding to the fitness advantages of different strategies, could determine if patterns of life history strategies are consistent across selective regimes, and could generate predictions of expected life history strategies given taxonomic group, habitat type, etc.

Recently, an analytical procedure has been developed that quantifies patterns of phenotypic change across multiple levels to test evolutionary hypotheses (Adams and Collyer 2007; Collyer and Adams 2007; Adams and Collyer 2009). This procedure uses a phenotypic change vector, defined as the trajectory across a set of evolutionary levels in multivariate phenotypic space. In the analysis, attributes of trajectories (magnitude, orientation, and shape) are quantified and statistically compared across pairs of taxa to determine if the patterns of phenotypic change differ across taxa and the extent to which the patterns are similar. The procedure is illustrated using morphological data, but it is suggested that the procedure can be used to quantify phenotypic evolution for other traits with multivariate data, such as life history traits (Collyer and Adams 2007; Adams and Collyer 2009). In this paper, we adapt the analytical procedure presented by Adams and Collyer (2009) to develop a life history trajectory analysis for

quantifying phenotypic trajectories of life history strategies. First, we summarize the analytical approach to demonstrate how life history trajectories are constructed and statistically compared. We then illustrate the approach with two examples, one comparing life history trajectories in the burying beetle *Nicrophorus orbicollis* and one comparing life history trajectories in the livebearing fish *Brachyrhaphis rhabdophora*. Finally, we discuss how this procedure can be used to test life history theory, particularly hypotheses that predict patterns of allocation in response to a change in age or state of individuals.

Trajectory Analysis

Evolutionary ecology research, which among other topics studies phenotypic variation and its causes, has made a recent shift to focus on the covariation of phenotypic traits (Pigliucci 2003; Hoverman et al. 2005; Collyer and Adams 2007). Analysis of multivariate phenotypic change can be accomplished using the multivariate analysis of variance (MANOVA) to test for differences between operational taxonomic units and another categorical variable, such as environmental condition (Adams 2004; Langerhans et al. 2004). However, unlike univariate analysis of variance, interpretation of a significant interaction in multivariate data is not intuitive (Collyer and Adams 2007). The framework for analysis of phenotypic trajectories (Adams and Collyer 2007; Collyer and Adams 2007; Adams and Collyer 2009) provides an appropriate method to test how phenotypic change varies in multivariate space when the interaction between operational taxonomic unit and categorical variable is significant in the MANOVA model.

Phenotypic change can occur across two or more levels that form a sequence, with phenotypic change being defined as the difference in phenotype that is expressed across the levels of the sequence. Levels can represent phenotypes in time (e.g. ancestor and descendent

phenotypes, pre- and post-disturbance phenotypes, age-based phenotypes) or in space (e.g. phenotypes in differing environments). For each case, phenotypic change is expressed as a vector or trajectory connecting the phenotypic means from each level in multivariate trait space defined by phenotypic traits. Phenotypic trajectories can be characterized by their mathematical attributes: magnitude or amount of phenotypic change, orientation or direction of phenotypic change, and shape or path of phenotypic evolution (only for trajectories with more than two levels; Adams and Collyer 2009). These attributes provide valuable insight on the pattern of phenotypic variation: does one population or taxon exhibit greater divergence over time or space; do the patterns of phenotypic change among populations or taxa represent parallelism, divergence, or convergence; does each population demonstrate a gradual, unidirectional change from one phenotype to another or a fluctuating or oscillating change in phenotype across levels? Thus, a trajectory analysis can quantitatively provide a description of patterns of phenotypic variation that can provide greater understanding to evolutionary patterns.

To assess differences in trajectory attributes, a multivariate analysis of variance (MANOVA) is used to test for differences in phenotypic change across trajectory levels of the taxonomic units (e.g. populations, species). The interaction of taxonomic unit and trajectory level in the MANOVA indicates if the attributes of the trajectories are significantly different (Collyer and Adams 2007). When this interaction is significant, the differences in trajectory attributes can be statistically tested using a residual randomization approach (Adams and Collyer 2007). The residuals from a reduced model of the MANOVA that lacks the taxonomic unit by trajectory level interaction are randomized and added to predicted values to produce random values. These random variables are then used in a MANOVA that has the taxonomic unit by

trajectory level interaction to generate a distribution of random values to compare to the observed values of trajectory attributes (Adams and Collyer 2009).

The procedure for analyzing trajectory attributes was originally proposed for comparing phenotypic change in a pairwise fashion (Adams and Collyer 2007; Collyer and Adams 2007). When more than two lineages or taxa are examined, the pairwise comparisons do not provide an overall assessment of the relative similarity of evolutionary responses across the set of trajectories being compared. Adams and Collyer (2009) provided an overall test that calculates summary statistics for each trajectory attribute that are evaluated using the same permutation procedure described above. These summary statistics will be significantly different from random chance if the variation in differences across the set of trajectories is greater than expected for a given attribute. Therefore, this procedure for quantitatively assessing the relative similarity of evolutionary responses provides an assessment of the predictability of evolutionary patterns across multiple lineages or taxa.

Life history trajectory analysis

As indicated by the authors, the analytical framework for analysis of multivariate phenotypic change can be used for analysis of any multivariate phenotypic trait (Collyer and Adams 2007; Adams and Collyer 2009). We modified the framework to develop the life history trajectory analysis, which can be used to compare phenotypic variation in life history strategies (see Appendix for example code for life history trajectory analysis).

Prior to using the life history trajectory analysis, it is essential to establish the appropriate levels for the trajectories to use in the analysis. Trajectories can be constructed for age-matched individuals with the number of levels determined by the maximum age of the individuals or a

subset of reproductive ages of interest given the research objectives. However, a limitation of the life history trajectory analysis and the framework for analysis of phenotypic change is that the trajectories must have an equal number of levels. This presents a challenge because variation in lifespan among individuals limits the number of levels that can be used in the trajectories to the maximum age of the individual with the shortest lifespan. Alternatively, the levels of a trajectory can be established from age classes, the simplest being a two-class trajectory of young and old, or early and late-reproducing, individuals. An individual's life history is not only influenced by age, but also by the individual's state, or condition (McNamara and Houston 1996; McNamara et al. 2009). Using age classes to set the levels for the trajectories provides the researcher the opportunity to construct life history trajectories that can compare age- and state-based cues simultaneously. For example, life history trajectories could be constructed by using levels based on similar periods in the organisms' lives, such as a three-level trajectory that represents early-, mid-, and late-life. In this manner, the life history response to both age- and state-based cues (i.e. changes in probability of survival and somatic deterioration) can be compared among individuals or organisms that vary in lifespan. Thus, establishing life history trajectories based on age classes provides the opportunity to broaden the diversity of taxa that can be included in a study, broadening the generalizations from life history hypotheses.

For organisms with indeterminate growth, reproductive output is positively correlate with body size and consequently with age (Sparkman et al. 2007; Belk and Tuckfield 2010). This presents a challenge of separating the relative effects of body size and age on life history strategy particularly if the selective regime being tested results in differences in body size patterns on operational taxonomic units (e.g. body size differences in livebearing fishes in predator versus no-predator environments; Reznick and Endler 1982; Johnson and Belk 2001; Jennions and

Telford 2002). Adjustment for variation in body size can be accomplished using a log-log regression of the life history trait on body size; the residuals from the regression can then be used in the subsequent analyses (Dobson and Oli 2008; Reisch et al. 2010). Additionally, we suggest that the slope of the regression be constrained to a value of one; this will account for isometric variation of life history traits with body size while maintaining allometric variation (e.g. due to terminal investment which would represent a hyperallometric relationship or slope greater than one, or due to senescence which would represent a hypoallometric relationship or a slope less than one).

The life history trajectory analysis also provides a mechanism to test observed patterns in life history strategies to predictions of the cost of reproduction hypothesis or to predictions of other cues that similarly affect within lifetime changes in life history. This is best demonstrated for organisms with indeterminate growth. Predictions of the cost of reproduction hypothesis and of other mechanisms cause changes in life history traits to deviate from isometric change with increasing body size as indicated above. Therefore, life history trajectories can be generated based on these predictions and incorporated into the analysis to provide a formal test of the hypotheses.

Prior to using the life history trajectory analysis, a principal components analysis of the life history data (transformed data or residuals as indicated above) should be used to obtain principal components for each individual. These principal components are then used as the response variable in the MANOVA. Life history data can be used as the response in the MANOVA; however, interpretation of differences in orientation between life history trajectories can not be interpreted in a straightforward manner. Additionally, principal components facilitates the visualization of life history trajectories on just two axes while still accounting for a

large portion of the variation in the multivariate data. Using principal components similarly provides the opportunity to reduce the number of variables used in the analysis if necessary while still incorporating all life history traits.

The life history trajectory analysis uses a MANOVA to compare differences in life history strategies between operational taxonomic units (Collyer and Adams 2007; Adams and Collyer 2009). Under most experimental designs, however, a mixed model MANOVA will be the most appropriate model for analysis because random effects will be included in the model. For example, longitudinal studies in which life history strategies of individuals are quantified through time will include random effects in the form of repeated measures (see burying beetle example below). We therefore describe how to incorporate a mixed model MANOVA in the life history trajectory analysis.

Differences in life history trajectories between operational taxonomic units can be assessed using a mixed model MANOVA with principal components as the response variables for each individual. The random effect will be determined by the experimental design. The main effects will be operational taxonomic units (e.g. populations, species, etc.), trajectory levels (i.e. ages or age classes that define life history trajectories), and their interaction. Additionally, an index variable that represents the ordering of the principal components should also be included as a main effect along with its interactions with the other main effects (Wesner et al. in press). Without including the index variable, the model will test if the direction and magnitude of differences between levels of the main effect are consistent on all principal components. However, principal components are orthogonal and ordered according to the amount of variance they explain; as a consequence, the magnitude and direction of differences between levels of main effects on one principal component have no bearing on the differences

(magnitude or direction) between levels on other principal components. By including the index variable and associated interactions in the model, we account for possibility that differences in direction and magnitude between levels of main effects will occur on some of the principal components. Therefore, the interaction of index variable with main effect(s) allows differences of magnitude and direction to be detected independently among principal components (Rencher 2002; Butler et al. 2009). In the MANOVA, the interaction of operational taxonomic units, trajectory levels, and the index variable indicates whether life history trajectories have differences in trajectory attributes. The life history trajectory analysis incorporates the residual randomization procedure as described in Adams and Collyer (2009) to determine whether the significant three-way interaction indicates differences in trajectory magnitude, orientation, or shape.

We provide two empirical examples that demonstrate the utility of the life history trajectory analysis for comparing phenotypic change in life history strategy.

Example 1: Burying beetle

In this example, we used life history data for a burying beetle (*Nicrophorus orbicollis*) from Creighton et al. (2009) who examined the cost of reproduction hypothesis and terminal investment hypothesis by measuring life history traits for females on successive reproductive bouts. The authors used three reproductive treatments to test these hypotheses: a 20-g control for which females were given a 20-g mouse carcass each reproductive bout; a 30-g control for which females were given a 30-g mouse carcass each reproductive bout; and an experimental or over-allocation treatment for which females were initially given a 30-g carcass that was switched for a prepared 20-g carcass after reproduction was initiated (Creighton et al. 2009). The results of this

study presented strong support to both hypotheses: all reproductive females had shorter life spans than nonreproductive females; reproductive allocation increased with age; females with larger carcasses invested more into current reproduction than did females with small carcasses; females that over-allocated had reduced fecundity and life span compared to controls (Creighton et al. 2009). Thus, these data present an excellent example to compare life history trajectories of individuals with different reproductive strategies.

Female burying beetles demonstrated the effects of the cost of reproduction through variation in their life span given the different treatments (Creighton et al. 2009). Females in the 20-g control lived the longest (approximately 5 reproductive bouts); 30-g control had intermediate lifespan (approximately 4 reproductive bouts); experimental treatment had shortest lifespan (approximately 3 reproductive bouts). Given these differences in life span, we could not compare life history trajectories among treatments with the full data. Instead, we used four comparisons to demonstrate different alternatives for creating life history trajectories. First, we analyzed life history strategy for all treatments as two-point trajectories characterized by the first two reproductive bouts for each individual. Next, we conducted an analysis of variation in life history strategy on the first three reproductive bouts for all treatments. However, the third reproductive bout represents the last bout for females in the experimental treatment, but represents mid-life for females in the 20-g control. Therefore, life history trajectories were also compared as a two-point trajectory of the first and last reproductive bouts for each female. Finally, life history strategies were compared using a three-point trajectory that represented the first three bouts for the experimental treatment, the first, second, and fourth bouts for the 30-g control, and the first, third, and fifth bouts for the 20-g control. These trajectories would

represent similar stages in the life of females in each treatment (either early and late or early, mid, and late life for the two-point and three-point trajectories, respectively).

Four life history traits were measured for each female for each reproductive bout: 1) brood size (number of third instar larvae at dispersal from carcass), 2) offspring size (mean mass [g] of larvae at dispersal), 3) female mass change (female mass [g] at start of bout minus female mass at end of bout), and 4) carcass use efficiency (combined mass of larvae at dispersal and female mass change divided by carcass mass). Prior to analyses, we transformed life history traits to accommodate for potential nonlinear relationships between variables; brood size was square root transformed, offspring size and female mass change were $\log_{10}(x+1)$ transformed, and carcass use efficiency was arcsine square root transformed. Four principal component scores for each individual were obtained from a principal components analysis of the four life history traits, and were used as response variables in subsequent analyses.

We described life history strategy in four quadrants on the first two principal components, which accounted for 73.3% of the variance, based on the combination of loadings of life history traits on each principal component (Figure 1) and predictions from age- and state-based life history hypotheses. A female that exhibited high allocation to current reproduction would have a small or negative mass change (quadrants I and II in Figure 1) because more of the carcass would be used to feed the offspring as opposed to the female. A female that had a high somatic condition would also be able to maximize other life history traits (i.e. many large offspring; quadrant I in Figure 1) while a female in poor somatic condition would not be able to maximize all traits if any at all (i.e. few large offspring; quadrant II in Figure 1). A female that allocated to future reproduction would exhibit a higher or positive mass change and would minimize all life history traits to further reduce costs of reproduction (quadrant III in Figure 1).

Quadrant IV in Figure 1 represents a strategy with high allocation to future reproduction with only offspring size minimized (i.e. many small offspring).

We assessed differences in life history strategies between treatments using a mixed model MANOVA. The main effects in the model were treatment (three levels), reproductive bout (two or three levels depending on comparisons; see description above), the index variable, and all possible interactions. The random effect was subject or female. Following a significant interaction of treatment, reproductive bout, and index variable, we applied the life history trajectory analysis with 10,000 permutations to determine whether the interaction indicated differences in trajectory magnitude, orientation, or shape. All analyses were conducted in R (R Core Development Team 2010); mixed model MANOVAs were conducted in ASREML-R version 3.00 (Butler et al. 200) within R.

With only the first two reproductive bouts, life history strategy was different among treatments (treatment by index variable interaction; Table 1). The interaction of treatment, reproductive bout, and index variable was not significant, and it was not necessary to use the life history analysis to compare trajectory attributes (magnitude and direction; Table 1 & 2). Females in the 20-g control exhibited a strategy consistent with allocation to future reproduction (quadrant 3; Figure 2a), while the 30-g control and experimental treatments exhibited a strategy consistent with allocation to current reproduction by beetles with high somatic condition (quadrant 1; Figure 2a).

When the first three reproductive bouts were compared among treatments, the significant interaction of treatment, reproductive bout, and index variable in the MANOVA indicated that life history strategies were different among treatments (Table 1). Females in the experimental treatment exhibited a greater amount of phenotypic change compared to the 20-g and 30-g

controls; the magnitude of life history change did not differ between the 20-g and 30-g controls (Table 2). All trajectories exhibited a shift towards the second quadrant, indicative of a shift towards allocation to current reproduction with a decrease in somatic condition, a pattern consistent with the cost of reproduction hypothesis (Figure 2b). This shift was the greatest for the experimental treatment in which females were forced to over-allocate and incur a higher cost of reproduction and subsequent deterioration in somatic condition. Additionally, females in the experimental treatment reproduced for a mean of three reproductive bouts before dying, indicative of large costs of reproduction on the first three reproductive bouts, while females in the 20-g and 30-g controls were able to reproduce a mean of five and four reproductive bouts, respectively, indicative of lower costs incurred on these first three reproductive bouts. Therefore, trajectory magnitude and costs of reproduction are positively correlated in these analyses, that is greater trajectory magnitude infers higher costs of reproduction incurred. The direction and shape of phenotypic change in life history strategy did not differ among treatments (Table 2).

The first three bouts represent different somatic states for females in each treatment as indicated by the differences in mean life span of females in each treatment. Therefore, trajectories constructed from reproductive bouts that represent similar states in a female's life (i.e. first and last reproductive bouts; first, mid-life, and last reproductive bouts) will provide a better description of phenotypic changes in life history strategy across an individual's lifetime. For both the two point (first and last reproductive bouts; Figure 2c) and the three point (first, mid-life, and last reproductive bouts; Figure 2d) state-based trajectories, the significant interaction of treatment, bout, and index variable indicated differences in life history trajectories. Both trajectory analyses demonstrated that these differences were the result of significant

differences in the orientation or direction of phenotypic change, with the experimental treatment having a significantly different direction through multivariate space than the 20-g or 30-g control treatments, which did not significantly differ from each other (Table 2). The magnitude and shape of trajectories were not significantly different among treatments. As indicated above, trajectory magnitude and costs of reproduction are positively correlated in these analyses; therefore, the accumulated cost of reproduction or deterioration of somatic condition across each female's lifetime was similar regardless of treatment.

Example 2: Costa Rican livebearing fish

The Costa Rican livebearing fish *Brachyrhaphis rhabdophora* is found in streams with divergent selective environments characterized by a correlated suite of ecological factors including the presence or absence of predators (Johnson 2002; Johnson and Zúñiga-Vega 2009). For consistency with previous research, we refer to streams as predator and no-predator (or predator-free) environments (Johnson and Belk 2001; Johnson 2002; Johnson and Zúñiga-Vega 2009). Predation acts as a strong selective agent for this fish, particularly on adult individuals; survival decreases significantly for adult fish in predator environments while increasing significantly in no-predator environments (Johnson and Zúñiga-Vega 2009). This difference in survival causes differences in morphology and life history for these fish (Johnson 2001; Johnson and Belk 2001; Johnson 2002; Langerhans and DeWitt 2004). Juvenile (both sexes) and adult male *B. rhabdophora* in predator streams have a more streamlined body with a larger caudal peduncle region, shape characteristics that offer greater burst swimming performance for predator avoidance (Langerhans and DeWitt 2004; Rasmussen 2010). Adult females, however, demonstrates a convergence on shape, an apparent morphological constraint due to pregnancy

(Wesner et al. in press). Predation also causes marked differences in life history traits between environments; female *B. rhabdophora* exposed to predators exhibit earlier size at maturity, increased clutch size, and smaller embryo size relative to females in predator-free environments (Johnson 2001; Johnson and Belk 2001).

Given these effects of predation, we can make predictions about the optimal life history strategy for female *B. rhabdophora* in each environment based on the cost of reproduction hypothesis. First-reproducing adult *B. rhabdophora* in predation environments will have a lower reproductive value because they have a lower probability of survival than those in no-predator environments. This is evident in the smaller size at maturity in predation environments, a strategy that increases the likelihood of successfully reproducing before falling prey to a predator (Johnson and Belk 2001). Therefore, we predict that first-reproducing or young adult females in predation environments will allocate more energy to current rather than future reproduction than first-reproducing adult females in no-predation environments. Additionally, we predict that females in no-predator environments will have a greater increase in reproduction late in life (terminal investment) because they demonstrated greater restraint (allocation to future rather than current reproduction) early in life.

We tested these predictions using life history traits collected from 541 females collected from 15 populations (8 predator environment; 7 no-predator environment) in Costa Rica; these populations represent a subset of the data reported by Johnson and Belk (2001). We measured standard length (mm), female body mass (dry mass of viscerated body), clutch dry mass, clutch size (number of embryos), and offspring size (dry mass) for each female. Dry mass was determined after drying specimen or material for 24 hr at 55°C. We classified females from each location as young or old based on standard length; young females were those from a population

that were smaller than the population median while old females were larger than the population median.

Life history traits are often positively correlated with female body size; therefore, it was necessary to account for variation in life history traits due to changes in body size (i.e. isometric change in traits with change in body size). In a log-log regression of a trait on female body size, a slope equal to one would indicate that the trait is changing proportionally or isometrically with the change in body size. We regressed clutch dry mass, clutch size, and offspring size (each \log_{10} transformed) on \log_{10} female dry mass, constraining the slope to one; residuals from each regression were used as trait variables in subsequent analyses. We also generated trajectories based on predictions of the cost of reproduction hypothesis to create four reference trajectories to further characterize the life history trajectories for *B. rhabdophora* in predator and no-predator environments. Because magnitude of life history trajectories is more difficult to predict, these reference trajectories are used to compare direction of phenotypic change to determine if life history strategies are consistent with predictions from life history theory. The first reference line represented a life history strategy with no age-related changes in allocation or with a constant rate of allocation proportional to changes in body size (i.e. isometric increase in each trait). The second reference line represented a life history strategy that demonstrated restraint early in life (allocation to future versus current reproduction) and terminal investment (allocation to current rather than future reproduction late in life). This line was characterized as a hyper-allometric increase (slope >1) in life history traits with equal slopes for clutch size and offspring size. A common life history tradeoff is the balance of clutch size and offspring size (Roff 2002). Therefore, the third and fourth reference lines also represented a life history strategy that demonstrated restraint early in life and terminal investment, but one represented greater

allocation to clutch size (slope of clutch size \gg slope of offspring size) while the other demonstrated greater allocation to offspring size (slope of clutch size \ll slope of offspring size).

Principal component scores were calculated for each individual (including reference data) from a principal components analysis. The dependent variables were the residuals generated above for clutch dry mass, clutch size, and offspring. With principle component scores as the response variables, we used a mixed model MANOVA to assess differences in life history strategy of female *B. rhabdophora* in predator and no-predator environments. We tested the main effects and interactions of predation environment, age (two levels: young and old), and index variable; the random effect was population. The interaction of predation environment, age, and index variable was significant; we therefore conducted a trajectory analysis using 10,000 permutations to compare differences in attributes (magnitude and direction) between the life history trajectories. All analyses were conducted in R; mixed model MANOVAs were conducted in ASREML-R version 3.00 within R.

The significant interaction among predation, age, and the index variable demonstrated that the life history strategies differed for females in different predator environments (Table 3). The life history trajectories for females in no-predator and predator environments differed in size ($MD_{1,2} = 0.368$; $P_{\text{size}} = 0.018$) and in orientation ($\theta_{1,2} = 50.17^\circ$; $P_\theta < 0.001$; Figure 3). Females in no-predator environments exhibited a greater magnitude in life history change from young to old. Also, the life history trajectory of females in the no-predator environment had a similar orientation to the reference trajectory that represented increased allocation with age with greater allocation to clutch size rather than offspring size ($\theta_{1,2} = 17.85^\circ$; $P_\theta = 0.666$; other reference trajectories $\theta_{1,2} > 64.64^\circ$; $P_\theta < 0.006$). Conversely, the life history trajectory for females in the predator environment had a similar orientation to the reference trajectory that represented no

age-related changes in reproductive allocation ($\theta_{1,2} = 28.71^\circ$; $P_\theta = 0.349$; other reference trajectories $\theta_{1,2} = 68.00^\circ$; $P_\theta < 0.002$).

The variation in life history strategies of female *B. rhabdophora* in predator versus no-predator environments was consistent with our predictions. In no-predator environments, females exhibited greater phenotypic change in life history strategy between ages along an orientation that characterizes life history characteristics consistent with predictions of the cost of reproduction hypothesis. Conversely, life history strategy of females in predator environments was characterized by less phenotypic change and a constant rate of reproductive allocation (i.e. no age-related changes in allocation). Young females in the predator environment may have exhibited less reproductive restraint as compared to females in the no-predator environment and therefore incurred greater costs of reproduction. A greater cost of reproduction incurred early in life will necessarily limit future reproductive potential (McNamara et al. 2009), and may have limited the ability to terminally invest. However, females in predator environments might be constrained morphologically and unable to have greater reproductive allocation; pregnancy reduces burst swimming performance, necessary to evade predators, an effect that increases with increased reproductive allocation (Ghalambor et al. 2004; Belk and Tuckfield 2010; Wesner et al. in press). Thus, terminal investment would not be an advantageous strategy, and females would instead minimize performance effects of pregnancy and increase the probability of carrying a clutch to birth.

Discussion

Life history trajectories must be established across sequential units to quantify the path of phenotypic change. In the empirical examples, we demonstrate how these trajectories can

represent patterns of change across time either by using absolute age or an age or state classification (e.g. young and old classification). The burying beetle example demonstrated how results of trajectory analysis might differ if trajectories are constructed based on absolute age or based on relative age classification. As predicted from the cost of reproduction hypothesis, beetles exhibited variation in mean life span under the different treatments (Creighton et al. 2009). Consequently, the analysis of phenotypic change in life history demonstrated different patterns among trajectories dependent on the age classification. Variation in life history strategy between selective environments often precludes the ability to generate trajectories based on age (e.g. *Brachyrhaphis rhabdophora*); therefore, a classification of age based on relative or biological age (e.g. young –old; young, middle-age, old) or based on state or condition will provide the set of sequential units that is necessary for generating life history trajectories and that is relevant for the evolutionary units. The state or condition of individuals (e.g. body size, fat reserves, foraging skills, immune system, etc.) can have a large effect on reproductive success, can be age-independent, and can vary both within and among populations (McNamara and Houston 1996; Wendeln and Becker 1999; Hamel et al. 2009; Cotter et al. 2010). Therefore, phenotypic change in life history can be compared using state or condition as sequential units as demonstrated in the burying beetle example, or can be compared among phenotypes that are characterized by variation in somatic condition.

A major goal of evolutionary biology is to understand how natural selection generates phenotypic diversity, particularly the extent to which phenotypic change is repeatable and therefore predictable (Langerhans 2008; Adams and Collyer 2009; Langerhans 2010). Life history theory provides general predictions concerning life history tradeoffs both across age and environmental or ecological gradients (Roff 2002). Given these predictions, theoretical

trajectories can be created for the life history analysis to provide a robust a priori approach for analyzing life history strategies as compared to post-hoc explanations of reproductive patterns. The example of *Brachyrhaphis rhabdophora* demonstrated how predictions from life history theory could be incorporated into the trajectory analysis to determine the extent that life history changes are predictable. Thus, life history trajectory analysis provides a powerful tool to test the predictions of life history theory, to evaluate the types of evolutionary responses (e.g. differences in rate or amount of phenotypic evolution, evolutionary convergence or divergence, etc.), and to determine if evolutionary patterns are repeatable.

Langerhans (2008, 2010) described generalized models of divergent selection, an approach to understand the causes and consequences of phenotypic evolution to determine the predictability of evolution. Using generalized models of divergent selection, a researcher derives a priori predictions of phenotypic or genetic change based on a set of assumptions (determined by the researcher). Comparative and/or experimental data are then examined to determine if the generalized models of divergent selection produced accurate evolutionary predictions. The generalized models of divergent selection approach represents a method to determine the predictability of evolutionary change and the extent that evolutionary patterns are repeatable and predictable across disparate groups of organisms (Langerhans 2008, 2010). Combining the life history trajectory analysis and the generalized models of divergent selection approach will provide a powerful tool to generate predictions of phenotypic change in life history strategy across selection regimes and statistically test if observed phenotypic change is repeatable and predictable. For example, livebearing fish have shown consistent life history shifts among predation regimes when comparing a single reproductive decision (Reznick et al. 1996; Johnson and Belk 2001; Jennions and Telford 2002). Given differences in survival, the generalized

models of divergent selection can be used to generate predictions of phenotypic change for two types of comparisons: 1) two-point phenotypic change trajectories for multiple species to characterize divergence in single reproductive decisions (i.e. population means) in predator and no-predator environments; 2) phenotypic change trajectories characterizing life history change among ages (e.g. *Brachyrhaphis rhabdophora* example) for each species by predation environment combination. These predictions could then be tested using the life history trajectory analysis to determine if the generalized models of divergent selection accurately predicted phenotypic evolution. When phenotypic evolution differs in magnitude or direction, further examination will determine constraints, either ecological (e.g. resource availability due to competitors, such as seen in *Poeciliopsis baenschi*; Scott and Johnson 2010) or organismal (e.g. morphological constraints, such as seen in *Alfaro cultratus*; personal observation), that drive the differences in phenotypic change.

The examples examined here with the life history trajectory analysis demonstrate how the approach can be used to make comparisons within a species. However, a major goal of evolutionary biology is to determine the generality of observed patterns of phenotypic evolution (Grime 1977; Winemiller 1992; Winemiller and Rose 1992; Charnov 2002). The life history trajectory analysis can be used to examine phenotypic change across disparate taxa only when similar life history traits can be quantified for each taxa. Additionally, life history traits must be standardized prior to analysis to control for inherent variation due to body size differences among taxa (see examples in Charnov 2002). When it is not possible to quantify similar life history traits, the life history trajectory analysis could be used to compare groups of similar organisms, and then observed patterns could be compared among disparate taxa using the generalized models of divergent selection (Langerhans 2008, 2010).

The life history trajectory analysis provides a method to quantitatively assess phenotypic evolution. We believe that this analysis provides a useful tool to for examining evolutionary change to understand how phenotypic evolution is similar or different across taxa. Additionally, this procedure can be used to explicitly test predictions of life history theory, to strengthen our understanding of the evolutionary consequences of life history strategies and tradeoffs, and to generate additional life history hypotheses. Finally, the life history trajectory analysis will help evolutionary biologists realize the goal of understanding the extent to which phenotypic evolution is repeatable, particularly when combined with other approaches.

Acknowledgments

We thank Bruce Schaalje for statistical help and support. Jerry Johnson, Steve Peck, Russ Rader, and Bruce Schaalje provided direction and valuable advice that improved the quality of this paper.

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Table 1. Analysis of variance table for the mixed model MANOVA for life history strategies (defined by principal components derived from four life history traits) of female burying beetles as a function of treatment, reproductive bout, and an index variable (accounts for ordering of principal components; see explanation in text).

Effect	Degrees of Freedom	Wald Statistic	P
Bouts 1 – 2			
Treatment	2	157.055	<0.001
Bout	1	3.717	0.054
Index variable	4	41.269	<0.001
Treatment x bout	2	0.641	0.726
Treatment x index variable	6	255.477	<0.001
Bout x index variable	3	0.641	0.187
Treatment x bout x index variable	12	7.404	0.285
Bouts 1 – 3			
Treatment	2	245.946	<0.001
Bout	2	4.203	0.122
Index variable	4	20.530	<0.001
Treatment x bout	4	6.713	0.152
Treatment x index variable	6	268.147	<0.001
Bout x index variable	6	28.998	<0.001
Treatment x bout x index variable	12	23.833	0.021
Bouts first and last			
Treatment	2	163.859	<0.001
Bout	1	33.028	<0.001
Index variable	4	9.242	0.055
Treatment x bout	2	42.039	<0.001
Treatment x index variable	6	136.221	<0.001
Bout x index variable	3	72.818	<0.001
Treatment x bout x index variable	6	34.818	<0.001
Bouts first mid last			
Treatment	2	210.533	<0.001
Bout	2	31.210	<0.001
Index variable	4	3.651	0.455
Treatment x bout	4	40.406	<0.001
Treatment x index variable	6	195.947	<0.001
Bout x index variable	6	86.709	<0.001
Treatment x bout x index variable	12	42.284	<0.001

Table 2. Statistical assessment of differences in life history trajectory size ($MD_{1,2}$), direction ($\theta_{1,2}$), and shape (D_{shape}) between three reproductive treatments for female burying beetles: 20-g control, 30-g control, and experimental treatment. Significant differences as generated empirically from 10,000 permutations are indicated in bold.

Comparison	$MD_{1,2}$	P_{size}	$\theta_{1,2}$	P_0	D_{shape}	P_{shape}
Bouts 1 – 2						
20-g, 30-g	0.0389	0.9127	55.0986	0.6210	-	-
20-g, experimental	0.5229	0.1569	111.6848	0.2780	-	-
30-g, experimental	0.4840	0.1959	61.3451	0.4882	-	-
Bouts 1 – 3						
20-g, 30-g	0.0468	0.9266	46.1876	0.2527	0.3205	0.5620
20-g, experimental	1.2129	0.0311	80.3320	0.1451	0.0489	0.9856
30-g, experimental	1.1661	0.0367	51.0379	0.2502	0.2979	0.6154
Bouts first and last						
20-g, 30-g	1.0119	0.0726	30.3599	0.1325	-	-
20-g, experimental	0.6413	0.2856	70.6060	0.0324	-	-
30-g, experimental	0.3706	0.5368	44.3570	0.0384	-	-
Bouts first mid last						
20-g, 30-g	1.0809	0.0570	30.5454	0.1007	0.0027	0.9991
20-g, experimental	0.7107	0.2289	66.8693	0.0305	0.1874	0.6244
30-g, experimental	0.3702	0.5268	43.2313	0.0359	0.1896	0.6094

Table 3. Analysis of variance table for the mixed model MANOVA for the life history strategies (defined by principal components derived from three life history traits) of female *Brachyrhaphis rhabdophora* as a function of predation environment, age, and an index variable.

Effect	Degrees of Freedom	Wald Statistic	P
Predation	1	0.000	1.000
Age	1	172.788	<0.001
Index variable	3	0.000	1.000
Predation x age	1	2.487	0.115
Predation x index variable	2	0.000	1.000
Age x index variable	2	278.786	<0.001
Predation x age x index variable	2	25.701	<0.001

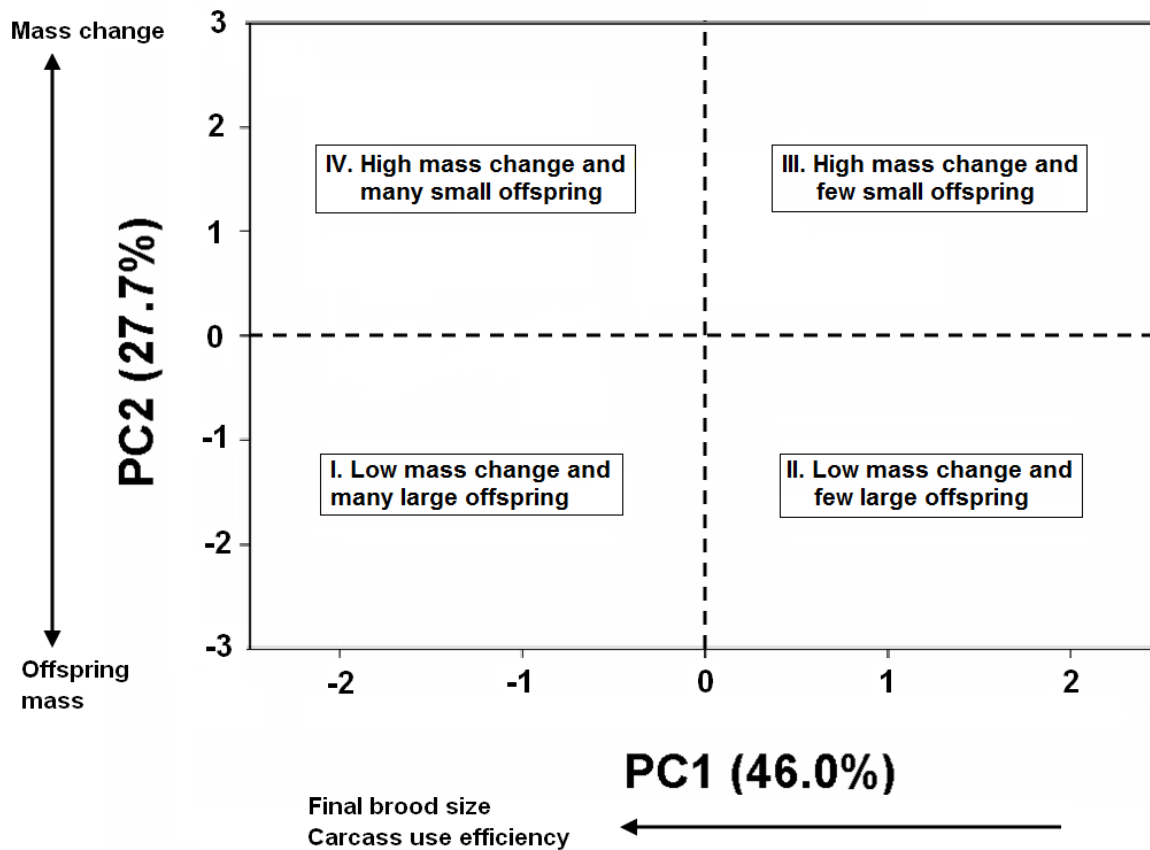


Figure 1. Summary of the four quadrants in multivariate trait space for burying beetle life history trajectories on the first two principle components based on loadings of life history traits. Interpretations of characteristic life history strategies for each quadrant can be made using life history theory (see text for interpretations).

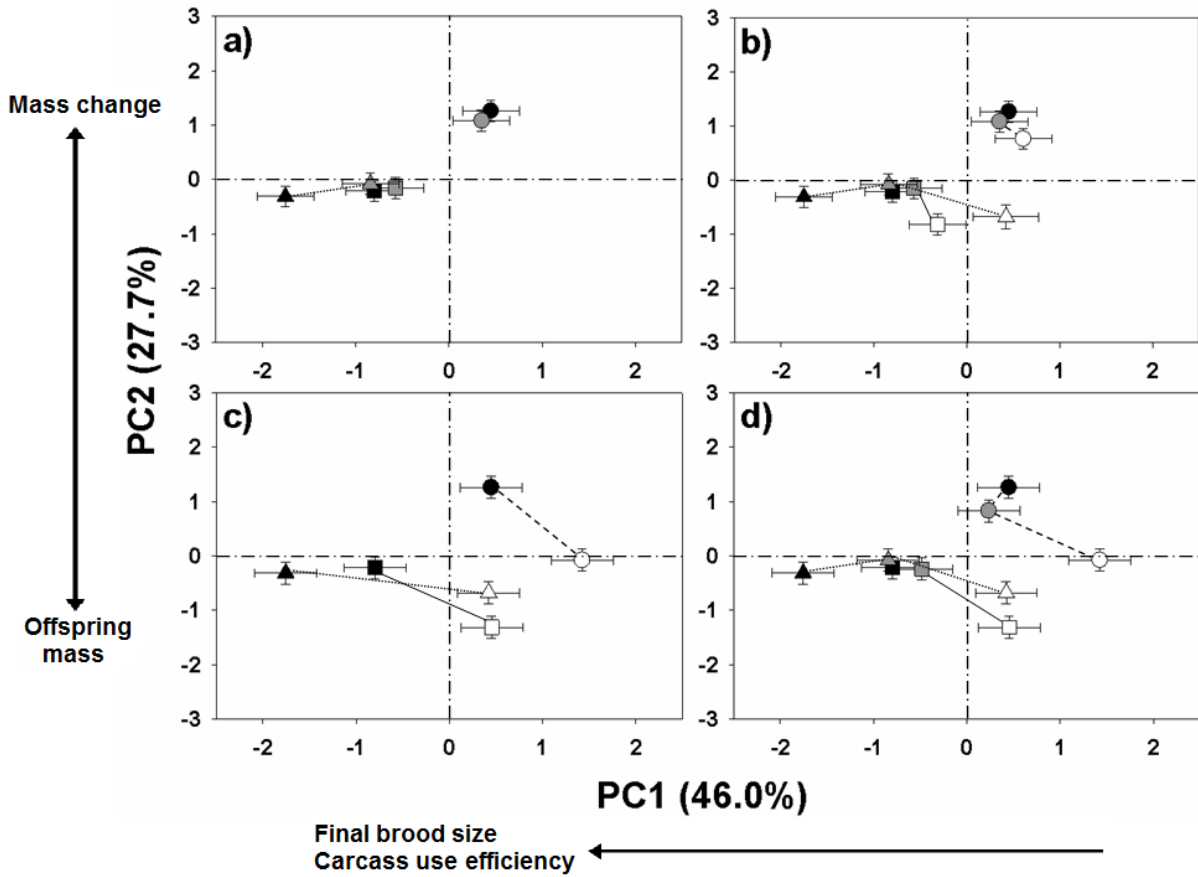


Figure 2. Least squares means (\pm SE) of principal component scores for life history trajectories for female burying beetles in three treatments: 20-g control (circles), 30-g control (squares), and experimental treatment (triangles). Life history trajectories were constructed in four ways: a) first and second reproductive bouts; b) first, second and third reproductive bouts; c) first and last reproductive bouts; d) first, mid-life, and last reproductive bouts. Black symbols represent first reproductive bouts, gray symbols represent second or mid-life reproductive bouts, and white symbols represent third or last reproductive bout.

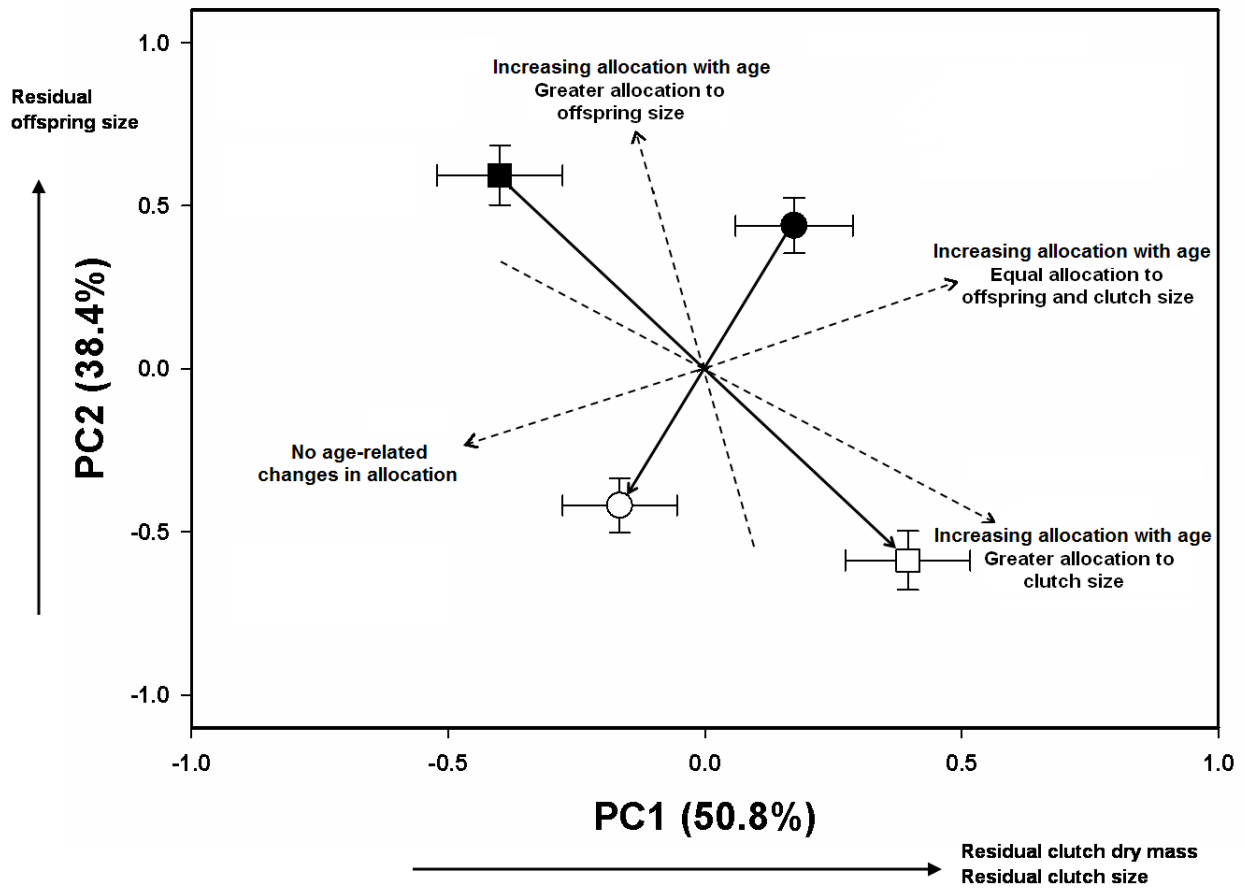


Figure 3. Least squares means (\pm SE) of principal component scores for life history trajectories for female *Brachyrhaphis rhabdophora* from predator (squares) and no-predator environments (circles). Filled symbols represent young females and open symbols represent old females. Arrows with dashed lines represent reference lines generated from life history predictions of age-based changes in reproductive allocation and the null hypothesis of constant allocation or no age-based changes in reproductive allocation.

Chapter 2: Appendix

R Code for Life History Trajectory Analysis

```
## Life History Trajectory Analysis ##
# Modified from Adams and Collyer 2009 #

# Code assumes first column = random variable, second column = taxa, third column = levels #
# This example shows code for burying beetle example #
# Random variable = female, taxa = treatment, levels = bout #

# Read and set-up data #
data<-read.csv("data.csv",header=T)
data$female<-as.factor(data$female)
data$treatment<-as.factor(data$treatment)
data$bout<-as.factor(data$bout)

# separates just the factor columns from the rest of the data;
# used in the resid randomization #
factors<-data[,1:3]

# Principal components analysis #
pc<-prcomp(data[,4:7],scale=TRUE)$x
data<-cbind(data,pc)

pcsummary<-summary(prcomp(data[,4:7],scale=TRUE))
plot(prcomp(data[,4:7],scale=TRUE)) #####scree plot
plot(prcomp(data[,4:7],scale=TRUE)$x) #####plot of data with no classifications

#Basic data parameters (n=# of taxa, p=# trajectory levels, k=# dimensions)
n<-length(levels(data$treatment))
p<-length(levels(data$bout))
k<-ncol(pc) #dimensions of phenotypic data

# Mixed Model Multivariate Analysis of Variance of Observed Data #
library(asreml)

#Full model#
data.full <- asreml(fixed=cbind(PC1,PC2,PC3,PC4)~trait*treatment*bout, random=~female,
rcov=~units:corh(trait), data=data)
update.asreml(data.full)
write.csv(wald(data.full),'Full.Wald.LHtrajectory.csv')
```

```

#Reduced model#
data.red <- asreml(fixed=cbind(PC1,PC2,PC3,PC4)~trait*(treatment+bout), random=~female,
rcov=~units:corh(trait), data=data)
update.asreml(data.red)
write.csv(wald(data.red),'Reduced.Wald.LHtrajectory.csv')

# Retrieve and format LS Means from Full Model #
data.pred <- predict.asreml(data.full,classify='trait:treatment:bout')

tmp <- data.frame(data.pred$predictions)
tmp <- tmp[,4]
tmp4 <- NULL #Parses LS Means
for (i in 1:k){
tmp5 <- tmp[(i*(n*p)-(n*p-1)):i*(n*p)]
tmp4 <-<- cbind(tmp4,tmp5)
}
lsmeans.obs <- as.matrix(tmp4)
dim(lsmeans.obs) <- dim(lsmeans.obs)

# Retrieve and format residuals from Reduced Model #
tmp2 <- NULL
for (i in 1:k){
tmp3 <- NULL
  for(x in 0:(nrow(data)-1)){
    tmp <- data.red$residuals[(i+k*x)]
    tmp3 <- rbind(tmp3,tmp)}
tmp2 <- cbind(tmp2,tmp3)
}
res.red <- as.matrix(tmp2)
dim(res.red) <- dim(res.red)

# Retrieve and format predicted values from Reduced Model #
tmp2 <- NULL
for (i in 1:k){
tmp3 <- NULL
  for(x in 0:(nrow(data)-1)){
    tmp <- fitted(data.red)[(i+k*x)]
    tmp3 <- rbind(tmp3,tmp)}
tmp2 <- cbind(tmp2,tmp3)
}
yhat.red <- as.matrix(tmp2)
dim(yhat.red) <- dim(yhat.red)

# Functions calculating Trajectory attributes #

#Ls means organized as 3-d arrays

```

```

arrayspecs<-function(A){
n.m<-NULL # n.m stands for "new" means
for(i in 1:n){
  temp<-as.matrix(A[((1+(i-1)*p):(i*p)),1:k])
  n.m<-cbind(n.m,temp)}
trajectories<-array(n.m,dim=c(p,k,n))
}

# Pathlength distance
pathdist<-function(M) { as.matrix(dist(M))}
trajsize<-function(M){
traj.pathdist<-array(0,dim=c(n,1))          #loop across trajectories
for (i in 1:n){
  temp<-pathdist(M[,i])
  for (j in 1:(p-1)){
    traj.pathdist[i]<-traj.pathdist[i]+temp[j,j+1]
  }
}
traj.size.dist<-as.matrix(dist(traj.pathdist))      #TrajSizeDiff
}

#trajectory direction
orient<-function(M) {(svd(var(M))$v[1:k,1])}      #find orientation
trajorient<-function(M){
traj.orient<-array(NA,dim=c(n,k))          #loop across trajectories
check.1<-array(NA,dim=c(n))
for (i in 1:n){
  temp<-orient(M[,i])
  traj.orient[i,]<-temp
  check.1[i]<-M[1,,i]%*%traj.orient[i,] #check startingpoint location
  check.1[i]<-check.1[i]/abs(check.1[i])
  if(check.1[i]==-1) traj.orient[i,]<-1*traj.orient[i,]
}
options(warn=-1)          #b/c acos of 1 (e.g. on diagonal) yields warning
traj.ang.diff<-(-180/pi)*acos(traj.orient%*%t(traj.orient))
#diag(traj.ang.diff)<-0
}

#trajectory shape
#### GPA: following J. Claude 2008: Morphometrics in R
trans<-function(A){scale(A,scale=F)}          ##TRANSLATION
csize<-function(A)          ##CSIZE
  {p<-dim(A)[1]
  size<-sqrt(sum(apply(A,2,var))*(p-1))
  list("centroid_size"=size,"scaled"=A/size)}
mshape<-function(A){apply(A,c(1,2),mean)}      #meanshape

```

```

pPsup<-function(M1,M2){
  k<-ncol(M1)
  Z1<-trans(csize(M1)[[2]])
  Z2<-trans(csize(M2)[[2]])
  sv<-svd(t(Z2)%**Z1)
  U<-sv$v; V<-sv$u; Delt<-sv$d
  sig<-sign(det(t(Z1)%**Z2))
  Delt[k]<-sig*abs(Delt[k]); V[,k]<-sig*V[,k]
  Gam<-U%**t(V)
  beta<-sum(Delt)
  list(Mp1=beta*Z1%**Gam,Mp2=Z2,rotation=Gam,scale=beta,
      df=sqrt(1-beta^2))}

pgpa<-function(A)
  {p<-dim(A)[1]; k<-dim(A)[2]; n<-dim(A)[3]
  temp2<-temp1<-array(NA,dim=c(p,k,n)); Siz<-numeric(n)#; Qm2<-numeric(n)
  for (i in 1:n)
    {Acs<-csize(A[,i])
    Siz[i]<-Acs[[1]]
    temp1[,i]<-trans(Acs[[2]])}
  Qm1<-dist(t(matrix(temp1,k*p,n)))
  Q<-sum(Qm1); iter<-0
  while (abs(Q)> 0.00001)
    {for(i in 1:n){
    M<-mshape(temp1[,,-i])
    temp2[,i]<-pPsup(temp1[,i],M)[[1]]}
    Qm2<-dist(t(matrix(temp2,k*p,n)))
    Q<-sum(Qm1)-sum(Qm2)
    Qm1<-Qm2
    iter=iter+1
    temp1<-temp2}
  list("rotated"=temp2,"it.number"=iter,"Q"=Q,"intereucl.dist"=Qm2,"mshape"=
      csize(mshape(temp2)[[2]]),"cent.size"=Siz)
}

## loop for GPA and shape distances
trajshape<-function(M){
  x<-pgpa(M)
  traj.shape.dist<-as.matrix(x$intereucl.dist)
}

## TrajectorySummaryStat
sumstat<-function(M){
  M<-as.dist(M)
  x<-if(length(M)>1)(x=var(M)) else 0
}

```

```
}
```

```
#####MAIN LOOP#####
```

```
traj.specs.obs<-arrayspecs(lsmmeans.obs)  
trajsize.obs<-trajsize(traj.specs.obs)  
trajdir.obs<-trajorient(traj.specs.obs)  
diag(trajdir.obs)<-0 #b/c some NA/N values on diagonal)  
trajshape.obs<-trajshape(traj.specs.obs)  
sumstatsize.obs<-sumstat(trajsize.obs)  
sumstatdir.obs<-sumstat(trajdir.obs)  
sumstatshape.obs<-sumstat(trajshape.obs)
```

```
#####
```

```
# Residual Randomization Procedure #
```

```
permute<-9999
```

```
line<-nrow(pc)
```

```
PSize<-POrient<-PShape<-array(1,dim=c(n,n))
```

```
PSumSize<-PSumOrient<-PSumShape<-1
```

```
for(i in 1:permute){
```

```
  line.rand<-sample(line,replace=FALSE)
```

```
  res.temp<-cbind(line.rand,res.red)
```

```
  z<-(order(line.rand))
```

```
  res.temp2<-as.matrix(res.temp[z,])
```

```
  res.p<-res.temp2[,-1] # Rows of residuals are now randomized
```

```
  y.rand<-yhat.red+res.p
```

```
  y.rand<-cbind(factors,y.rand)
```

```
  data.full.rand <- asreml(fixed=cbind(V1,V2,V3,V4)~trait*treatment*bout,  
    random=~female, rcov=~units:corh(trait),  
    control=asreml.control(trace=F), maxiter=100, data=y.rand)
```

```
  if(data.full.rand$converge=="TRUE"){print("TRUE")}
```

```
  else{print("FALSE")}
```

```
  data.pred.rand <- predict.asreml(data.full.rand,classify='trait:treatment:bout')
```

```
  if(data.pred.rand$converge=="TRUE"){
```

```
    tmp <- data.frame(data.pred.rand$predictions) # Format LS Means
```

```
    tmp <- tmp[,4]
```

```
    tmp4 <- NULL #Parses LS Means
```

```
    for (x in 1:k){
```

```
      tmp5 <- tmp[(x*(n*p)-(n*p-1)):(i*n*p)]
```

```
      tmp4 <<- cbind(tmp4,tmp5)
```

```
    }
```

```

lsmeans.rand <- as.matrix(tmp4)
dim(lsmeans.rand) <- dim(lsmeans.rand)

traj.specs.rand<-arrayspecs(lsmeans.rand)
trajsize.rand<-trajsize(traj.specs.rand)
trajdir.rand<-trajorient(traj.specs.rand)
diag(trajdir.rand)<-0 #b/c some NA/N values on diagonal)
trajshape.rand<-trajshape(traj.specs.rand)
sumstatsize.rand<-sumstat(trajsize.rand)
sumstatdir.rand<-sumstat(trajdir.rand)
sumstatshape.rand<-sumstat(trajshape.rand)
for(j in 1:n){
  for(jj in 1:n){
    PSize[j,jj]<-if(trajsize.rand[j,jj]>=trajsize.obs[j,jj])
      (PSize[j,jj]+1) else PSize[j,jj]
    POrient[j,jj]<-if(trajdir.rand[j,jj]>=trajdir.obs[j,jj])
      (POrient[j,jj]+1) else POrient[j,jj]
    PShape[j,jj]<-if(trajshape.rand[j,jj]>=trajshape.obs[j,jj])
      (PShape[j,jj]+1) else PShape[j,jj]
  }
}
PSumSize<-if(sumstatsize.rand>=sumstatsize.obs) (PSumSize+1) else PSumSize
PSumSOrient<-if(sumstatdir.rand>=sumstatdir.obs) (PSumOrient+1) else PSumOrient
PSumShape<-if(sumstatshape.rand>=sumstatshape.obs) (PSumShape+1) else
PSumShape
}
else{permute<-permute+1}
print(i)}

for(j in 1:n){
  for(jj in 1:n){
    PSize[j,jj]<-PSize[j,jj]/(permute+1)
    POrient[j,jj]<-POrient[j,jj]/(permute+1)
    PShape[j,jj]<-PShape[j,jj]/(permute+1)
  }
} ### End Permute ###

# Calculate P-values for summary statistics #
PSumSize<-PSumSize/(permute+1)
PSumOrient<-PSumOrient/(permute+1)
PSumShape<-PSumShape/(permute+1)

# Trajectory pairwise comparisons and P-values #
trajsize.obs
PSize

```

trajdir.obs
POrient
trajshape.obs
PShape

#####NOTE: summary statistics only valid for 2+ trajectories!!

sumstatsize.obs
PSumSize
sumstatdir.obs
PSumOrient
sumstatshape.obs
PSumShape

Chapter 3: Effect of Prior Experience on Allocation to Current Reproduction in a Burying Beetle

Coauthors: J. Curtis Creighton and Mark C. Belk

Abstract

The cost of reproduction hypothesis predicts that the level of reproductive investment to current reproduction is determined by age-based cues or the time left for future reproduction. Young individuals should have a low level of investment to current reproduction to minimize costs to future reproduction while old individuals should have a high level of investment to current reproduction because future opportunities are not available. However, recent theoretical work suggests that age-specific fecundity is determined by the state, or level of the body's deterioration, of the individual. We evaluated the reproductive investment of same-aged female burying beetles (*Nicrophorus orbicollis*) that differed in their prior reproductive experience to determine the effect of age- and state-based cues. Consistent with previous research, females reproducing on low-quality carcasses allocated more to future reproduction by producing smaller offspring and gaining more mass than females on high-quality carcasses. When prior experience was manipulated, females that initially reproduced on a low-quality resource exhibited an accentuated response to a high-quality carcass by producing significantly larger broods of offspring compared to control females reproducing on high-quality carcasses. Conversely, females that initially reproduced on a high-quality carcass and were subsequently presented a low-quality carcass exhibited a decrease in offspring size and an increase in female mass change, indicative of a switch in allocation from current to future reproduction. The effect of prior experience resulted in a terminal investment or accentuated response to age-based cues, or

resulted in a conservative investment strategy or reproductive restraint. Our results demonstrate that the level of reproductive investment in burying beetles is the result of the combined effects of age- and state-based cues.

Keywords: life history evolution, ageing, reproductive restraint, state-dependent, resource availability, *Nicrophorus*

Introduction

Organisms exhibit great diversity in their age-related patterns of reproductive investment. Life history theory attempts to account for this diversity by explaining the age-specific patterns of fecundity that will maximize an individual's lifetime fitness (Roff 2002). Due to competing energetic demands, reproductive investment in current reproduction comes at a cost to future reproductive potential (Williams 1966; Reznick 1985). Therefore, in determining the level of reproductive investment, organisms must balance the fitness benefits of investment to current reproduction with the potential costs to future reproductive opportunities. The cost of reproduction hypothesis predicts that the level of reproductive investment to current reproduction will be determined by an individual's future reproductive potential or residual reproductive value (Fisher 1930; Williams 1966; Clutton-Brock 1984; Reznick 1985). Individuals that are young and that have a high residual reproductive value should be less willing to incur a high reproductive cost and thus should have a low level of investment to current reproduction to ensure future reproductive potential. Conversely, old individuals that have a low residual reproductive value should terminally invest, or have a high level of investment to current reproduction, because future reproductive opportunities are not available (Clutton-Brock 1984).

These predicted patterns of allocation to current versus future reproduction assume that same-aged individuals are equivalent in quality or state (McNamara and Houston 1996; Wilson and Nussey 2010). We refer to state as a suite of traits that contributes to reproductive success including but not limited to body size, immune function, fat reserves, parasite load, foraging skills, territory size, prior experience, etc. Differences that occur in state among individuals are important for determining reproductive success and result in variation in reproductive success

among individuals (Wendeln and Becker 1999; Beauplet and Guinet 2007; Hamel et al. 2009; McNamara et al. 2009; Lescroël et al. 2010). Variation in individual state may have a genetic basis, may be environmentally induced, or may be the result of an individual's genotype and the unique combination of environments experienced over the individual's lifetime (Wilson and Nussey 2010).

Early life conditions and prior reproductive experience can greatly influence state-based cues that will influence an individual's reproductive investment for current and future reproductive bouts (Creighton et al. 2009; Cotter et al. 2011). For seasonal breeders, age and state should be strongly correlated because the passage of time will indicate the number of reproductive opportunities an individual will be afforded. Therefore, age-based and state-based life history models should have similar predictions for life history patterns across an organism's life. However, age and state will be more weakly correlated in opportunistic breeders because the passage of time does not accurately predict the extent of prior reproductive investment (i.e. number of reproductive bouts) and the consequent deterioration in state. Thus, state-based cues may have greater influence than age-based cues for determining the extent of reproductive investment in opportunistic breeders (Cotter et al. 2011).

Burying beetles (Coleoptera: Silphidae) are an excellent model system for evaluating the effect of state-based cues on current reproductive investment. These beetles are opportunistic breeders, utilizing the carcasses of small vertebrates to breed. Bi-parental care is provided to offspring; parents bury and preserve the small vertebrate carcass which serves as the sole food source for parents and offspring during the reproductive bout (Scott and Traniello 1990). Thus, carcass size represents resource quality or the amount of resource that is available to allocate for current reproduction and for somatic investment for future reproduction for the parents. During

burial, parents estimate carcass size by assessing carcass volume and regulate brood size to match carcass size through filial cannibalism (Trumbo and Fernandez 1995; Trumbo 2006). These behaviors result in a positive correlation between brood size and carcass size (Creighton 2005) and a positive correlation between carcass size and costs of reproduction (Creighton et al. 2009). Carcasses become available at unpredictable intervals; consequently reproductive opportunities are unpredictable both in terms of finding carcasses and in terms of carcass quality. Therefore, burying beetles that are the same age will have potentially experienced different levels of reproductive investment and consequent deterioration in state.

We conducted an experiment on the burying beetle *Nicrophorus orbicollis* to determine the effect of previous reproductive experience on the level of current reproductive investment. Reproductive patterns in *N. orbicollis* allow us to predict expected levels of reproductive investment on the current bout given previous experience (Creighton 2005; Creighton et al. 2009). Female *N. orbicollis* respond differently to carcasses of different size, consistently demonstrating restraint (allocation to future versus current reproduction) on small carcasses, which represent poor quality resources (Creighton et al. 2009). This reproductive restraint on poor carcasses may be an adaptive response for females in an attempt to increase potential of future reproduction assuming more and larger carcasses will be available. Therefore, we would predict that a female first given a small carcass and subsequently a large carcass will increase her level of reproductive investment (shift from low to high investment to current reproduction) to reflect the higher-quality carcass. Conversely, we predict that a female first given a large carcass and subsequently a small carcass will decrease her level of reproductive investment (shift from high to low allocation to current reproduction) to reflect the lower-quality resource and to conserve her state given the possibility of finding another high-quality carcass in the future. In

this experiment, we manipulated the order and quality of available resources for female *N. orbicollis* to test these predictions of the effect of previous reproductive experience on current reproductive investment.

Methods

Nicrophorus orbicollis reproduces on small vertebrate carcasses which serve as the sole food source for both parents and offspring during the reproductive bout. Once a carcass is secured, parents prepare the carcass by burying it, cleaning it of fur or feathers, shaping it into a ball, and preserving it with oral and anal secretions. Females lay eggs in the surrounding soil; first instar larvae usually arrive at the carcass 5 d after carcass burial. After larvae arrive at the carcass, parental care continues in the form of feeding larvae regurgitated carcass tissue, maintenance of the carcass, and defense of the carcass and young from predators. Parental care is provided until larvae disperse into the soil to pupate, approximately 15 – 20 d after carcass burial. In the laboratory, females compensate for the absence of males when caring for young, which allows us to measure individual reproductive investment and costs (Creighton 2005; Creighton et al. 2009).

Wild burying beetles were captured in central Wisconsin during summer 2008, using pitfall traps baited with aged chicken. Male-female pairs were placed on 30-g mouse carcasses and allowed to reproduce to generate a laboratory population at Purdue University at Calumet, Indiana. First-generation beetles (n = 65 individuals) representing 13 wild-pairs were transported to the Evolutionary Ecology Laboratory at Brigham Young University in Provo, Utah. Individuals were reared individually in small plastic containers (14.7 cm x 10.3 cm x 6.9

cm), fed raw chicken liver twice per week, and maintained on a 14L:10D light cycle. For age determination of adult beetles, the date of eclosion was designated as day 0.

We evaluated the effects of prior reproductive experience on current reproduction using the following experimental design. When 28 days old, virgin females were randomly assigned to one of four treatments (two experimental treatments and two control treatments); treatments differed in the order of carcass sizes presented to females over two reproductive bouts. For the experimental treatments, females were given either a 20-g or 30-g (± 1 -g) carcass for the first reproductive bout, and the carcass size was switched for the second reproductive bout (20-30 treatment and 30-20 treatment, respectively). For control treatments, females were given either 20-g or 30-g carcasses for both the first and second reproductive bouts (20-20 control and 30-30 control, respectively).

At the start of each reproductive bout, females were weighed, and then paired with a randomly selected male that was virgin, sexually mature, and unrelated to the female. Breeding pairs were placed in a plastic container (21.1 cm x 14.8 cm x 9.6 cm) with 6 cm of moist commercially purchased topsoil, and provided with a newly defrosted mouse carcass. After carcass preparation (48 hours), males were removed to isolate reproductive patterns of females. Females were removed when the carcass was consumed and larvae dispersed from the burial chamber into the surrounding soil to pupate. Females were weighed and returned to individual small plastic containers and fed raw chicken liver. After 72 hours, the female was placed on a new carcass, again with a male that was virgin, sexually mature, and unrelated to the female. Pupae from each reproductive bout were checked daily until eclosion, at which point the offspring were counted and weighed.

Statistical analyses

In this experiment, we were interested in the differences in life history response to resource availability given prior reproductive experience. We first compared differences in individual life history traits (univariate analyses) that were exhibited between bouts and among treatments. While these univariate analyses demonstrate whether reproductive investment is similar among treatments on a trait by trait basis, it is the coordinated or multivariate life history strategy that occurs in both reproductive bouts in each treatment that is the most interesting. Therefore, we also analyzed the data using the life history trajectory analysis, a modified multivariate approach for comparing phenotypic change (Adams and Collyer 2007; Collyer and Adams 2007; Adams and Collyer 2009).

Univariate analyses

Life history traits were compared among treatments using a mixed model analysis of covariance (ANCOVA; Proc Mixed, SAS Institute Inc. 2008). Response variables for four separate analyses were final brood size (number of adult offspring produced), mean mass of offspring (g), female mass change (g), and carcass use efficiency. Female mass change was determined by subtracting the mass of the female at the start of a reproductive bout from her mass at larval dispersal. A gain in mass represents investment to future reproduction because any mass increase represents food not available to current offspring. Carcass use efficiency was calculated by dividing the combined mass of all offspring plus the female mass change by the carcass size for each reproductive bout. Prior to analyses, we transformed life history traits to accommodate for potential nonlinear relationships between variables; final brood size was square root transformed, offspring size was \log_{10} transformed, female mass change were $\log_{10}(x+1)$

transformed, and carcass use efficiency was arcsin square root transformed. For each analysis, female body size (mass at start of reproductive bout, \log_{10} transformed) was the covariate. The main effects in the model were treatment, bout, and their interaction. We used $\alpha = 0.05$ to determine statistical significance and α between 0.05 and 0.1 to determine marginal significance of main effects in the univariate models.

For these analyses, we were interested in determining how life history traits changed on the second reproductive bout given prior experience (i.e. carcass size for first reproductive bout). Therefore, the treatment by bout interaction is the term of greatest interest in the model. In the event of a significant treatment by bout interaction, post-hoc mean comparisons of each treatment by bout combination result in a large number of pairwise comparisons ($n = 28$). Multiple comparison procedures (e.g. Tukey's HSD) use table-wide adjustments of the P -value to decrease the probability of Type I errors; however, these methods can obscure biologically significant results and increase Type II errors (Quinn and Keough 2002; Nakagawa 2004). Therefore, we followed Douglass et al. (2008) and Allen and Vaughn (2011) and opted to not apply a table wide adjustment to P -values. For post-hoc pairwise comparisons, we instead used $\alpha = 0.01$ to minimize the probability of Type I errors but still maintain power to detect biologically significant contrasts. Additionally, we considered P -values between 0.01 and 0.05 as marginally significant and examined mean differences to determine if these represent biologically significant mean differences.

Life history trajectory analysis

We evaluated the differences in the coordinated or multivariate life history strategy among treatments. The phenotypic change in life history that occurs from the first to the second bout can be characterized by a trajectory in multivariate trait space that is characterized by the

magnitude or amount of change (*MD*) and the direction of change (θ ; Adams and Collyer 2009). To construct life history trajectories, we first obtained principal component scores ($n = 4$) for each female from a principal components analysis of the four life history traits (final brood size, offspring size, female mass change, and carcass use efficiency) to use as response variables in subsequent analyses.

Differences in life history trajectories among treatments were assessed using a mixed model multivariate analysis of variance (MANOVA). The principal component scores for each female were the response variables; female was the random effect in the model. We included treatment (four levels), reproductive bout (two levels), an index variable, and all possible interactions in the MANOVA. The index variable accounts for the ordering of the principal components. Without including the index variable, the model will test if the direction and magnitude of differences between levels of the main effects are consistent on all principal components. However, principal components are orthogonal and ordered according to the amount of variance they explain; as a consequence, the magnitude and direction of differences between levels of main effects on one principal component have no bearing on the differences (magnitude or direction) between levels on other principal components. By including the index variable and associated interactions in the model, we account for possibility that differences in direction and magnitude between levels of main effects will occur on some of the principal components. Therefore, the interaction of index variable with main effect(s) allows differences of magnitude and direction to be detected independently among principal components (Rencher 2002; Butler et al. 2009); the interactions of the index variable and main effects therefore are the most direct test of our hypothesis. Following a significant interaction of treatment, reproductive bout, and index variable, we applied the life history trajectory analysis with 10,000 permutations

to determine whether the interaction indicated differences in trajectory magnitude or orientation. All analyses were conducted in R (R Core Development Team 2010); mixed model MANOVAs were conducted in ASREML-R version 3.00 (Butler et al. 2009) within R.

Results

Univariate analyses

Final brood size was significantly affected by bout and the interaction of treatment and bout, but was not significantly affected by treatment (Table 4). For the first reproductive bout, there were not significant differences in brood sizes among treatments (Figure 4a). However, females in the 20-30 treatment exhibited a large increase in brood size on the second reproductive bout, averaging almost five more offspring than any other treatment by bout combination (Figure 4a). As a result, final brood size in the second bout for the 20-30 treatment was significantly greater than all other treatment by bout combinations (for five pairwise comparisons: $P < 0.010$, mean difference > 5.59 offspring; for two pairwise comparisons: $P < 0.031$, mean difference > 4.71 offspring).

Analysis of covariance comparing differences in offspring mass revealed that treatment was a significant effect, the interaction of treatment by bout was marginally significant, and bout was not a significant effect (Table 4). Offspring mass was consistently greater for females breeding on 30-g carcasses as compared to 20-g carcasses (Figure 4b). Both reproductive bouts in the 30-30 control had significantly larger offspring mass than the reproductive bouts for the 20-20 control ($P < 0.007$). Females in the 20-30 treatment exhibited an increase in offspring mass on the second bout that was marginally significant ($P < 0.023$) compared to the first reproductive bout and compared to the 20-20 control reproductive bouts (Figure 4b; Appendix

1). Females in the 30-20 treatment exhibited a decrease in offspring mass in the second reproductive bout (Figure 4b). The reduced offspring mass was only marginally significant when compared to the first reproductive bout of the 30-30 control ($P = 0.050$), and was not significantly different ($P > 0.063$) compared to offspring mass in other reproductive bouts with 30-g carcasses.

Female mass change was significantly affected by bout and the interaction of treatment and bout, but was not significantly affected by treatment (Table 4). Female mass change decreased from the first to the second reproductive bout in all treatments except for the 30-20 treatment. Females that were given a 20-g carcass on the first reproductive bout (i.e. 20-20 control and 20-30 treatment) exhibited the greatest female mass change in the reproductive bout, exhibiting significance ($P < 0.010$ for six pairwise comparisons) or marginal significance ($P < 0.034$ for two pairwise comparisons) in all comparisons ($n = 8$) with females given 30-g carcasses. Females in the 20-30 treatment exhibited a significant decrease in female mass change from the first to second reproductive bout. Females in the 30-20 treatment exhibited an increase in female mass change from the first to second bout; however, female mass change on the second reproductive bout was not significantly different from the first bout ($P = 0.096$).

Carcass use efficiency was significantly affected by treatment, bout, and their interaction (Table 4). Carcass use efficiency increased significantly ($P < 0.004$) in the second bout compared to the first bout in the 20-30 and 30-20 treatments, and the increase in carcass use efficiency from the first to the second bout was marginally significant ($P = 0.047$) in the 20-20 control (Figure 4d). In the 30-30 control, females did not exhibit a significant change in carcass use efficiency.

Life history trajectory analysis

Life history strategies were significantly affected by the interaction of treatment and the index variable and by the interaction of treatment, bout, and the index variable (Table 5), the latter indicating that the trajectory attributes (magnitude and amount of phenotypic change) were significantly different among treatments. Summary statistics from the life history trajectory analysis revealed a lack of concordance in life history strategy across treatments as demonstrated in the significant variation in the amount, or magnitude, of phenotypic change among treatments ($\text{Var}_{\text{size}} = 0.273, P = 0.0156$) and the significant variation in the direction of phenotypic change among treatments ($\text{Var}_{\text{orient}} = 579.492, P < 0.0001$). Pairwise comparisons of the magnitude of trajectories revealed that the 30-30 control treatment exhibited significantly less change in life history strategy compared to the other treatments (Table 6; Figure 5). Additionally, the 20-30 treatment exhibited the greatest amount of change, but only significantly more than the two control treatments (Table 6; Figure 5). Despite significant variation in the direction of phenotypic change among treatments, there were no significant pairwise differences in the direction of phenotypic change (Table 6).

Discussion

Variation in age-specific fecundity may be determined by age-based cues, i.e. the time remaining for future reproduction, or by state-based cues (Williams 1966), i.e. the level of deterioration that has resulted from previous reproductive experience or other biological causes (e.g. injuries, immune function, etc.; McNamara and Houston 1996; McNamara et al. 2009). However, age- and state-based hypotheses are not mutually exclusive. Instead, individuals likely assess multiple cues to determine the level of reproductive investment to balance the benefits of

current reproduction with costs to future reproductive opportunities. By manipulating the quality and order of carcasses, we demonstrated that previous experience interacts with age to determine the level of reproductive investment to current reproduction, i.e. whether a female demonstrated a terminal investment or a conservative strategy.

Previous research has indicated that *N. orbicollis* exhibits patterns of reproductive investment consistent with predictions of the cost of reproduction hypothesis (Creighton et al. 2009). Additionally, females exhibit different patterns of investment dependent on carcass size, with females reproducing on a high quality resource (i.e. 30-g carcass) investing more to current reproduction consistently across their lifetime compared to females on a low quality resource (i.e. 20-g carcass). In this study, the life history strategies of females in the 20-20 and 30-30 controls were consistent with the combined effects of age and carcass quality (Creighton et al. 2009). Females in the 30-30 control demonstrated high investment to current reproduction in both reproductive bouts resulting in only a small amount of phenotypic change in life history strategy (Figure 5). Females in the 20-20 control initially had a low reproductive investment on the first bout, but had a greater reproductive investment on the second bout. The amount of phenotypic change in life history strategy was greater for females in the 20-20 control reflecting a greater increase in investment with age compared to females in the 30-30 control. Thus, females in both controls exhibited patterns of reproductive investment consistent with the cost of reproduction hypothesis, but the degree of increased reproductive investment from the first to second bout was dependent on carcass quality, or previous experience.

The life history strategies of females in the 20-30 and 30-20 treatments also demonstrated the combined effects of age and previous experience on the level of reproductive investment. Females in the 20-30 treatment greatly increased reproductive investment from the first to

second bout consistent both with age-based cues and carcass quality. The amount of phenotypic change in life history trajectory for females in the 20-30 treatment was significantly greater than that observed for females in the control treatments. Additionally, greater reproductive investment was achieved using a different strategy than females in the 30-30 control; females in the 20-30 treatment had many large offspring compared to fewer large offspring in the 30-30 control. The age-related increase in reproductive investment predicted by the cost of reproduction hypothesis was much greater in the 20-30 treatment compared to the controls because of the female's previous experience. Conversely, females in the 30-20 treatment change their pattern of reproductive investment from high to low investment to current reproduction.

This study demonstrates that an individual's state as determined by prior experience can interact with age-based cues leading an individual to terminally invest or can counteract age-based cues leading an individual to invest conservatively. Previous experience therefore affects how an organism assesses the balance of fitness benefits from investment to current reproduction with costs to future reproduction. For females in the 20-30 treatment, the balance shifts towards the benefits from increased investment to current reproduction. Similar patterns are predicted for old individuals with a low reproductive value (Williams 1966; Clutton-Brock 1984) or for individuals with a perceived decrease in reproductive value (e.g. individuals presented with an immune challenge; Norris and Evans 2000; Bonneaud et al. 2004; Velando et al. 2006; Cotter et al. 2011). For females in the 30-20 treatment, the benefits from increased investment to current reproduction are not high enough to outweigh the costs to future reproduction; therefore, the balance shifts towards a reduced investment to current reproduction, or reproductive restraint. Organisms are predicted to exhibit reproductive restraint early in life when reproductive value is high (Williams 1966). Additionally, recent research has demonstrated that reproductive restraint

late in life is a mechanism to reduce damage accumulation and deterioration of somatic condition to gain further breeding attempts (McNamara et al. 2009; Cotter et al. 2011).

An organism that exhibits reproductive restraint must assume that the future will be at least as good as current conditions. For organisms such as burying beetles that reproduce on discrete resources, reproductive restraint will allow those individuals that initially secure suboptimal carcasses to conserve energy and condition so they can capitalize on high-quality resources in future reproductive bouts. Some observations of female reproductive behavior (e.g. utilization of suboptimal carcasses and satellite females) suggest that opportunities to secure a carcass for reproduction are limited (Müller et al. 1990; Trumbo 1990; Trumbo 2006; Trumbo 2009). However, evidence of carcass availability for *N. investigator* support the assumption of the reproductive restraint hypothesis that burying beetles will have future reproductive opportunities. Densities of small rodents are sufficient such that only a small percentage (1-2%) would be required to die to support the beetle population (Smith and Merrick 2001).

Additionally, reproductive investment in this study and in others (Creighton et al. 2009; Cotter et al. 2011) suggest that beetles may have a high probability of future reproduction; otherwise the response to resource acquisition should be to maximize reproduction on the current bout despite reproductive costs. For example, in some arthropods, the probability of successfully mating is sufficiently low such that an individual will maximize investment on the current reproductive bout despite the high survival costs (Toda and Kimura 1997; Andrade 2003; Barry and Kokko 2010). An extreme example is the male redback spider (*Latrodectus hasselti*) which exhibits an adaptive behavior of offering itself as a nuptial gift to his mate who cannibalizes the male during copulation; high mortality during mate searching and increased paternity benefits ensures that self-sacrifice is an adaptive strategy (Andrade 2003).

Reproductive restraint on low-quality carcasses allowed female burying beetles to exhibit an accentuated response in reproductive investment, or apparent terminal investment, to a subsequent high-quality resource. This adaptive response likely provides a lifetime fitness advantage to females under conditions of variable resource availability despite the increased cost incurred. Adaptive responses to resource availability have been demonstrated in other organisms as well. Other insect species that reproduce on a discrete resource exhibit similar patterns of increased allocation to current reproduction on high quality resources (Heimpel and Rosenheim 1995; Heimpel and Collier 1996). For species that have one sex with more variable reproductive success (e.g. males in polygynous species), the Trivers-Willard hypothesis predicts that females in good condition (e.g. during years of high resource availability) would receive a fitness advantage by producing more of the sex with variable reproductive success (Trivers and Willard 1973). While studies testing the Trivers-Willard hypothesis with a variety of condition measures provide inconsistent results, Cameron (2004) reported that 73% of 23 studies examining food availability for mammalian species provided evidence in support of this hypothesis. For some bird species, high resource availability increases reproductive investment in the current reproductive period manifest either in larger clutch sizes and/or more broods within the reproductive season (Martin 1987; Grant et al. 2000; Christman 2002; Preston and Rotenberry 2006). In fishes in the family Poeciliidae, incipient matrotrophy allows small females to have larger broods and offspring under high resource availability, but the pattern is less obvious for large females presumably because greater energy reserves in larger fish allow them to sufficiently provision embryos even under low resource availability (Reznick and Yang 1993; Trexler 1997; Marsh-Matthews and Deaton 2006). Thus, in a variety of taxonomically distinct

species, increased investment to current reproduction during periods of high resources provides fitness benefits that outweigh costs to future reproduction.

This study successfully demonstrated that burying beetles exhibit an adaptive response to carcass size based on their previous reproductive experience. However, because we only tested for the adaptive response over two reproductive bouts, it is unclear what the consequence of this adaptive response will be for the lifetime fitness of a female burying beetle. When provided a constant supply of carcasses for reproduction, female *N. orbicollis* exhibit different life history strategies depending on carcass quality (Creighton et al. 2009). Despite the different life history strategies, females in the two controls (20-g or 30-g carcasses for each reproductive bout, respectively) produced the same number of offspring over their lifetime (Creighton et al. 2009). This pattern occurred because females reproducing on 20-g carcasses had smaller brood sizes but more reproductive attempts due to greater allocation to future reproduction. Under variable resource availability, females that initially reproduce on a suboptimal carcass and subsequently on an optimal carcass may receive a lifetime fitness advantage by exhibiting an accentuated reproductive investment as observed in this study. However, the increased reproductive costs incurred could limit the number of reproductive attempts such that the lifetime number of offspring would not be significantly different than for females reproducing continually on suboptimal carcasses (Creighton et al. 2009; McNamara et al. 2009). Why then would the adaptive response observed in this study be exhibited? Under laboratory conditions, costs of reproduction are reduced compared to natural conditions because we remove the costs of securing a carcass; burying beetles compete for the opportunity to reproduce on a carcass, receiving injuries that reduce their state and probability of future reproduction (Cotter et al. 2011). Additionally, burying beetles under laboratory conditions do not have to seek for food or

carcasses, both of which can be spatially and temporally variable, further reducing costs to somatic conditions in laboratory studies. Therefore, measures of lifetime fitness from laboratory studies represent the maximum potential an individual can achieve. Under natural conditions, we predict that the adaptive responses observed in this study will provide a lifetime fitness advantage for burying beetles; however, this prediction has not been tested.

Life history theory provides predictions for the patterns of life history allocation. Previous research has demonstrated that burying beetles use both age-based and state-based cues to determine the extent of reproductive investment (Creighton et al. 2009; Trumbo 2009; Cotter et al. 2011). In this study, we also demonstrated that burying beetles use previous experience and current carcass size to determine reproductive investment. Thus, individuals use a variety of cues to adjust their schedule of reproduction, and predictions of reproductive investment across an organism's lifetime must account for the interaction of these cues.

Acknowledgments

We are grateful to Stefan and Jane Shoup who provided access to their property to allow us to collect beetles. Funding was provided to M. C. Belk through a Mentoring Environment Grant from Brigham Young University. We are grateful to Peter Meyers who helped care for beetles and collect data and to Sandra Garrett and Megan Durrant of Central Animal Care at Brigham Young University for providing mouse carcasses (IACUC number 080403). Jerry Johnson, Steve Peck, Russ Rader, and Bruce Schaalje provided reviews of earlier drafts of the manuscript.

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Table 4. Analysis of covariance tables for mixed models comparing brood size, offspring mass, female mass change, and carcass use efficiency of female burying beetles as a function of treatment, reproductive bout, and the covariate female mass.

Effect	Num DF	Den DF	F-statistic	P-value
Brood size				
Treatment	3	51.1	0.44	0.724
Bout	1	53.6	7.04	0.011
Female mass	1	61.5	0.72	0.400
Treatment x bout	3	52.1	5.92	0.002
Offspring mass				
Treatment	3	51.1	4.77	0.005
Bout	1	53.3	0.27	0.606
Female mass	1	58.4	0.00	0.996
Treatment x bout	3	52.1	2.25	0.093
Female mass change				
Treatment	3	48.3	1.96	0.133
Bout	1	50.2	3.80	0.057
Female mass	1	54.1	31.33	<0.001
Treatment x bout	3	49.3	4.17	0.010
Carcass use efficiency				
Treatment	3	51.2	4.87	0.005
Bout	1	53.8	20.60	<0.001
Female mass	1	62.5	1.66	0.202
Treatment x bout	3	52.1	4.79	0.005

Table 5. Mixed model multivariate analysis of variance for the life history strategy (defined by principal components derived from four life history traits) of female burying beetles as a function of treatment, reproductive bout, and an index variable (accounts for ordering of principal components; see Methods for explanation).

Effect	Degrees of Freedom	Wald Statistic	P
Treatment	2	157.055	<0.001
Bout	1	3.717	0.054
Index variable	4	41.269	<0.001
Treatment x bout	2	0.641	0.726
Treatment x index variable	6	255.477	<0.001
Bout x index variable	3	0.641	0.187
Treatment x bout x index variable	12	41.296	<0.001

Table 6. Statistical assessment of differences in life history trajectory size (MD) and direction (θ) for four reproductive treatments for *Nicrophorus orbicollis* females: 1) 20-20 control, 2) 20-30 treatment, 3) 30-20 treatment, and 4) 30-30 control. Bold indicates significant differences as generated empirically from 10,000 permutations (see Methods for explanation).

Comparison	MD	P_{size}	θ	P_{θ}
1, 2	0.853	0.078	38.705	0.661
1, 3	0.106	0.830	69.746	0.219
1, 4	0.884	0.069	90.234	0.126
2, 3	0.747	0.119	94.900	0.149
2, 4	1.737	<0.001	87.296	0.201
3, 4	0.989	0.041	45.874	0.504

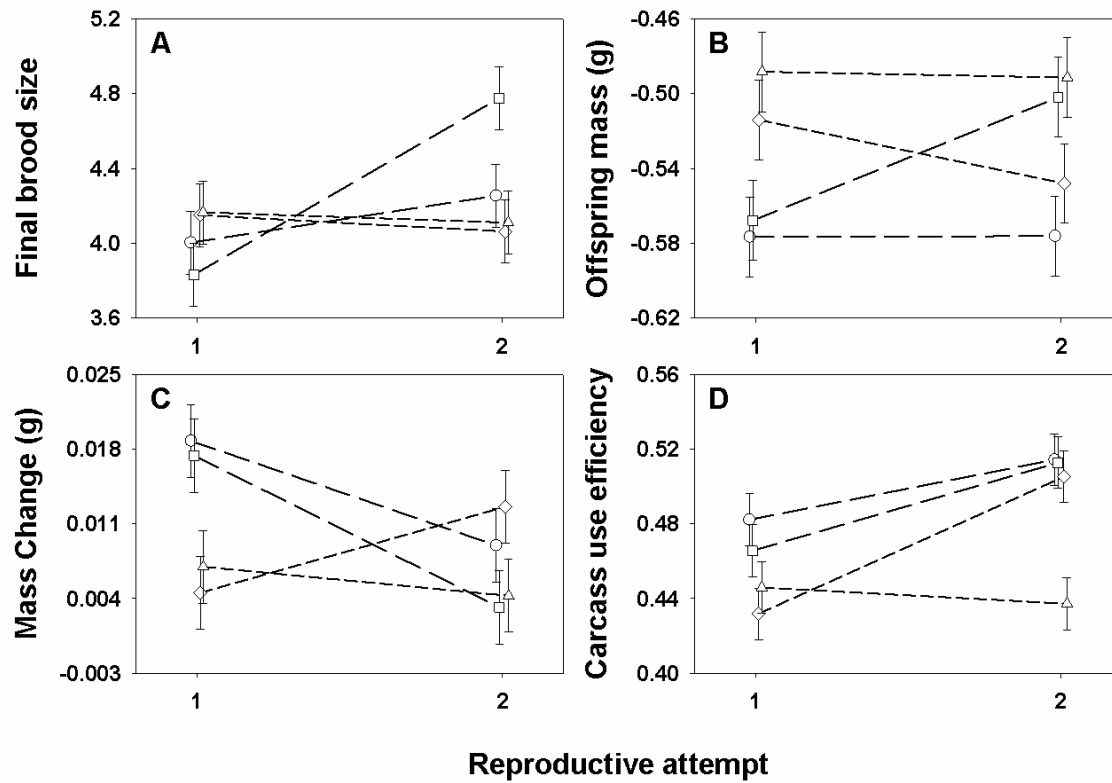


Figure 4. Mean (\pm SE) life history traits for female burying beetles from four treatments: 20-20 control (circles), 20-30 treatment (squares), 30-20 treatment (diamonds), and 30-30 control (triangles). The four life history traits are a) final brood size (square root transformed), b) offspring mass (g; \log_{10} transformed), c) female mass change (g; $\log_{10}(x+1)$ transformed), and d) carcass use efficiency (arcsine square root transformed).

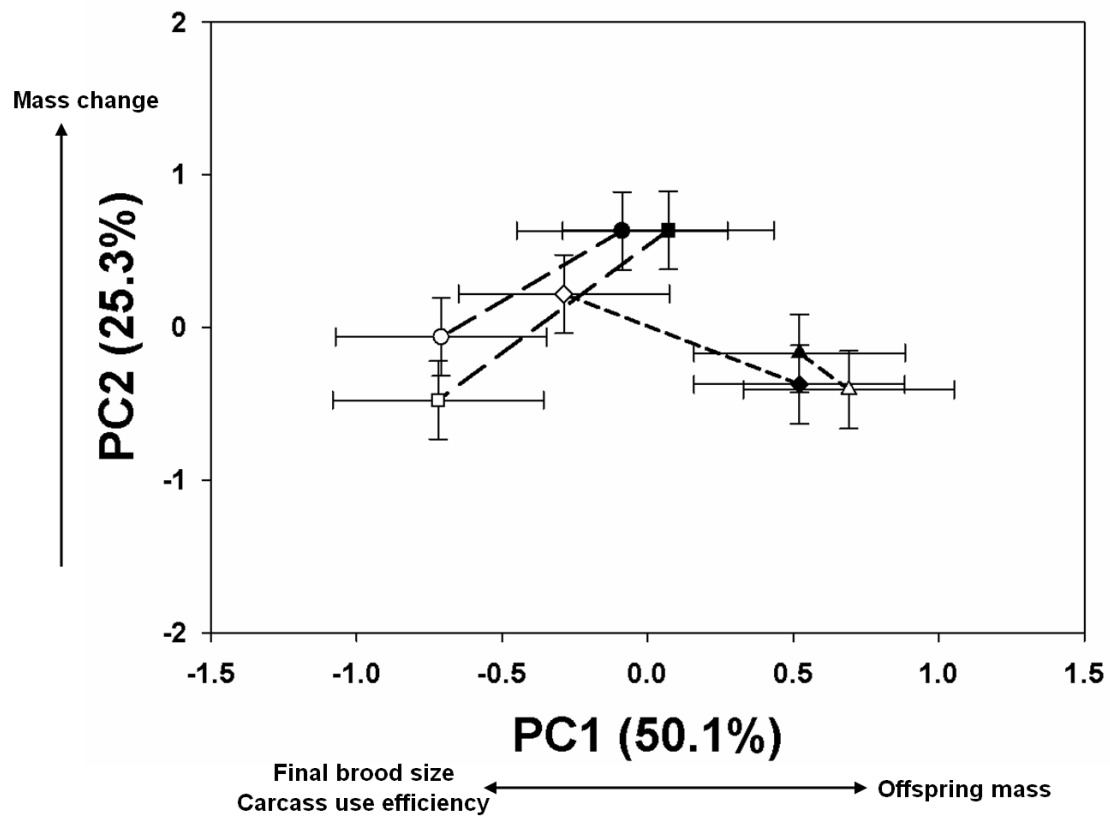


Figure 5. Least squares means (\pm SE) of principal component scores for life history trajectories defined by two reproductive bouts for female burying beetles in four treatments: 20-20 control (circles), 20-30 treatment (squares), 30-20 treatment (diamonds), and 30-30 control (triangles). Filled symbols = first reproductive bout; open symbols = second reproductive bout.

Chapter 3: Appendix

Summary of post-hoc contrasts from mixed analysis of covariance for four life history traits of female burying beetles as a function of treatment ($n = 4$) and reproductive bout ($n = 2$). The four treatments are 1) 20-g control, 2) 20-g:30-g treatment, 3) 30-g:20-g treatment, and 4) 30-g control. We used $\alpha = 0.01$ to minimize the probability of Type I errors but still maintain power to detect biologically significant contrasts to minimize the likelihood of Type II errors.

Additionally, we considered P -values between 0.01 and 0.05 as marginally significant. **Bold** indicates both significant and marginally significant contrasts.

Contrast				Final brood size		Offspring size		Female mass change		Carcass use efficiency	
Treatment	Bout	Treatment	Bout	$t_{51.9}$	<i>P</i>	t_{52}	<i>P</i>	$t_{49.2}$	<i>P</i>	t_{52}	<i>P</i>
1	1	1	2	-1.27	0.209	-0.02	0.987	2.05	0.045	-2.03	0.047
1	1	2	1	0.72	0.471	-0.29	0.769	0.28	0.777	0.85	0.397
1	1	2	2	-3.24	0.002	-2.47	0.015	3.22	0.002	-1.56	0.123
1	1	3	1	-0.61	0.541	-2.07	0.041	2.94	0.004	2.57	0.012
1	1	3	2	-0.25	0.806	-0.94	0.348	1.27	0.207	-1.17	0.244
1	1	4	1	-0.68	0.498	-2.92	0.004	2.44	0.017	1.86	0.067
1	1	4	2	-0.44	0.657	-2.81	0.006	2.98	0.004	2.29	0.024
1	2	2	1	1.77	0.080	-0.28	0.782	-1.74	0.085	2.48	0.015
1	2	2	2	-2.2	0.031	-2.46	0.016	1.2	0.233	0.08	0.935
1	2	3	1	0.44	0.661	-2.06	0.042	0.92	0.360	4.22	<0.001
1	2	3	2	0.81	0.421	-0.93	0.354	-0.75	0.454	0.47	0.643
1	2	4	1	0.37	0.709	-2.91	0.004	0.42	0.679	3.5	0.001
1	2	4	2	0.61	0.545	-2.81	0.006	0.97	0.332	3.95	0.000
2	1	2	2	-4.78	<0.001	-2.35	0.023	2.98	0.004	-2.98	0.004
2	1	3	1	-1.33	0.185	-1.77	0.079	2.65	0.009	1.72	0.088
2	1	3	2	-0.97	0.337	-0.65	0.517	0.99	0.326	-2.02	0.047
2	1	4	1	-1.4	0.165	-2.62	0.010	2.15	0.034	1.01	0.317
2	1	4	2	-1.16	0.248	-2.51	0.014	2.7	0.008	1.44	0.152
2	2	3	1	2.64	0.010	0.4	0.691	-0.28	0.779	4.14	<0.001
2	2	3	2	3	0.003	1.53	0.130	-1.95	0.054	0.38	0.702
2	2	4	1	2.57	0.012	-0.45	0.651	-0.79	0.434	3.42	0.001
2	2	4	2	2.8	0.006	-0.35	0.725	-0.23	0.822	3.87	0.000
3	1	3	2	0.45	0.658	1.22	0.228	-1.7	0.096	-4.68	<0.001
3	1	4	1	-0.07	0.948	-0.85	0.396	-0.5	0.615	-0.72	0.473
3	1	4	2	0.17	0.867	-0.75	0.454	0.06	0.956	-0.27	0.785
3	2	4	1	-0.43	0.665	-1.98	0.050	1.17	0.246	3.04	0.003
3	2	4	2	-0.2	0.841	-1.88	0.063	1.73	0.087	3.48	0.001
4	1	4	2	0.28	0.779	0.11	0.914	0.57	0.572	0.56	0.581

Chapter 4: Effect of Age-based and Environment-based Cues on Reproductive Investment

in *Gambusia affinis*

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Abstract

The cost of reproduction hypothesis predicts that an individual will balance fitness benefits with costs to future reproductive opportunities when determining the level of reproductive investment to current reproduction. Age, or the expected time to death, has been considered the main cue that determines an individual's level of reproductive investment; however, environment-based cues can also influence the balance of fitness benefits and costs to future reproduction. We examined the life history trajectories of female *Gambusia affinis* in Utah Lake, Utah, USA, to determine whether age-based or environment-based cues have the greatest effect on reproductive investment. In this system, female *G. affinis* only live for two reproductive seasons, reproducing in the first summer as age-0 individuals and in the second as age-1 individuals. Using the life history trajectory analysis, we demonstrated that age-0 females decreased the level of reproductive investment to current reproduction in late summer prior to the onset of fall and winter months. Old females, on the other hand, increased the level of reproductive investment as the summer progressed. The reproductive restraint and terminal investment patterns exhibited by age-0 and age-1 females, respectively, were consistent with the predictions from the cost of reproduction hypothesis. Age-0 females responded to environment-based cues (i.e. the onset of winter) and decreased investment to current reproduction to increase the probability of over-winter survival and the ability to reproduce the following summer. Age-1 females responded to age-based cues, or the proximity of death, and increased investment to current reproduction as

future reproductive opportunities decreased late in life. Our results show that individuals use multiple cues to determine the level of reproductive investment and that the strength of the effect of each cue will depend on the age of an individual.

Keywords: reproductive value, reproductive restraint, terminal investment, cost of reproduction, mosquitofish

Introduction

Combinations of life history traits and behaviors over an organism's lifetime determine an individual's reproductive success, or evolutionary fitness. A compelling question of life history evolution is determining how age or changes in the environment affect reproductive allocation (Fisher 1930; Williams 1966; Roff 2002). Age-based life history theory predicts patterns of reproductive allocation based on the interaction of age and the probability of survival. The prominent age-based hypothesis, the cost of reproduction hypothesis, predicts the level of reproductive allocation based on future reproductive opportunities assuming that reproduction comes at a cost (e.g. reduction in body condition or survival) that reduces future reproductive opportunities (Williams 1966; Clutton-Brock 1984; Reznick 1985). The cost of reproduction hypothesis predicts that young individuals that have a high reproductive value, or high future reproductive potential (Fisher 1930), should allocate less to current reproduction to ensure future reproductive opportunities. Conversely, old individuals that have low reproductive values should allocate more to current reproduction (i.e. terminal investment; Clutton-Brock 1984), accepting the greater costs of reproduction because future opportunities may not be available (Williams 1966). Reproductive strategies that support the predictions of the cost of reproduction hypothesis have been reported in organisms that have seasonal reproduction (Pärt et al. 1992; Berteaux and Boutin 2000; Descamps et al. 2007) and in short-lived organisms that reproduce multiple times in a single reproductive season (Poizat et al. 1999; Poizat et al. 2002; Baker et al. 2008; Creighton et al. 2009).

Environmental conditions can similarly affect the balance of the cost of reproduction tradeoff by altering the availability of resources, the rates of physiological processes, or the

probability of survival independent of age (McNamara and Houston 1996). Temporal variation in environmental conditions can lead to adaptive life history responses in organisms to maximize reproduction when environmental conditions are favorable and to conserve energy by reducing or abstaining from reproduction during poor environmental conditions (Winemiller 1993; Ohbayashi-Hodoki and Shimada 2005; Sockman et al. 2006; Lake et al. 2008; Bårdsen et al. 2010). Seasonality can likewise affect patterns of reproductive allocation, particularly if survival through the poor or selective season is dependent on somatic energy storage. Therefore, the level of reproductive investment should be affected by environment-based cues that indicate changes in season.

When should an individual be more likely to respond to age-based rather than environment-based cues? *Gambusia affinis* provides a good model species to examine life history responses to both age-based and environment-based cues. *Gambusia affinis* is a livebearing fish in the family Poeciliidae which is characterized by small-bodied, short-lived fishes. Endemic to southeastern United States, *G. affinis* has been introduced worldwide for mosquito abatement programs (Bay 1972; Rupp 1996; Pyke 2005). In temperate climates, environmental seasonality results in two distinct age classes (age-0 and age-1), both of which are reproductive (Krumholz 1948; Haynes and Cashner 1995; Belk and Tuckfield 2010). Because winter acts as a strong selective agent, fish must balance age-based and environmentally-based cues to determine the extent of reproductive investment (Daniels and Felley 1992). We examined life history responses to the interaction of age and environmental seasonality in female *G. affinis* from an introduced population in Utah Lake, Utah, USA. We hypothesize that age-0 and age-1 females should exhibit divergent patterns of reproductive investment due to the relative influence of age- versus environment-based cues.

Methods

Study site

Utah Lake is a large freshwater remnant of ancient Lake Bonneville in the Great Salt Lake watershed in central Utah (Figure 6). Utah Lake has a large surface area (~38,800 ha) but reaches a maximum depth of only about 4 m. Provo Bay is a large (~1,800 ha) southeast extension of Utah Lake (Figure 6). It is fringed almost entirely by emergent vegetation including *Scirpus validus*, *Typha latifolia*, and *Phragmites sp.* (Miller and Crowl 2006). *Gambusia affinis* was introduced into Utah in the early 1930s, and likely dispersed into Utah Lake between 1934 and 1945 after it was introduced into freshwater springs near the northwest shore of the lake (Rees 1934, 1945). *Gambusia affinis* has since become abundant in the lake. High densities of *G. affinis* occur around the emergent vegetation in Provo Bay where the fish seeks refuge from avian predators and introduced piscivorous fishes (*Morone chrysops*, *Micropterus salmonoides*, *Lepomis macrocephalus*, and *L. cyanellus*).

In this temperate environment, *G. affinis* reproduction is strongly seasonal (Krumholtz 1948; Hughes 1985; Haynes and Cashner 1995) producing distinct age classes (Belk and Tuckfield 2010). Summer water temperatures along the margins of Provo Bay range between 15°C to 28°C from the end of April to September (Billman unpublished data), during which months *G. affinis* are reproductively active. No reproduction and little growth occurs during the other seven months of the year because water temperatures are too low (Vondracek et al. 1998; Priddis et al. 2009). Female *G. affinis* survive for only two reproductive seasons in this environment. This results in a distinct separation between old and young females. Old females

(age-1) at the end of their lives reproduce primarily in the early to mid portion of the reproductive season. Young females (age-0) reproduce from mid-summer to end of the reproductive season determined decreases in water temperature due to the transition from warm summer to cold winter months. Therefore, age-0 and age-1 females should use different cues to determine the extent of reproductive investment. Age-0 females should base the level of reproductive investment on environmental cues indicating the onset of fall and winter months while age-1 females should use age-based cues associated with the end of life to determine the level of reproductive investment.

Population sampling

Gambusia affinis were collected along a 1-km section of the north shore of Provo Bay during 2008 and 2009. Samples were collected monthly between the end of April or beginning of May and the middle of September for a total of six samples per summer (Table 7): three samples that consist mainly of age-1 reproductive females (end of April to beginning of July) and three samples that consist mainly of age-0 reproductive females (mid-July to mid-September). Females in each age were classified into three time periods representing early, mid, and late reproduction (time periods 1, 2, and 3, respectively; Table 7). The first three collections each year represented time periods 1 – 3 for age-1 females, and the last three collections each year represented time periods 1 – 3 for age-0 females.

Gambusia affinis were collected using large D-nets with 3-mm mesh. Habitat utilized by *G. affinis* (emergent vegetation) precluded using seine nets because there were few areas of open water large enough to use seine nets, and *G. affinis* remained close to emergent vegetation where individuals quickly retreated for refuge. Water depth ranged from 3 – 100 cm where fish were

collected. Fish were euthanized with an overdose of tricaine methanesulfonate (MS-222) and immediately placed into 70% ethanol.

Female *G. affinis* from each sample were measured to the nearest mm standard length (SL) and weighed to the nearest 0.1 mg. Sex of fish less than 13 mm SL could not be determined. Females were dissected to determine pregnancy. Ovaries were removed and weighed to the nearest 0.1 mg. Additionally, the eviscerated bodies of the females were weighed to the nearest 0.1 mg. The number of embryos in the ovaries for each individual was counted, and the stage of development of the embryos was determined according to Haynes (1995). . Females and their clutches were dried for 24 hr at 55°C, after which they were weighed to the nearest 0.1 mg. Female size at maturity was determined as the size (SL mm) at which greater than 50% of females contained developing embryos (Johnson and Belk 2001), defined as stage 3 or greater according to Haynes (1995).

Statistical analyses

We used three life history traits for reproductive females in both age classes: 1) clutch dry mass, 2) clutch size, and 3) offspring dry mass. Clutch dry mass represents the reproductive allotment of a female to her current clutch of offspring (Johnson and Belk 2001; Scott and Johnson 2010). Clutch size equals the total number of developing embryos in the female. Offspring dry mass equals the per capita dry weight of the developing offspring (clutch dry mass divided by clutch size).

Univariate analyses

We used a mixed model analysis of covariance (ANCOVA; Proc Mixed; SAS Institute, Inc. 2008) to test for differences between age-0 and age-1 females for each of the three life

history traits. Restricted maximum likelihood was used to fit the model. The main effects in the model were age, time period, and their interaction. For each analysis, female dry mass (\log_{10} transformed) was the covariate in the model; additionally, embryonic stage of development (hereafter stage) was used as an additional covariate in the analyses for clutch dry mass and offspring dry mass. Prior to analyses, we transformed life history traits (\log_{10} transformed) to accommodate for potential nonlinear relationships between variables. A Tukey test was used for post-hoc mean comparisons for all three tests.

Life history trajectory analysis

Collyer and Adams (2007) and Adams and Collyer (2009) recently described a general framework to analyze multivariate phenotypic trajectories in evolutionary studies. In studies of life history evolution, the life history strategies of evolutionary groups across multiple time periods can be described by a trajectory in life history trait space. Using a modified approach called life history trajectory analysis, attributes of life history trajectories (i.e. magnitude of phenotypic change, direction of phenotypic change, and shape of the trajectory) can be statistically compared to determine the extent to which lifetime life history strategies are parallel, convergent, or divergent. Additionally, life history trajectories can be compared to age-based life history predictions to determine the extent to which lifetime life history strategies support life history theory.

Life history traits must be transformed prior to life history trajectory analysis to account for variation in traits due to embryonic stage of development and female dry mass. Clutch characteristics for lecithotrophic species (i.e. clutch dry mass and offspring dry mass) will be negatively correlated with stage of development (Marsh-Matthews et al. 2005). *Gambusia affinis* has been described as a strict lecithotrophic species, although it has recently been reported

that *G. affinis* exhibits maternal provisioning typical of matrotrophic species (Marsh-Matthews et al. 2005). However, this maternal provisioning is not sufficient to preclude loss of mass across the entire gestation of the embryos as demonstrated in this study (Table 8). Because mass loss accumulates with embryonic stage of development, we used the coefficients for stage from the ANCOVA models for clutch dry mass and offspring dry mass to adjust these life history traits (\log_{10} transformed) to stage 3 for each individual. To account for the positive relationship between life history traits and female dry mass, we regressed each life history trait (\log_{10} transformed) separately on female dry mass (\log_{10} transformed) with the slope constrained to one. By constraining the slope, we account for isometric changes in life history traits but maintain patterns of reproductive restraint (hypoallometric changes in life history traits) and terminal investment (hyperallometric changes in life history traits) if they are present. The residuals of these regressions are used as life history variables for the life history trajectory analysis.

Additionally, we generated four reference trajectories based on predictions of allocation due to age-based and environment-based cues and the life history tradeoff between offspring size and number. Two trajectories represented life history strategies that reflect a change in reproductive investment due to age-based cues (i.e. increased allocation to current reproduction with increase in time period; terminal investment). The other two trajectories represented life history strategies that represent predictions of reproductive investment due to environment-based cues (i.e. increased allocation to future reproduction with increase in time period; reproductive restraint). For each pair of trajectories, one trajectory represented a life history strategy with greater allocation to offspring size (i.e. offspring dry mass) while the other represented a strategy with greater allocation to offspring number (i.e. clutch size).

We calculated principal component scores for each individual (including reference data) from a principal components analysis with life history traits (i.e. residuals generated above) as the dependent variables. Principal component scores were used as response variables in a mixed model multivariate analysis of variance (MANOVA; Proc Mixed; SAS Institute, Inc. 2008) to assess differences in life history strategy of age-0 and age-1 females. Year of collection was the random effect in the model. In addition to the main effects of age and time period, we included an index variable that accounts for the ordered nature of the principle components (i.e. PC1, PC2, PC3). Principle components are orthogonal; therefore, the magnitude and direction of differences between levels of main effects on one principal component have no bearing on the magnitude and direction of differences between levels on the other principal components. We included all interactions of the main effects in the MANOVA. The interactions of the index variable with main effect(s) test differences in the levels of the main effect(s) while allowing the magnitude and direction of differences to vary independently among principal components; these interactions are the terms of interest in the MANOVA (Rencher 2002; Butler et al. 2009).

Following a significant interaction of age, time period, and index variable, we applied the life history trajectory analysis. Differences in trajectory attributes were calculated and statistically tested using a residual randomization approach (Adams and Collyer 2007). The residuals from a reduced model of the MANOVA that lacked the age, time period and index variable interaction were randomized and added to the predicted values to produce a random data set. We then used the full MANOVA to analyze the random data set. This procedure was repeated 10,000 times to generate a distribution of random differences of trajectory attributes to compare to the observed differences in trajectory attributes. The life history trajectory analysis

was conducted in R (R Core Development Team 2010); mixed model MANOVAs were conducted in ASREML-R version 3.00 (Butler et al. 2009) within R.

Results

Growth patterns of female *G. affinis* as determined by length-frequency histograms were similar in both summers and resulted in two distinct age classes (2008 collections shown in Figure 7). The most robust measure of size at maturity for females in this population was determined for time periods 1 and 2 for age-0 females (mid to late July and August) when the greatest range of females was captured. For both of these time periods across both years, the estimated size at maturity was consistently 25 mm SL. Age-1 females initially had a low percentage of pregnant females (63% and 41% for time period 1 in 2008 and 2009, respectively). However, all or nearly all (>97%) of age-1 females were pregnant in time periods 2 and 3 in both years. For age-0 females, the percentage of pregnant females was high for time periods 1 and 2 for both years (93 – 96% and 89 – 93% in 2008 and 2009, respectively); however the percentage of pregnant females declined greatly in time period 3 to 32% in 2008 and 15% in 2009.

Clutch dry mass was significantly affected by age, time period, and their interaction after controlling for variation due to female dry mass and embryonic stage of development (Table 8). Age-0 females had relatively high clutch dry mass for the first two time periods, but clutch dry mass significantly declined in the third time period (Figure 8a). For age-1 females, clutch dry mass significantly increased with each time period. However, clutch dry mass at time period 3 for age-1 females was not significantly different than clutch dry mass for age-0 females in the first two time periods (Figure 8a). Clutch dry mass was positively related to female dry mass

(coefficient = 1.153; $P < 0.001$), but negatively related to embryonic stage of development (coefficient = -0.021; $P < 0.001$).

Clutch size was significantly affected by age, time period, and their interaction after controlling for variation due to female dry mass (Table 8). For age-0 females, clutch size significantly decreased with each time period (Figure 8b). Age-1 females also had a significant but smaller decrease in clutch size from time period 1 to time period 2; however, clutch size significantly increased from time period 2 to time period 3 such that clutch size at time period 3 was the largest for age-1 females (Figure 8b). Clutch sizes for age-0 females at time period 1 and age-3 females at time period 3 were not significantly different. Clutch size was positively related to female dry mass (coefficient = 0.587; $P < 0.001$).

Offspring dry mass was significantly affected by age, time period, and their interaction after controlling for variation due to female dry mass and embryonic stage of development (Table 8). Both age-0 and age-1 females had a similar pattern of small offspring at time period 1 with a significant increase in offspring dry mass at time period 2 (Figure 8c). For age-0 females, offspring dry mass was reduced in time period 3 and was not significantly different than offspring dry mass at time period 1. For age-1 females, offspring dry mass was not significantly different between time periods 2 and 3 (Figure 8c). The largest offspring were produced by age-0 females in time period 2. Offspring dry mass was positively related to female dry mass (coefficient = 0.500; $P < 0.001$) and negatively related to embryonic stage of development (coefficient = -0.011; $P = 0.002$).

Using the MANOVA in the life history trajectory analysis, we found significant life history differences between ages (age by index variable interaction) and among time periods (time period by index variable interaction), as well as in their interaction (age by time period by

index variable; Table 9). The significant three-way interaction suggested that there were significant differences in one or more life history trajectory attributes between the trajectories for age-0 and age-1 females. Life history trajectories had significant differences in the magnitude of phenotypic change ($MD_{1,2} = 1.204$; $P_{\text{size}} < 0.001$) as age-0 females exhibited a greater amount of life history change compared to age-1 females (Figure 9). The direction of the trajectories were significantly different between the ages ($\theta_{1,2} = 117.45^\circ$; $P_\theta = 0.003$). Age-0 females followed a pattern of change consistent with increased allocation to future reproduction while age-1 females followed a trajectory of increased allocation to current reproduction (Figure 9). Finally, the shape of the trajectories were significantly different ($D_{\text{Shape}} = 1.470$; $P_{\text{Shape}} < 0.001$), mainly in the balance of the tradeoff between offspring size and number. Age-0 females initially allocated more to offspring number but switched to greater allocation to offspring size compared to offspring number in time periods 2 and 3 (Figure 9). Age-1 females switched from greater allocation to offspring number at time period 1 to greater allocation to offspring size at time period 2; in time period 3, age-1 females had greater allocation to offspring number (Figure 9).

Discussion

Age-based life history theory assumes that individuals of the same age have the same condition and experience the same environmental conditions (McNamara and Houston 1996). In this study, we documented an example of how age- and environment-based cues interact to determine the pattern of reproductive investment in *G. affinis*. The comparisons of age-0 and age-1 life history trajectories to reference trajectories in the life history analysis demonstrated that age-0 females followed a pattern of decreased investment to current reproduction, or reproductive restraint. On the other hand, age-1 females followed a pattern similar to the

reference trajectories indicating an increase in the level of investment to current reproduction, or terminal investment. Age-0 females demonstrated a pattern of reproductive restraint in late summer in response to environment-based cues indicating the onset of winter. This life history response likely increases a female's probability of overwinter survival; therefore, age-0 females demonstrated a shift towards allocation to future reproduction. For age-1 females, the extent of reproductive investment across time periods was consistent with predictions from age-based life history theory: females exhibited increased allocation to current reproduction (i.e. terminal investment) as summer progressed.

The patterns of reproductive investment in age-0 female *G. affinis* demonstrated a shift in the balance of fitness benefits of reproductive investment to current reproduction and the costs to future reproduction (Williams 1966). This change in life history strategy is consistent with predictions of the cost of reproduction hypothesis; however the change in costs and benefits is driven largely by environment-based cues rather than age-based cues. By exhibiting reproductive restraint late in summer, age-0 females should be able to increase their probability of overwinter survival, thus increasing future reproductive potential. The long winter period in central Utah (at least 7 months) acts as a strong selective agent for *G. affinis* such that only individuals with sufficient somatic storage will be able to overwinter. The amount of somatic storage necessary for an individual to overwinter increases with the length of winter (Schultz and Conover 1997), and the rate of storage depletion is inversely related to size (Shultz and Conover 1999). Therefore, females that exhibit reproductive restraint late in the summer and allocate more energy to growth and somatic storage will increase their probability of overwinter survival. Reznick and Braun (1987) documented a similar reduction in reproductive investment in age-0 *G. affinis* in late summer in a population at the northern extent of the species' native range; this

reduction in reproduction corresponded to an increase in somatic storage. Similar patterns of reproduction and somatic storage have also been documented in *G. holbrooki*, the sister species of *G. affinis* (Meffe and Snelson 1993; Pérez-Bote and López 2005). A reduction in either clutch size or reproductive investment based on environmental cues has been documented in other fish species (Hatch and Elias 2002; Fox et al. 2011), birds (Sockman et al. 2006) and mammals (Bårdsen et al. 2010).

Despite the reproductive restraint exhibited late in summer, age-0 females had a high level of reproductive investment (clutch dry mass) in the first two time periods similar to the extent of reproductive investment in age-1 females (Table 8; Figure 8a). Age-0 females appear to be allocating more to current reproduction in the first two time periods, and then switching to allocation to future reproduction by time period 3. An alternative strategy for age-0 females that we might predict from age-based life history theory would be a strategy with low investment or abstinence during all time periods to grow and store energy. This alternative strategy would allow females to have an increased probability of over-winter survival and to benefit from increased reproductive potential as age-1 fish due to their larger body size. However, patterns of reproductive investment observed in age-0 females suggest that winter mortality is high regardless of body size. By increasing reproductive investment early in the summer, an age-0 female increases the probability that at least some of her offspring, if not herself, will survive the winter to reproduce in the following summer. That there is high winter mortality is evident in the greatly reduced densities of *G. affinis* in April compared to the previous September (based on catch per unit effort; Billman unpublished data).

As summer progresses and winter approaches, the reproductive value of newly born offspring should decline due to winter mortality; the life history strategy exhibited by age-0

females reflects this reduction. Depletion of somatic storage occurs more rapidly in small fish leading to size-dependent winter mortality (Schultz and Conover 1999). In this population, the smallest individual (female or male) collected in the April collection for both years was 18 mm SL, indicating a potential minimum size for overwinter survival. For the first two time periods, age-0 females shifted the balance of the offspring size versus number tradeoff; age-0 females had large clutches of poorly provisioned embryos in time period 1 and smaller clutches of well provisioned embryos in time period 2 (Figure 8b and c). This shift in the balance of the tradeoff is likely an adaptive strategy: females have many small offspring early when individuals have sufficient time to reach a minimum size for winter survival and switch to having fewer larger offspring later. By producing better provisioned offspring at time period 2, females can ensure that offspring are adequately provisioned increasing the probability of reaching the minimum size for winter survival despite the shorter time until the onset of this selective season (Heins et al. 2004). Offspring born in late summer will have insufficient time to reach the minimum size with enough somatic storage to survive winter, will have a low reproductive value (Sockman et al. 2006), and will not likely contribute to the mother's lifetime fitness (i.e. representation in subsequent generations). The pattern of reproductive restraint in time period 3 by age-0 females reflects the marginal returns of offspring born late in the season.

Reproductive investment by age-1 *G. affinis* females was consistent with predictions of the cost of reproduction hypothesis. As the summer progressed, these females increased reproductive investment characteristic of individuals that are terminally investing (Clutton-Brock 1984). At the beginning of the reproductive season, *G. affinis* females utilize remaining somatic storage for the first reproductive bout (Reznick and Braun 1987). The low reproductive investment of age-1 females and high proportion of non-reproductive females suggests that few

somatic stores remain after the long winter season. Alternatively, these females might be demonstrating reproductive restraint to allocate more energy to growth to receive the increase in reproductive output afforded by a larger body size. Belk and Tuckfield (2010) also reported evidence demonstrating that reproductive allocation in *G. affinis* is consistent with predictions from the cost of reproduction hypothesis. In their study, age-1 females had higher reproductive allocation compared to age-0 females, and consequently experienced a significant decline in escape performance, evidence that older females experienced a greater cost of reproduction due to higher reproductive allocation (i.e. terminal investment).

The results of this study demonstrated that *G. affinis* females determine the level of their reproductive investment based on age and environmental cues. However, alternative mechanisms might generate or contribute to the observed patterns. We assumed that the two ages observed in this population were sequential cohorts. Alternatively, the population could consist of two distinct life history phenotypes that represent a short, fast reproductive life and a long, slow reproductive life (Roff 2002; Belk and Tuckfield 2010). Age-0 females had high reproductive allocation in time periods 1 and 2 that was similar to reproductive allocation by terminally investing age-1 females, a pattern that seems consistent with the difference in mortality and expected lifespan if two life history phenotypes existed (Reznick and Endler 1982; Johnson and Belk 2001). However, we would expect that the age-0 cohort would include both reproductive and non-reproductive individuals in each time period if there were two life history phenotypes. In this study, the vast majority (> 89%) of age-0 females larger than 24 mm SL had developing embryos in time periods 1 and 2; those without developing embryos were primarily small females that had just reached reproductive maturity (25 – 27 mm SL). Fewer age-0 females had developing embryos in time period 3, a pattern that is expected as females reduce

and cease reproduction due to environment-based cues indicating the end of summer. Therefore, the data in this study are not consistent with predictions for a population with two life history phenotypes.

Seasonal variation in resource availability provides another explanation for the differences in reproductive allocation observed between age-0 and age-1 females. Resource availability necessarily affects rates of resource acquisition which in turn affects the amount of energy available for allocation into competing demands. Therefore, rates of resource acquisition provide more or less energy for investment into both current and future reproduction resulting in changes in investment that can occur without changing the proportional allocation (Winemiller 1993; Reznick et al. 2000; Jennions et al. 2006; Belk and Tuckfield 2010). For age-1 females, resource availability may increase from April to June, while for age-0 females resource availability may decrease with the onset of fall and winter months. Under this resource availability scenario, we would predict reproductive investment to increase for age-1 females and decrease for age-0 females as was observed in this study. However, Vondracek et al. (1988) demonstrated that larger (older) females allocated increasingly more of their available energy to reproduction and less to growth compared to smaller (younger) females regardless of the level of resource availability. Additionally, Reznick and Braun (1987) demonstrated that age-0 females allocated more energy to somatic storage and less to reproduction in fall despite a decline in resource availability. Thus, the life history strategies observed in age-0 and age-1 females are not consistent with the pattern expected from seasonal resource availability alone.

In this study, we were unable to follow individual females, but instead used population means for each age to characterize life history trajectories. While this methodology is the standard for life history evolution studies of poeciliids (Reznick and Braun 1987; Daniels and

Felley 1992; Johnson and Belk 2001; Jennions et al. 2006), it necessarily assumes that individuals of each age have the same condition or state, i.e. they have had the same reproductive history and have the same future reproductive potential (McNamara and Houston 1996). Because poeciliids can have multiple clutches in a reproductive season, age classes that are established on an annual basis will include individuals that have large variation in birth date. This pattern can be observed in the size distribution of over-wintered females in the April samples (Figure 7). Age-1 females in April include mature females that had reproduced the previous year as well as females that have not yet reached reproductive maturity. Given this variation in age-1 females, we would predict that small over-wintering females would live longer and reproduce more times than large over-wintering females (Haynes and Cashner 1995). This does not invalidate the results of our study; we still predict that small over-wintering females will determine the extent of reproductive investment given age-based cues considering there is no evidence that female *G. affinis* in this population live to age-2. The effect of variation in birth date within age-0 and age-1 females on the observed life history trajectories could be further examined with an extensive mark-recapture study. Females could be captured and marked according to size multiple times across two reproductive seasons to determine growth and survival patterns. This would not only verify patterns of overwinter survival but could also determine how birth date affects patterns of reproductive investment.

In this study, we observed life history trajectories of two age classes of *G. affinis* demonstrating that females use multiple cues to determine the level of reproductive investment. Additionally, we demonstrated that the relative strength of age- and environment-based cues depended on age of the females. Environment-based cues had their greatest effect on reproductive investment in age-0 females while age-based cues had their greatest effect on

reproductive investment in age-1 females. For this population of *G. affinis*, we were able to determine that the effect of age- and environment-based cues in causing a shift, whether increase or decrease, in optimal reproductive investment depended on the age of the female. This demonstrates the importance of examining multiple cues in life history studies to determine how cues interact across an organism's life (Fisher 1930; Cotter et al. 2011)

Acknowledgments

Funding for this research was provided by a Mentoring Environment Grant from Brigham Young University to Mark Belk. We are grateful to Peter Meyers and a host of undergraduate students that helped with fish collections and sample processing while participating in the undergraduate mentoring research program in the Department of Biology. Josh Rasmussen provided assistance with statistical analyses. *Gambusia affinis* were collected under permits from the Utah Division of Wildlife Resources, and collecting procedures were approved by the Institutional Animal Care and Use Committee at Brigham Young University.

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Table 7. Summary of collection information for *Gambusia affinis* females in Utah Lake, Utah. Sample sizes indicate the number of females with developing embryos (stage > 2) out of the total number of females (immature and mature) collected.

Year	Sample date	Time period: age-0	Time period: age-1	<i>n</i> age-0 females	<i>n</i> age-1 females
2008	9 May	-	1	0	91/151
	6 June	-	2	0	146/146
	3 July	-	3	0/63	38/39
	18 July	1	-	117/177	6/6
	18 August	2	-	84/218	4/4
	9 September	3	-	31/223	0
2009	28 April	-	1	0	34/75
	29 May	-	2	0	58/58
	26 June	-	3	0/35	43/44
	30 July	1	-	63/92	2/2
	26 August	2	-	74/100	1/1
	17 September	3	-	8/192	½

Table 8. Analysis of covariance tables for mixed models comparing clutch dry mass, clutch size, and offspring dry mass for female *Gambusia affinis* as a function of age, time period, and the covariates female dry mass and stage of embryonic development.

Effect	Num DF	Den DF	F-statistic	P-value
Clutch dry mass				
Age	1	773	10.86	0.010
Time period	2	773	22.22	<0.001
Female dry mass	1	773	424.56	<0.001
Stage	1	774	25.97	<0.001
Age x time period	2	773	88.11	<0.001
Clutch size				
Age	1	775	49.97	<0.001
Time period	2	774	46.69	<0.001
Female dry mass	1	775	152.76	<0.001
Age x time period	2	775	68.92	<0.001
Offspring dry mass				
Age	1	773	4.28	0.039
Time period	2	773	84.76	<0.001
Female dry mass	1	773	113.01	<0.001
Stage	1	774	9.72	0.002
Age x time period	2	773	5.96	0.003

Table 9. Multivariate analysis of variance table for the mixed model comparing the life history strategy (defined by principal components derived from three life history traits) of female *Gambusia affinis* as a function of age, time period, and an index variable (accounts for ordering of principal components; see Methods for explanation).

Effect	Degrees of Freedom	Wald Statistic	P
Age	1	772.62	<0.001
Time period	2	199.83	<0.001
Index variable	3	0.09	0.993
Age x time period	2	88.92	<0.001
Age x index variable	2	9.84	0.007
Time period x index variable	4	354.76	<0.001
Age x time period x index variable	4	204.93	<0.001

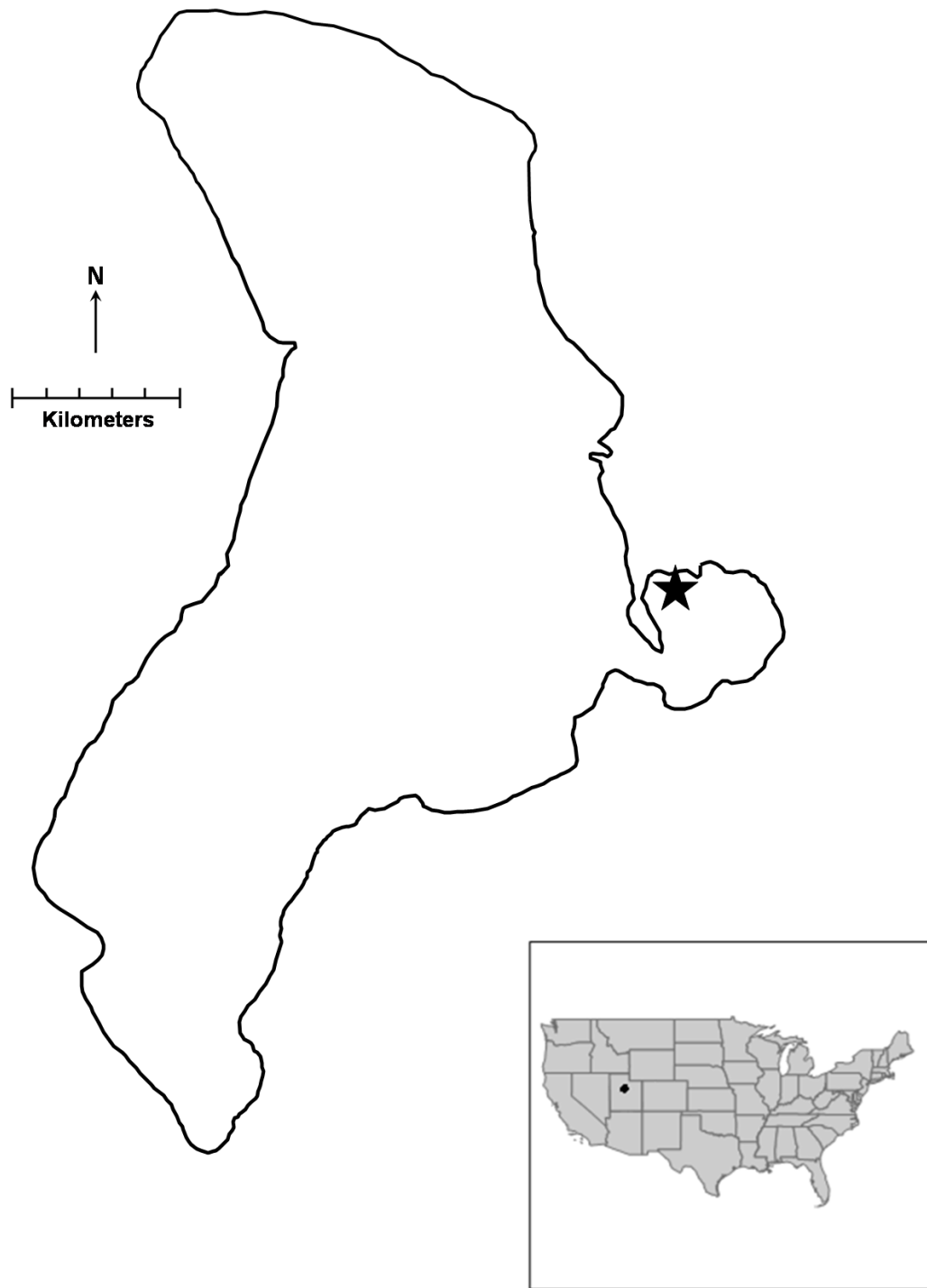


Figure 6. Map of Utah Lake with the star indicating the location of *Gambusia affinis* collections in Provo Bay. Inset shows the location of Utah Lake in Utah, USA.

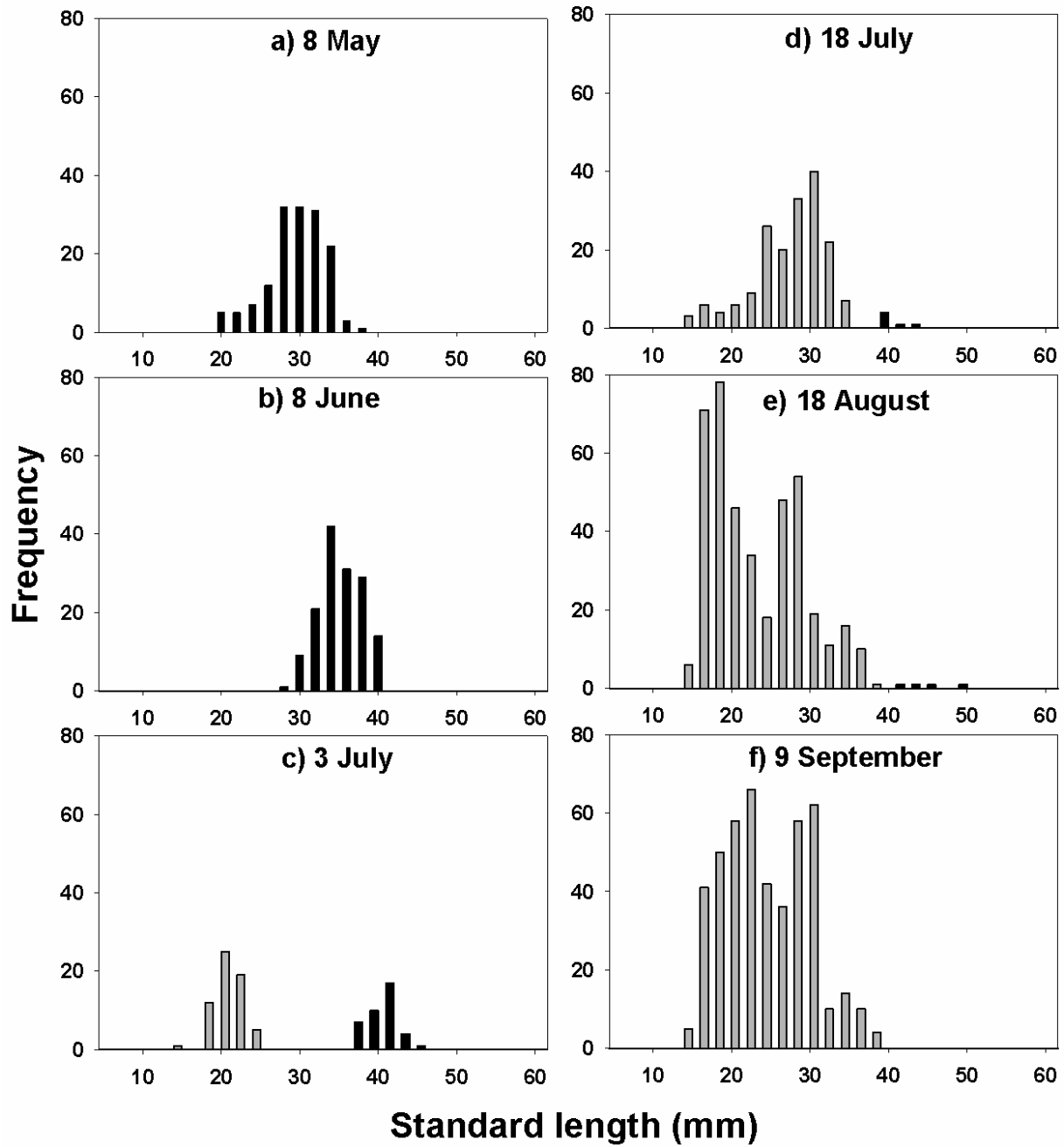


Figure 7. Length histograms of *Gambusia affinis* in Provo Bay of Utah Lake, Utah for the six collecting periods in 2008. Black bars indicate age-1 females and gray bars indicate age-0 females.

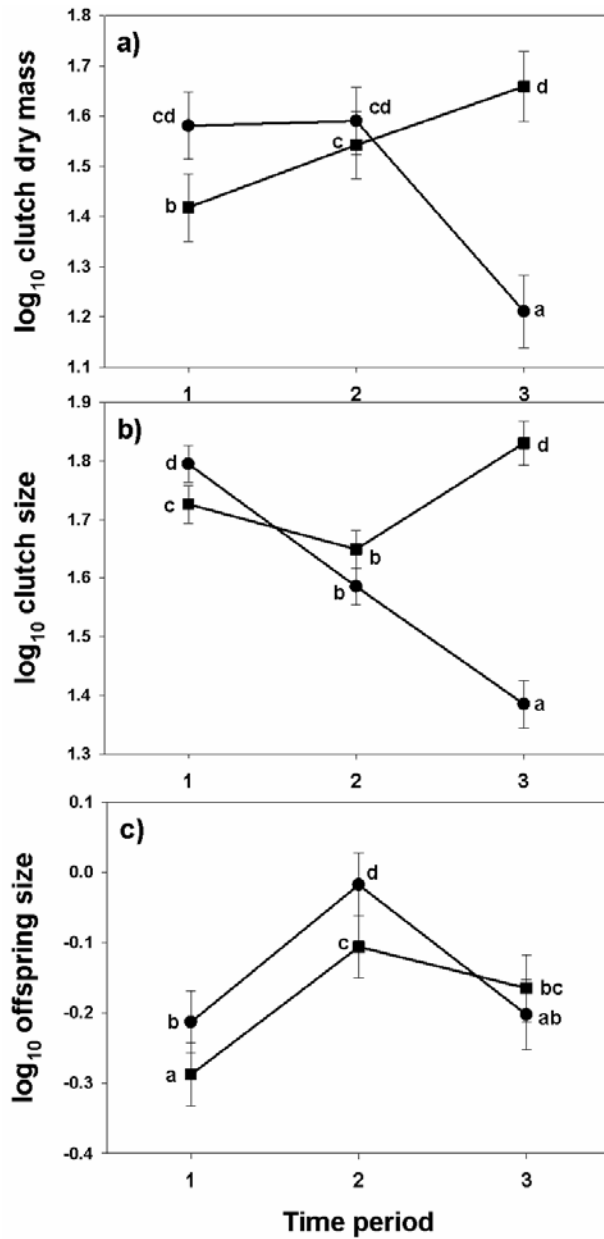


Figure 8. Least squares means (\pm SE) of life history traits for female *Gambusia affinis* in Provo Bay of Utah Lake, Utah: a) clutch dry mass (mg), b) clutch size, and c) offspring dry mass (mg). Circles represent age-0 females and squares represent age-1 females. Means have been adjusted to account for inherent differences in female body size between the two ages. Different letters indicate significant ($\alpha = 0.05$) differences in means.

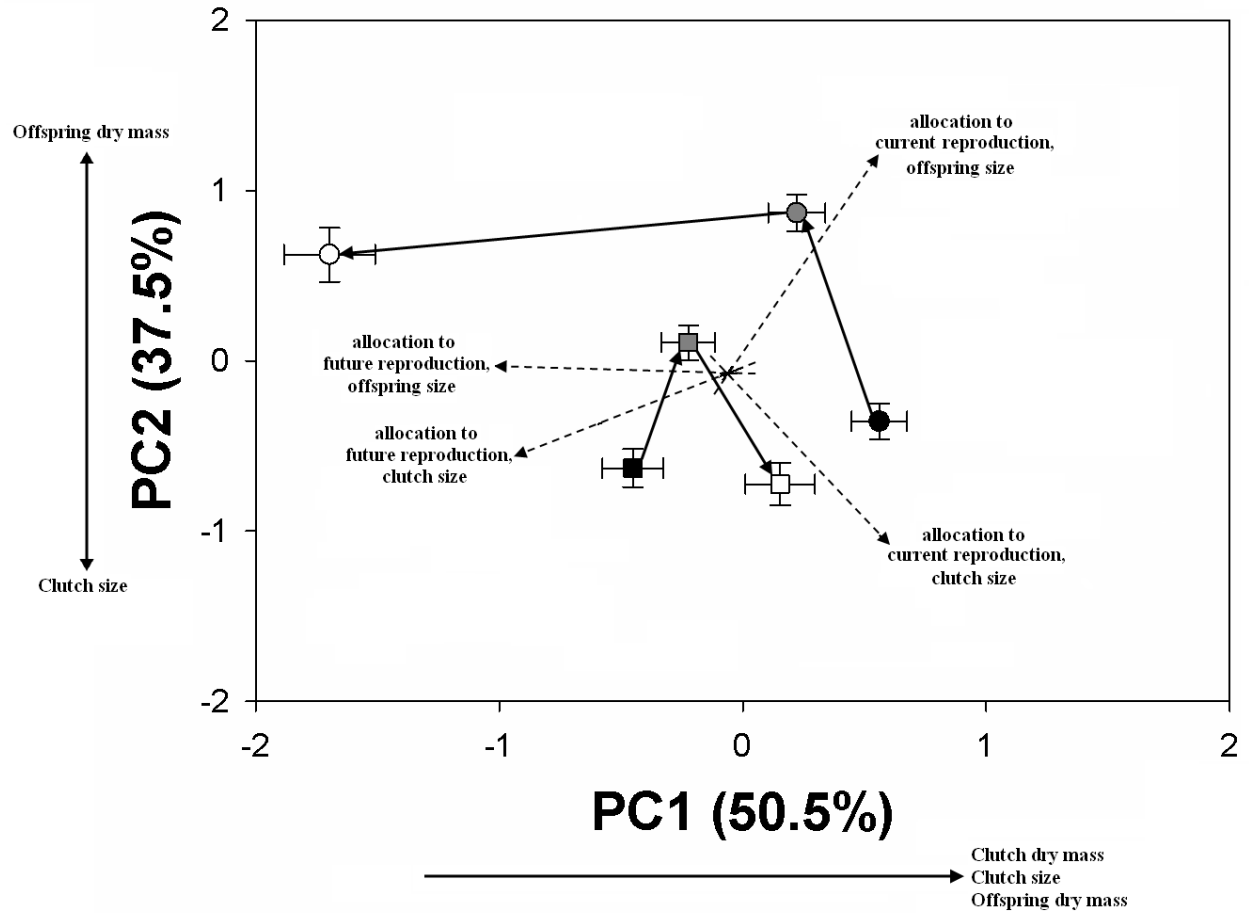


Figure 9. Least squares means (\pm SE) of principal component scores for life history trajectories defined by three time periods for age-0 (circles) and age-1 (squares) female *Gambusia affinis*. Black symbols = time period 1; gray symbols = time period 2; open symbols = time period 3.