

ENVIRONMENTAL, GENETIC, AND MATERNAL EFFECTS  
ON LIFE-HISTORY TRAITS OF THE  
STRIPED GROUND CRICKET,  
*ALLONEMOBIUS SOCIUS*

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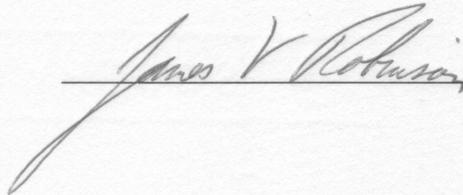
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ON LIFE-HISTORY TRAITS OF THE  
STRIPED GROUND CRICKET,  
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by

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In loving memory of Frances S. Wood and K. Frederick Huestis.

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ABSTRACT

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STRIPED GROUND CRICKET,  
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Organismal fitness is affected by both environmental and genetic factors. More importantly, an interaction between an individual's genotype and the current environmental conditions influences the success of that individual in its environment. These genotype-by-environment (GxE) interactions provide a useful system for evolutionary biologists to study factors which affect fitness.

Fitness effects due to differential performance along environmental gradients are often used to explain clines in allozyme allele frequencies seen in nature. A cline exists in the allele frequencies for isocitrate dehydrogenase (*Idh-1*) in the striped ground

cricket, *Allonemobius socius*, and I found significant geographic structure to allele frequencies of this gene which were mirrored by temperature and moisture gradients. These correlations, together with a significant GxE between *Idh-1* genotype and temperature which affected fitness in a laboratory study, support the hypothesis that allele distributions are the result of natural selection on performance of different genotypes at different temperatures.

Temperature also affects the survival of offspring once eggs are laid, as direct-developing individuals will die if conditions are too cold. Therefore, the ability of a female to produce eggs which will be successful in future, unknown conditions should be under selection, potentially resulting in another GxE. I found that an interaction between parental diapause history and egg-incubation temperature affected the proportion of diapause eggs produced by *A. socius*. Furthermore, the offspring of non-diapause parents were more likely to respond to the effects of temperature changes than were offspring of diapausing parents.

The results of these studies suggest a complex interaction between genotype, maternal effects, and the environment affects fitness in this species. My results provide hypothesis for further tests of GxE in *A. socius* and in other insect species.

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## CHAPTER 1

### INTRODUCTION

#### 1.1 Background

One of the central questions in evolutionary biology is “what influences organismal fitness?”. Fitness is typically defined as the total reproductive output during an organism’s life, and this reproductive output can vary widely. For example, an individual must live to reproductive age in order to produce any offspring, and the ability to do so is affected by many factors, including predation levels, nutrient availability, and habitat suitability. Once reaching reproductive age, the individual must have enough energy reserves to produce eggs or sperm and to mate. In species with parental care, the parents must also be able to provide for their young after reproducing. Therefore, successfully producing any offspring requires survival to sexual maturity with enough energy reserves to produce young. However, the number of young produced also varies depending on many factors, including energy reserves, environmental conditions, and an individual’s genotype. The rest of this chapter will outline two large areas of research that contribute to our knowledge of organismal fitness: 1) how genotype-by-environment interactions affect adult fitness and 2) the ability to enter a dormant state during unfavorable conditions or to break dormancy during favorable conditions, increasing the probability of survival to reproduction. The

end of this chapter will briefly summarize the specific research I conducted within these areas, to be discussed in depth in Chapters 2 and 3.

## 1.2 Allozyme Alleles and Organismal Fitness

### *1.2.1 Definition of an allozyme*

Enzymes consist of chains of amino acids that fold into a particular shape, known as a conformation, depending on charge and interactions between certain amino acids (Li, 1997). This overall folding of the enzyme results in the formation of an active site, where the reaction takes place. Enzymes are a specific type of protein produced by cells to catalyze metabolic processes (Harris, 1966; Campbell and Reece, 2002), such as the breakdown of glucose and the production of ATP.

Allozymes are different alleles of the same protein-coding gene, at the same location on homologous chromosomes, and have the same metabolic function (Li, 1997). Changes in the original DNA sequence, such as a point mutation, may lead to an amino acid substitution, particularly if the mutation occurs at the first or second codon position, as third-codon changes often code for the same amino acid, leaving the protein unchanged (Li, 1997). Amino acid substitutions may change the charge, weight, and folding of the protein, thus changing the relative electrophoretic mobility of the enzyme, particularly if the charge of the protein is changed (Hubby and Lewontin, 1966). These amino acid changes may also affect the function of the protein, changing optimal ranges for temperature, pH, or substrate concentration. Thus, most amino acid changes will likely be deleterious and quickly eliminated by purifying selection (Harris, 1966; Lewontin and Hubby, 1966). However, some amino acid shifts may not affect the

function of the protein, and will therefore be selectively neutral, while others may improve enzyme function, and will be favored by selection.

Originally, evolutionary biologists and geneticists thought that genetic diversity in populations would be quite low (Lewontin and Hubby, 1966; Li, 1997). However, early studies of protein polymorphism (allozymes) showed a lot of unexpected diversity, revealing polymorphisms at most loci studied (Harris, 1966; Hubby and Lewontin, 1966; Lewontin and Hubby, 1966; Li, 1997). As a result of the high diversity revealed, allozymes were thought to be neutral (Kreitman and Akashi, 1995). However, some researchers believed that allozymes would be subject to selection, and subsequent studies have supported selection in many cases (Watt, 1994; Kreitman and Akashi, 1995). Thus, a debate over whether allozymes were neutral or were subject to selection began soon after their discovery, and it has been stated that “few subjects in biology have been more strongly debated than the evolutionary significance of protein polymorphisms” (Powers et al., 1991).

### *1.2.2 Evidence for allozyme neutrality*

Neutral theory states that most mutations are neutral (Kimura, 1983; Li, 1997; Freeman and Herron, 2004), and that genetic drift is the major force behind allele frequency shifts (Kreitman and Akashi, 1995). The “simple neutral” theory states that allozyme genotypes do not differ in function (Watt, 1992), and thus, allozymes are a selectively neutral form of genetic diversity. Neutral theory further states that although many mutations will be deleterious and are removed from the population, some are beneficial (but such mutations are rare), and many mutations will be neutral (Li, 1997;

Freeman and Herron, 2004). These neutral mutations will appear, be lost, or accumulate and increase in frequency over time, and spread throughout populations. The number of alleles for a given locus is predicted to be a function of population size, according to neutral theory, such that larger populations will have more alleles for a given locus (Kimura, 1983). As a result of neutral theory, most allozymes were thought to be selectively neutral, and allozymes were widely used in systematics to study relationships between populations and species (Watt, 1995; van der Bank et al., 2001).

Neutrality is often used as the null model for many studies investigating allozymes in natural systems (Watt, 1994). Neutral theory can be tested using allozyme data by comparing allele frequencies against Hardy-Weinberg predictions, or performing tests of neutrality on the allele frequency data (van der Bank et al., 2001). Also, if selection is not acting on allozymes, there should be no association between allozymes and any environmental conditions. In support of neutral theory, many allozymes show no correlations with environmental parameters, such as latitude, temperature, or moisture (e.g. Hoffmann et al., 1995; Jaramillo-Correa et al., 2001).

For example, all allozymes studied in European white spruce populations showed no correlation with environmental parameters (Jaramillo-Correa et al., 2001). Significant differences were found between all environmental parameters studied, indicating that populations are exposed to different environmental conditions and that selection could possibly act on allozyme loci due to this environmental variation. However, each of the 14 loci studied appeared to be neutral and frequencies were not significantly different from the expectations of Hardy-Weinberg equilibrium. All

relationships between allele frequencies and bioclimatic variables were also non-significant (Jaramillo-Correa et al., 2001), indicating no correlation between any allozyme locus and environmental conditions. Overall, all allozyme data from white spruce indicated that no selection is acting on these loci, despite evidence of selection on other genetic markers studied in these populations (Jaramillo-Correa et al., 2001).

Many allozymes (30 of 37 studied) in mice of the genus *Peromyscus* also appeared to be neutral, and followed predictions of genetic drift; however, the others showed significant departures from neutral expectations (Storz and Nachman, 2003). Therefore, allozyme allele frequencies at certain loci may often change simply due to genetic drift or other stochastic processes. Or, there may be little change in frequencies over a wide area. In fact, many patterns of enzyme polymorphisms fit predictions of neutral theory (Hoffmann et al., 1995; Kreitman and Akashi, 1995).

### *1.2.3 Evidence for selection on allozymes*

In contrast to neutral theory, selection theory states that many mutations are deleterious, few are neutral, and many are beneficial (Li, 1997; Freeman and Herron, 2004). Beneficial mutations will be selected for and spread throughout populations, increasing in frequency over time, while deleterious mutations will be removed. Since enzyme function depends on temperature, pH, substrate concentration, and other environmental factors, some amino acid substitutions will lead to an enzyme that functions best under certain conditions, and these may be favored locally by natural selection, depending on environmental factors. Thus, allozymes may not be neutral, and instead are subject to selection pressures.

Unlike neutral theory, evidence on a case-by-case basis for selection on allozymes continues to grow as studies examine relationships between allozymes and fitness. Many studies have been conducted on individual allozyme loci, often initiated by an observation of natural populations. Evidence for allozyme selection includes the presence of clines in allozyme allele frequencies, correlations between environmental variables and allozyme allele frequencies, differences in chemical properties between allozyme alleles, and differential performance and/or fitness differences between individuals with different allozyme genotypes. These types of evidence are often combined within a system, and several lines of evidence together provide support for the hypothesis that selection is acting on certain allozyme loci.

#### *1.2.4 Clines in allozyme allele frequencies*

A natural cline of allele frequencies exists for many loci in *Drosophila melanogaster* (Ochando and Ayala, 1999), with the best-studied being that of alcohol dehydrogenase (*Adh*) along the east coast of North America (Berry and Kreitman, 1993). Clines also exist for many allozymes in the fish *Fundulus heteroclitus*, with 4 main patterns: 1) 2 alleles, 1 fixed at each end and clinal in between, 2) 1 allele fixed at one end, but variable everywhere else, 3) fixed for same allele at both extremes, but variable in between, and 4) no change with latitude (Powers et al., 1991). Clines may indicate changes in allele frequencies due to natural selection of environmental conditions. Since temperature varies with latitude along the east coast, it is inferred that temperature effects may have caused selection on certain loci in this species of fish.

A cline in the frequency of two alleles of isocitrate dehydrogenase (*Idh*) also occurs in European silver fir: one nearly fixed to the north, one fixed in the south, and clinal in between (Bergmann and Gregorius, 1993), a type I cline (see above description of the 4 classes of clines). Additionally, spatially varying selection was found to account for departures from neutrality noted in surveys of allozyme polymorphism in mice of the genus *Peromyscus* (Storz and Nachman, 2003). Specifically, albumin (*Alb*) repeatedly appears to have undergone local adaptation through selection in these mice (Storz and Nachman, 2003; Storz and Dubach, 2004). Thus, clines may point to the possibility of selection, although they do not demonstrate or measure the action of selection directly.

#### *1.2.5 Environmental correlates of allozyme allele frequencies*

In a survey of over 100 species, Riddoch (1993) found a large number of correlations with phosphoglucose isomerase (*Pgi*) in a wide range of species, from bacteria to plants to many animal phyla. In most cases, the fast allele appears to be favored under stressful conditions, such as increased temperature, desiccation risk, salinity, and reduced oxygen (Riddoch, 1993). The correlations are statistically significant, and indicate strong selection under stressful conditions. Interestingly, a reduction in overall charge should increase protein stability, which supports findings that the more stable allele is more common in stressful, unstable environments, but this is balanced by lower efficiency. Heterozygosity at the *Pgi* locus was also found to increase in uncertain environments (Riddoch, 1993). Additionally, allele frequencies of *Pgi* shifted due to climate change (cooler temperatures in the mid-1990's) in leaf beetles

(Rank and Dahlhoff, 2002). Individuals with the *Pgi*-1 allele respond to cold better than individuals with the *Pgi*-4 allele, and the frequency of the *Pgi*-1 allele increased significantly (11%) during a cold period (Rank and Dahlhoff, 2002). Other examples include correlations between certain allozyme allele frequencies and summer temperatures in the fish *F. heteroclitus* (Powers et al., 1991; Schulte, 2001), indicating selection on performance at high temperatures. Thus, many studies have revealed correlations between allozyme allele frequencies, particularly for *Pgi*, and certain environmental correlates (especially temperature), potentially indicating strong selection at these loci imposed by the environment. However, environmental correlates, as with clines, do not measure selection directly.

#### *1.2.6 Differences in chemical properties*

Thermostability differences and other differences in kinetic properties have been found between alleles of a given allozyme; these differences may lead to selection for or against enzyme performance (Fields, 2001). For example, in *Colias* butterflies, the most heat-stable allele for *Pgi* is the least kinetically favored, so a trade-off exists between protein stability at high temperatures and enzyme performance (Watt, 1992). Stability and kinetic performance differences were seen in at least 7 allozymes studied in the fish *F. heteroclitus*, including lactate dehydrogenase (*Ldh*), malate dehydrogenase (*Mdh*), and *Idh* (Powers et al., 1991). As a result, changes in gene expression may compensate for differential performance between genotypes during acclimation in this fish (Schulte, 2001), such that those proteins which are more heat stable, but perform worse kinetically, are produced in increased concentrations during temperature stress.

In silver fir, the allele for *Idh* that is found most often in southern populations was found to have higher thermostability, and is therefore better able to function at high temperatures, than proteins produced by trees with the northern-type allele (Bergmann and Gregorius, 1993). Thus, the observed cline between the two alleles in this species seems to reflect selection on this locus by high summer temperatures to the south. Increased thermostability may lower overall enzyme performance, so the north-type allele is more beneficial if there is no selection pressure imposed by hot temperatures. In leaf beetles, individuals with the *Pgi-1* allele produced heat shock proteins better than *Pgi-4* individuals, and were thus better able to tolerate and survive extreme temperatures (Rank and Dahlhoff, 2002). Individuals homozygous for the *Pgi-1* allele (1-1 genotype) had a lower induction temperature for heat shock proteins than heterozygous individuals with the 1-4 genotype, which produced heat shock proteins at lower temperatures than 4-4 individuals homozygous for the *Pgi-4* allele (Rank and Dahlhoff, 2002). In summary, many studies have revealed differences in enzyme performance between alleles of many loci in several species, and a trade-off between thermostability and enzyme performance often exists, leading to selection for stability in hot conditions, but selection for performance in cooler conditions. Again, differences in thermal stability may translate to differences in fitness, but need to be tested directly.

### *1.2.7 Differential performance*

In some species, differences in survival, feeding ability, mobility, or other important processes were found to exist between individuals differing in genotype for specific allozyme loci. For example, survival, flight performance, and mating success

differences in *Colias* butterflies were related to *Pgi* genotype (Watt, 1992, 1994). Proportions of metamorphs, time to metamorphosis, and metamorphic size depended on *Ldh* genotype in pond frogs (Hotz and Semlitsch, 2000). All three factors are thought to affect adult fitness in frogs, so although larval traits were measured, the observed performance differences are likely to translate into adult fitness differences. In the pond frog study, one genotype was superior in all 3 measures, raising the question of why this allele is not fixed in populations; one possibility postulated by the authors was that balancing selection with other alleles later in life maintains diversity at this locus (Hotz and Semlitsch, 2000). The development rate of *D. melanogaster* depends on allozyme alleles, particularly *Adh* (Ochando and Ayala, 1999), providing possible advantages to faster-developing individuals, depending on genotype. Survival after exposure to extreme cold depended on *Pgi* genotype in beetles, such that individuals with the 1-1 genotype survived better than individuals with genotypes 1-4 or 4-4 (Rank and Dahlhoff, 2002). Lastly, swimming ability, metabolic ability, developmental rates, and hatching times vary with *Ldh* genotype in *F. heteroclitus* (Powers et al., 1991), indicating selection on this locus. Performance differences between individuals that depend on allozyme genotype may translate to fitness differences between these individuals, allowing selection to act on these allozyme loci. As in the previous examples, these hypothesized fitness differences need to be tested directly to provide well-supported evidence for selection.

### 1.2.8 Fitness differences

The best test of selection on allozyme loci are studies which actually examine fitness differences between individuals differing in genotype for specific allozyme loci. One case where allozyme genotype was found to affect fitness was in *D. melanogaster*, where *Adh*, phosphoglucose mutase (*Pgm*), esterase (*Est*), and glycerol-3-phosphate dehydrogenase (*Gpdh*) genotypes affect fecundity, development rate, and survivorship (Ochando and Ayala, 1999). The greatest observed difference was between *Adh* genotypes, where individuals differed greatly in the number of progeny produced; overall, *Adh* had more significant genotype-by-temperature interactions (Ochando and Ayala, 1999), potentially indicating strong temperature-driven selection on this locus.

Laboratory selection experiments have shown differential survival for *Ldh* genotypes in the fish *F. heteroclitus*; these survival differences were both dependent on temperature and reflective of the observed cline in genotype frequencies (Powers et al., 1991). Additionally, in *Colias* butterflies, individuals with the *Pgi* allele that is most heat-stable have lower fecundity than those with the kinetically favored, less heat-stable allele (Watt, 1992). *Pgi* genotype also affects male mating success in these butterflies (Watt, 1995). In particular, the results of numerous studies on *Pgi* in *Colias* butterflies provide strong evidence of selection on this locus, and also provide hypotheses for future studies. For example, the fact that the most heat-stable allele confers the lowest fitness indicates that global warming may have a large impact on this species, as the genotype that is most successful in hot conditions confers the lowest fitness, possibly

resulting in an increase in the probability of extinction of this species as a result of selection for thermostability (Watt, 1992, 1995).

### 1.3 Genotype-by-Environment Interactions

Phenotypic plasticity can be adaptive because it allows the individual to develop a phenotype that will be advantageous under the range of environmental conditions that it experiences. Individuals that differ in genotype will often differ in phenotype, but a single genotype can also result in a range of phenotypes if that trait is plastic (Via and Lande, 1985; Schlichting and Pigliucci, 1998). Additionally, phenotypic bet-hedging may occur when a female produces offspring with a range of phenotypes from a given genotype, often in response to environmental uncertainty (Philippi and Seger, 1989).

Since phenotypic plasticity is generally thought to be advantageous, it is likely to be shaped by selection (Via and Lande, 1985), resulting in the evolution of a reaction norm. Thus, a reaction norm can be defined as “the set of phenotypes that can be produced by an individual genotype that is exposed to different environmental conditions” (Schlichting and Pigliucci, 1998, p. 51). The existence of a reaction norm is evidence for a genotype-by-environment (GxE) interaction, in which one genotype produces a specific phenotype depending on the environment. Selection may then act on this GxE interaction (Via and Lande, 1985), either increasing or decreasing the level of plasticity and the sensitivity of the organism to environmental changes. Therefore, the study of GxE interactions in laboratory settings enables one to examine if phenotypic plasticity exists, if it is adaptive, and can potentially allow for the discovery of the genetic basis for plastic traits.

#### 1.4 Insect Dormancy as an Adaptation

Harsh environmental conditions often make it impractical or impossible for organisms to be active year-round, particularly at high latitudes or in arid regions. Insects are adapted to seasonal patterns of environmental extremes, as most are able to spend some portion of their life-cycle in a dormant state when the habitat is unusable (Danks, 1987). Dormancy allows individuals to survive harsh conditions and emerge later during more favorable conditions (Philippi and Seger, 1989; Mousseau and Dingle, 1991; Mousseau and Fox, 1998; Hockham et al., 2001). Most species can enter dormancy in only one stage of the life cycle, and most enter dormancy in the egg stage (Danks, 1987; Mousseau and Dingle, 1991), a condition known as egg or embryonic diapause. In species that experience egg diapause, the initiation of diapause is typically thought to be under maternal control (Tanaka, 1986a; Mousseau and Dingle, 1991; Bradford and Roff, 1993; Olvido et al., 1998; Roff and Bradford, 2000; Hockham et al., 2001), although environmental conditions may directly influence embryonic development (Tanaka, 1986a; Shiga and Numata, 1997; Olvido et al., 1998; Hockham et al., 2001). Determining what environmental conditions lead to diapause initiation has been the focus of dozens of studies (e.g. Mousseau and Dingle, 1991; Mousseau and Fox, 1998), and the mechanism of diapause induction has also been the subject of much research (Mousseau and Dingle, 1991; Rossiter, 1996), although it is still not known in most species. In this section, I will summarize recent literature in these two areas.

#### *1.4.1 Environmental and maternal effects on diapause*

As temperature and photoperiod are linked to seasonality at most latitudes (Mousseau, 1991; Mousseau and Dingle, 1991; Mousseau and Fox, 1998; Olvido et al., 1998), these two environmental variables are ideal indicators of future environmental conditions. Most studies of environmental effects on diapause have focused on photoperiod and/or temperature, with the majority focusing on photoperiod (Mousseau and Dingle, 1991; Rossiter, 1996). However, the relative importance of these 3 factors (photoperiod, temperature, or their interaction) has been under much debate in the literature. Some studies have provided evidence that photoperiod is primarily responsible for diapause induction (Bradford and Roff, 1993; Shiga and Numata, 1997; McWatters and Saunders, 1998; Roff and Bradford, 2000), others have supported temperature (Tanaka, 1984, 1986a), while still others have pointed to an interaction between photoperiod and temperature (Mousseau and Dingle, 1991; Olvido et al., 1998); the relative importance of these factors may also depend on the geographic origin or genetic background of the species being studied (McWatters and Saunders, 1998).

Season length and female age have also been postulated as important in determining diapause occurrence. As the amount of time left in the growing season decreases, the probability that a direct-developing individual will complete development before death also decreases. As a result, diapause proportions typically increase throughout the season (Philippi and Seger, 1989), such that few diapausing eggs may be produced early in the year and the last batches of eggs produced will all initiate

diapause. As length of time remaining in the growing season typically decreases with increasing female age, diapause incidence is also predicted to increase with increasing female age. This result has been found in several studies (Mousseau, 1991; Roff and Bradford, 2000; Hockham et al., 2001), although the effects of female age may depend on genetic background (Mousseau, 1991).

Voltinism (the number of generations that can be completed per year) often varies with latitude, with more generations being produced at lower latitudes than at higher latitudes. For example, in North America, populations of striped ground crickets (genus *Allonemobius*) at high latitudes are univoltine, those at mid-latitudes are bivoltine, and those farthest south are multivoltine (Howard and Furth, 1986; Mousseau and Roff, 1989a). Heritability of diapause has been postulated or demonstrated to explain this variation in several instances (Mousseau and Roff, 1989a; Bradford and Roff, 1993, 1997; McWatters and Saunders, 1998; Roff and Bradford, 2000; Bégin and Roff, 2002). However, the mechanism of transmission of environmental information from mothers to offspring is still not known.

#### *1.4.2 Mechanisms of diapause induction*

Inherited environmental effects occur when offspring phenotype is altered by non-nuclear inheritance from the parents (Rossiter, 1996). These indirect environmental effects occur when the environment experienced by one individual affects the phenotype of another individual, such that the final offspring phenotype is the product of 3 factors: its own genotype, the environment it experiences, and indirect genetic effects of the environment experienced by its parents (Wolf and Brodie, 1998; Wolf et

al., 1998). Maternal effects are a specific example of indirect inheritance in which variation in the environment experienced by the mother affects offspring phenotype (Mousseau, 1991; Mousseau and Dingle, 1991; Wolf et al., 1998). A literature survey by Mousseau and Fox (1998) found evidence for environmentally-induced maternal effects in more than 70 insect species, emphasizing the importance of this mode of inheritance in this group of organisms.

The mode of transmission for maternal effects varies, but may include timing of reproduction, yolk amount, oviposition site, nutrients, parental care, and extra-nuclear factors (Kirkpatrick and Lande, 1989; Mousseau and Dingle, 1991; Rossiter, 1996; Mousseau and Fox, 1998). Cytoplasmic factors such as proteins, hormones, and mRNA's have been suggested as mechanisms for a range of maternal effects on early development (Rossiter, 1996; Mousseau and Fox, 1998). Hormones are most often suggested as the exact mode of diapause initiation (Mousseau, 1991; Mousseau and Dingle, 1991; Olvido et al., 1998; Hockham et al., 2001), although DNA methylation (Rossiter, 1996) and RNA transcripts (Kirkpatrick and Lande, 1989) have also been suggested. One putative example of a diapause hormone has been identified in silkworms; this hormone was probably derived from another developmental hormone and has evolved a new function to initiate diapause (Mousseau and Dingle, 1991). However, the true mechanism of diapause induction is still not known in most species (Mousseau and Dingle, 1991), even in well-studied systems like crickets in the genera *Gryllus* (Bégin and Roff, 2002) and *Allonemobius* (Olvido et al., 1998), and remains a very interesting question in evolutionary and developmental biology.

### 1.5 *Allonemobius* as a Model System

Striped ground crickets (genus *Allonemobius*) are well-studied, and have become a model system within evolutionary biology within the last few decades. They make an ideal lab organism because they are relatively easy to capture, breed, and maintain, as well as having a short generation time of about 2 months (e.g., Mousseau and Roff, 1989a). In this section, I review some of the primary research from the last two decades utilizing crickets of the genus *Allonemobius* as a model organism, covering a range of areas within evolutionary biology, from hybrid zones to mating strategies to life-history evolution.

#### *1.5.1 Taxonomy and species distributions*

Crickets of the genus *Allonemobius* were originally described under the genus *Nemobius*, and descriptions were originally made on the basis of morphology, calling song, mating preferences and behavior, and habitat type (Fulton, 1931). More recently, morphologically cryptic species have been identified by allozymes (Howard, 1983; Howard and Furth, 1986), and by DNA sequencing (Marshall, 2004). In the near future, 1 or 2 additional cryptic species within the *Allonemobius fasciatus*–*A. socius* complex will be described on the basis of allozyme allele frequency, DNA sequence differences, and reproductive isolating barriers (J.L. Marshall, pers. comm.). Hybrid populations and frequency of hybridization have also been explored using allozyme (Howard and Waring, 1991; Chu and Howard, 1998; Britch et al., 2001), RAPD (Chu et al., 1995; Chu and Howard, 1998), and AFLP markers (Howard et al., 2002).

### 1.5.2 Hybrid zone dynamics

The ranges of *Allonemobius fasciatus* and *A. socius* meet in the northern U.S., and a mosaic hybrid zone exists between these two species where they meet (Britch et al., 2001). This hybrid zone has been used as a model for many studies of hybrid zone dynamics in a range of areas, including: genetics of speciation, reproductive isolation and sperm precedence, calling song differences, and ecological divergence (e.g. Benedix and Howard, 1991; Chu et al., 1995; Howard et al., 2002; Marshall, 2004). These studies have explored what several factors contribute to isolation of these two species and the potential success of hybrids.

The discovery that at least 2 species exist within this complex was originally made using allozymes (Howard, 1986); more recently, fixed or nearly fixed differences between these two species has been studied using RAPD (Chu et al., 1995; Chu and Howard, 1998) and AFLP (Howard et al., 2002) markers. A long-term study of the hybrid zone between *A. socius* and *A. fasciatus* revealed that the frequency of *A. socius* allozyme alleles is increasing along the zone, and that this species appears to be moving northward into the range of *A. fasciatus* (Britch et al., 2001). Thus, the location of the hybrid zone may be shifting northward over time, and gene flow is occurring primarily from *A. socius* into *A. fasciatus*. Within mixed-species populations, allele distributions appear primarily bimodal, indicating limited gene flow and strong reproductive isolation between the two species (Britch et al., 2001).

Early laboratory crosses between these two species revealed that viable hybrids were produced from heterospecific matings, but that the number of eggs produced was

lower than in conspecific matings, as was hatchability (Tanaka, 1991). Developmental and life-history differences were also noted between the two species and their hybrids, including diapause proportions, time to hatching, developmental rate, adult morphology, and egg size (Tanaka, 1986a, 1991). In contrast, a later study by Gregory and Howard (1993) revealed differences in the number of eggs produced by heterospecific matings, but not differences in hatching success, development time, or survival in hybrid offspring. However, differences in development time were noted in the backcross F<sub>2</sub> generation (Gregory and Howard, 1993). Lastly, studies comparing the survival of hybrids to those of parental species revealed that *A. socius* individuals have significantly higher survival than either *A. fasciatus* or hybrids, and that *A. fasciatus* and hybrids have approximately equal survivorship (Howard et al., 1993).

Typically, male crickets use calling songs to attract mates, and females rely on calling songs to recognize potential mates. Therefore, it was hypothesized that differences in calling song may contribute to reproductive isolation between *A. socius* and *A. fasciatus*. In one study, no calling song displacement was observed in *A. socius* males when songs were compared inside and outside of the hybrid zone (Benedix and Howard, 1991). However, calling song displacement was noted for *A. fasciatus* in 3 mixed-species populations (Benedix and Howard, 1991). Further studies found no evidence for calling song displacement between the species in mixed populations, although temporal variation was present (Veech et al., 1996). Variation over the geographic range of each species was greater than variation between species in the hybrid zone, again providing evidence against character displacement for calling song

in these two species (Veech et al., 1996). However, calling song appears to be a plastic trait, such that the environment experienced during development influences the traits of the call produced as an adult (Olvido and Mousseau, 1995).

More recent studies have revealed significant differences in calling song attributed between the two species, and demonstrated a heritable genetic basis to these differences (Mousseau and Howard, 1998). However, these genetic differences in calling song were attributed to drift between the two species rather than sexual selection for increased differences (Roff et al., 1999), as the only study of female preference at that time showed no preference for calling song (Doherty and Howard, 1996). More recently, however, it was found that individuals showed variation for one attribute of calling song (chirp duration), revealing the potential for selected differences among populations (Olvido and Wagner, 2004); however, discrimination between *A. socius* and *A. fasciatus* females for their own species' mating calls was not measured in that study.

Conspecific sperm precedence (CSP) occurs when more eggs are fertilized by conspecific sperm than heterospecific sperm when both are present in the reproductive tract. Sperm precedence has been identified as a barrier to heterospecific fertilization between *A. socius* and *A. fasciatus* (Gregory and Howard, 1994; Howard et al., 1998, 2002). Additionally, females may be more likely to mate with males of their own species than with a heterospecific (Gregory et al., 1998), although these results were only weakly supported by Howard et al. (1998). Therefore, sperm precedence is a strong isolating mechanism between these two species (Howard et al., 1998), as conspecific matings produce more offspring than heterospecific matings, and the

conspecific will fertilize more eggs when a female has mated with both types of male. In fact, CSP has been proposed as the only barrier to hybridization between these two species (Howard et al., 1998), as many other common isolating mechanisms are absent. Differences in habitat utilization may also provide barriers to hybridization and the maintenance of distinct species of *Allonemobius* (Howard and Harrison, 1984a,b; Howard and Waring, 1991).

In summary, the hybrid zone between *Allonemobius fasciatus* and *A. socius* in the eastern U.S. has provided a very dynamic system for studying a range of factors that contribute to reproductive isolation within hybrid zones, including both pre- and post-zygotic isolation; further studies of hybrid zones within this complex will continue to advance our knowledge of hybrid zone dynamics.

### *1.5.3 Mating strategies and sexual conflict*

The elaborate courtship behavior of *Allonemobius* was described by Mays (1971), and it was noted that females gain nuptial gifts in the form of tibial spur chewing and eating the spermatophore. These observations set up an interesting system in which females gain nutrition through mating while males incur a high cost. Recently, this system has been used in several studies examining mate choice and the cost of reproduction to both sexes. For example, Sadowski et al. (2002) showed that females would mate more frequently and more rapidly with larger males, while males did not alter courtship behaviors in response to female size. Fedorka and Mousseau (2002a) also showed that females were more likely to mate with larger males. Female preference for larger males is likely due to the correlation between male body size and the size of

the nuptial gift, such that larger males produce larger gifts (Fedorka and Mousseau, 2002a). Thus, in both field and laboratory settings, male body size in *A. socius* is under both sexual selection, because females are more likely to mate with larger males, and natural selection, since larger males produce more offspring (Fedorka and Mousseau, 2002b, 2004).

*Allonemobius* are highly promiscuous, with males and females mating multiply. Females derive many benefits from multiple matings, as they receive a nuptial gift from each mating that allows for greater egg production (Fedorka and Mousseau, 2002c). Furthermore, mating with multiple males decreases the risk of genetic incompatibility, and hatching success and survivorship of offspring increases with multiple matings (Fedorka and Mousseau, 2002c). However, mating multiply results in decreased immune function in both males and females, and increases age-specific mortality in both species (Fedorka et al., 2004). Fedorka et al. (2004) postulated that the reduction in immune ability of males results from a loss of hemolymph through the nuptial gift, and that the wound incurred by tibial feeding may increase the risk of infection, leading to an overall increase in mortality. Females may receive harmful male substances during copulation that decrease immune function and increase mortality risk (Fedorka et al., 2004).

Thus, there appears to be a trade-off for females between the genetic and nutritional benefits of multiple mating and an increase in mortality risk in *Allonemobius*, making it an ideal system for studying the evolution of sexual conflict. Furthermore, successful males produce successful sons but unsuccessful daughters,

while less successful males produce more successful daughters but less successful sons, providing evidence of parent-offspring conflict in this system (Fedorka and Mousseau, 2004). In summary, crickets of the genus *Allonemobius* have recently become a model system for studying mating strategies, sexual conflict, and parent-offspring conflict, and will likely continue to be studied, as many questions remain unanswered.

#### *1.5.4 Morphology*

In insect species which possess ovipositors, females use the ovipositor to deposit eggs in the substrate at an appropriate depth. As it is hypothesized that increased egg depth will increase egg survival during overwintering in cold climates, ovipositor length should increase along a cline from south to north (Bradford et al., 1993; Mousseau and Roff, 1995). This predicted cline is seen in nature during collections made in the fall and has a genetic basis, as laboratory-reared individuals from several populations develop ovipositor lengths similar to field-caught adults from their own population (Mousseau and Roff, 1995). The genetic basis of variation in ovipositor length has been studied theoretically, and selection on this trait was supported (Roff and Mousseau, 1999).

Since the first generation of a bivoltine population does not overwinter, there may be selection for phenotypic plasticity in ovipositor length, whereby the first generation develops shorter ovipositors than the second (Bradford et al., 1993; Mousseau and Roff, 1995). However, Bradford et al. (1993) found that increased depth decreased hatchling survival in *A. socius*. Furthermore, very high variation in egg-laying depth was documented; such variation was not correlated with ovipositor length,

but rather was a behavioral response to current environmental conditions, with eggs being laid deeper in fall vs. summer environments (Bradford et al., 1993). Despite having a genetic basis, ovipositor length itself is a phenotypically plastic trait, such that crickets raised in an environment which simulates that experienced by second-generation crickets in a bivoltine population develop longer ovipositors than their siblings raised in an environment simulating that experienced by first-generation crickets (Mousseau and Roff, 1995). In summary, ovipositor length is under both genetic and environmental control in *Allonemobius*, and actual depth of eggs laid appears to be mainly influenced by environmental effects on egg-laying behavior (Bradford et al., 1993; Mousseau and Roff, 1995).

Striped ground crickets, as in many other crickets species, have two distinct wing morphs, long-winged (macropterous) and short-winged (micropterous). This dimorphism allows macropterous individuals to fly to new locations, while micropterous individuals do not fly. However, macroptery has a reproductive cost for female *Allonemobius*, such that macropterous individuals delay egg production and lay fewer total eggs, relative to micropterous individuals (Roff, 1984). This trade-off between flight ability and egg production has been noted in several cricket species (Roff, 1984; Mole and Zera, 1993; Roff and Bradford, 1996; Tanaka and Suzuki, 1998; Stirling et al., 1999; Bégin and Roff, 2002).

Removal of the wing pad in 6<sup>th</sup> instar females, prior to final ecdysis, results in 100% short-wing morphology, relative to an approximately 1:1 ratio of long- to short-wing morphology in the control (Tanaka, 1985). Additionally, removal of the wings

after ecdysis has been completed by adult females results in the breakdown of wing muscles and an increase in oocyte development (Tanaka, 1986b). Therefore, the natural removal of wings that has been observed in *Allonemobius* (e.g., Roff, 1984) may be an adaptive behavior that stimulates egg production once dispersion is complete. Wing morphology has been shown to be heritable (Mousseau and Roff, 1989b; Roff and Bradford, 1996; Roff and Bradford, 1998), to vary significantly between populations (Mousseau and Roff, 1989b), and to be influenced by environmental experienced during development (Olvido et al., 2003). Thus, *Allonemobius* are a model system for studying the progression of wing development, environmental and genetic factors that affect wing development, and the well-known oogenesis-flight syndrome (Mole and Zera, 1993) that represents a trade-off between dispersal ability and fertility.

#### *1.5.5 Life-history strategies*

Diapause is a very important stage of insect life histories, and crickets of the genus *Allonemobius* have been the subject of numerous studies on factors that affect diapause occurrence. Diapause incidence is clinal in crickets of the *Allonemobius fasciatus* – *A. socius* complex, as northern populations produce very high proportions of diapause eggs and are univoltine while southern populations produce fewer diapausing eggs and are bivoltine or multivoltine (Howard and Furth, 1986; Mousseau and Roff, 1989a). Adult body size (Mousseau and Roff, 1989a) and maternal age effects on diapause (Mousseau, 1991) also vary along a north-south cline in *Allonemobius*. Therefore, diapause incidence in these species appears to have a genetic basis, but may

also be influenced by environmental factors such as temperature and/or photoperiod, leading to the establishment of *Allonemobius* as a model system for studying diapause.

Tanaka (1984, 1992) studied temperature effects on diapause in *A. fasciatus* from Oregon, and found that low temperatures stimulated a “winter” diapause in which appendage formation was completed before diapause was initiated, while high temperatures initiated a “summer” diapause prior to the appendage formation stage. These two types of diapause are also linked to differences in water uptake at different temperatures (Tanaka, 1986c). In contrast to the two types of diapause observed in *A. fasciatus*, *A. socius* only entered diapause in early development, similar to “summer” diapause observed in *A. fasciatus* (Tanaka, 1986a). Additionally, some individuals of *A. socius* responded to long photoperiod and high temperature by developing directly, a characteristic not seen in *A. fasciatus* (Tanaka, 1986a). Overall, the numerous studies of Tanaka (1984, 1986a,c, 1987, 1992) indicate that an interaction between temperature and photoperiod is important in determining diapause incidence in *A. fasciatus* and at what stage in development diapause will occur.

The frequency of egg diapause in *A. socius* has also been demonstrated to have both a genetic and environmental basis. *Allonemobius socius* are univoltine in the northern part of their range, have a transition zone at about 37 to 35°N latitude, and are bivoltine south of 35°N (Mousseau and Roff, 1989a; Mousseau, 1991). Laboratory rearing experiments have shown that the variation observed in field populations is maintained in common-garden laboratory conditions, indicating a genetic basis for diapause occurrence in *A. socius* (Mousseau and Roff, 1989a; Bradford and Roff, 1995).

Indeed, diapause occurrence in *A. socius* has been recently demonstrated to be heritable (Roff and Bradford, 2000), as in at least one other cricket species (Bégin and Roff, 2002).

Additionally, diapause incidence typically increases with increasing maternal age; however, females from univoltine populations lay diapausing eggs almost exclusively, while females from bivoltine populations show increased diapause proportions during their lifetime, again supporting a genetic basis for diapause incidence (Mousseau, 1991, Bradford and Roff, 1997). In bivoltine populations of *A. socius*, the production of mixed batches of diapausing and nondiapausing eggs, combined with the gradual increase in the production of diapausing eggs, is viewed as a bet-hedging strategy for this species (Bradford and Roff, 1993, 1997).

The production of diapause eggs in *A. socius* is also affected by environmental influences such as temperature and photoperiod (Olvido et al., 1998; Roff and Bradford, 2000), as is the number of eggs laid (Olvido and Mousseau, 1998). For example, more eggs were laid by female *A. socius* under “summer” conditions than under “fall” conditions, but egg viability was higher for eggs reared in the “fall” environment than those reared in “summer” conditions (Olvido and Mousseau, 1998).

The incidence of embryonic diapause also appears to be under some control of the embryo as well as the mother, as a cross-fostering experiment resulted in different diapause proportions being produced when siblings were reared in different environmental conditions (Olvido et al., 1998). Specifically, eggs laid in “summer” conditions that were switched to “fall” conditions experienced higher diapause

proportions than their counterparts reared in “summer” conditions, and those eggs laid in “fall” conditions but switched to “summer” conditions experienced less diapause than their “fall”-reared siblings (Olvido et al., 1998). Moving eggs from 20 to 27°C was also found to terminate diapause in a small proportion of eggs laid by *A. fasciatus* (Tanaka, 1987, 1992). Therefore, the factors that lead to diapause initiation in *Allonemobius* appear to be a complex combination of genetic, maternal, and environmental influences. In summary, striped ground crickets of the *A. fasciatus*–*socius* complex are an ideal model organism for studying the evolution of life-history traits and many other aspects of evolutionary biology.

## 1.6 Areas of Exploration

This section will briefly describe the two experiments I conducted to examine genotype-by-environment interactions affecting fitness and development in the striped ground cricket, *Allonemobius socius*.

### *1.6.1 Allozyme allele clines in Allonemobius socius*

Allozyme allele frequencies at the locus isocitrate dehydrogenase (*Idh-1*) in the *A. fasciatus*–*socius* complex show apparent geographic structure (see Figure 2.1), yet this structure has never been explained or explored experimentally. I compiled long-term environmental data available from sites near collection localities for several dozen populations for which allele frequency data were available for this locus. Using these data, I looked for environmental correlates of allele frequencies in *A. socius* using regression modeling, and used the relationships found between allele frequencies and

temperature to set up hypotheses for testing in a laboratory setting. The results of the regression analyses and the results of this exploratory study are provided in Chapter 2.

### *1.6.2 Diapause induction in Allonemobius socius*

Striped ground crickets (*Allonemobius socius* and *A. fasciatus*) have been used as a model system to study environmental and genetic factors influencing the occurrence of embryonic diapause for about 2 decades. The majority of studies have focused on the effects of maternal photoperiod or the interaction between photoperiod and temperature in determining the proportion of diapausing eggs produced. Other studies have described patterns of voltinism in this complex. However, the effect of parental diapause history has not yet been studied as a main-order effect, yet is known to vary in field populations. Additionally, as temperature is typically studied in conjunction with photoperiod, studies examining temperature alone are lacking. I utilized a cross-fostering approach to examine the effects of parental diapause history, egg-laying temperature, and egg-incubation temperature in determining the proportion of diapause eggs produced, the results of which are discussed in Chapter 3.

## CHAPTER 2

### GEOGRAPHIC AND ENVIRONMENTAL CORRELATES OF ALLELE FREQUENCIES AT THE *IDH-1* LOCUS IN *ALLONEMOBIUS SOCIUS* WITH RESULTS FROM A PRELIMINARY FITNESS EXPERIMENT

#### 2.1 Abstract

Allozyme alleles in natural populations have been proposed as either neutral markers of genetic diversity or the product of natural selection on enzyme function, since amino acid substitutions that change electrophoretic mobility may also alter enzyme performance. Empirical studies examining effects on physiological performance or fitness resulting from possessing distinct allozyme alleles may support natural selection if differences result or neutral evolution if there are no significant effects of genotype. Here, I show that geographically-structured variation of the enzyme isocitrate dehydrogenase (*Idh-1*) exists in the striped ground cricket *Allonemobius socius*. The distributions of these alleles appear to be related to latitudinal and longitudinal gradients of temperature and moisture, respectively, as allele frequencies showed significant relationships with mean annual temperature and mean annual precipitation. Specifically, the slower mobility allele (1.8) was more frequent at colder temperatures, while the faster mobility alleles (2.0 and 2.2) were more frequent at warmer temperatures. An exploratory experiment was performed to examine fitness effects of possessing different *Idh-1* alleles at two temperatures (consistent with cooler

and warmer average summer temperatures) to test the hypothesis that the geographic structure of this locus may reflect environmental adaptation. Results showed a significant interaction between temperature and *Idh-1* genotype affected the number of eggs laid, as predicted from geographic structure. Individuals homozygous for the slower (1.8) allele laid more eggs at the cooler temperature while individuals homozygous for the faster (2.0 and 2.2) alleles laid more eggs at the warmer temperature. These results show that 1) variation in the frequency of *Idh-1* alleles is significantly related to moisture and temperature gradients in the eastern United States and 2) that alternative alleles appear to influence the egg-laying ability of individuals differently depending on environmental temperature. Taken together, my results suggest that natural selection is a plausible mechanism underlying the distribution of *Idh-1* alleles in this species, although more detailed studies are still needed. The results of this exploratory study also set up hypotheses for future tests of genotype-by-environment interactions.

## 2.2 Introduction

Variation in genetic diversity has been observed throughout the ranges of many species, but it is often impossible to determine if this variation resulted from adaptation to varying environmental conditions or neutral processes. For example, latitudinal clines in allozyme allele frequencies have been observed in taxa as diverse as mollusks, fish, insects, frogs, and plants (Powers et al., 1991; Berry and Kreitman, 1993; Riddoch, 1993), and appear to run counter to the assumption that allozyme allele differences represent neutral evolution within populations, an assumption which has been

questioned for decades (Powers et al., 1991; Kreitman and Akashi, 1995). Interestingly, some studies of natural populations have provided evidence for the action of natural selection on allozyme alleles (Berry and Kreitman, 1993; Storz and Nachman, 2003), while others have provided evidence that allozyme alleles may behave neutrally in populations regardless of environmental conditions (Jaramillo-Correa et al., 2001). Therefore, it would appear that the importance of natural selection in structuring allozyme variation depends on the locus, the organism, and the environmental context.

Empirical studies investigating fitness effects of possessing certain allozyme alleles in a range of environmental conditions can directly examine potential effects of selection on allozyme alleles. No fitness differences are expected if alleles are neutral, but are hypothesized if observed clines or correlations with environmental variables resulted from natural selection. Such experiments have revealed differences between particular alleles in growth and survival of pond frogs (Hotz and Semlitsch, 2000), a fitness differential in fruit flies (Ochando and Ayala, 1999), and fecundity differences in butterflies (Watt, 1992). These experiments, combined with latitudinal clines in allele frequencies, provide evidence that, in many cases, natural selection may act on allozyme alleles. Therefore, the hypothesis that allozyme allele clines seen in nature may have resulted from environmental selection pressures can be tested by measuring fitness of individuals possessing each allele combination at environmental conditions reflecting those observed along the cline in nature. One cline which lends itself to such a study is found in the striped ground cricket, *Allonemobius socius*.

In addition to being a model system within evolutionary biology, striped ground crickets (*Allonemobius fasciatus-socius* complex) show clines in various life-history traits (Mousseau and Roff, 1989a; Bradford and Roff, 1993, 1995) and morphological characteristics (e.g., ovipositor length; Mousseau and Roff, 1995; Roff and Mousseau, 1999) in response to latitudinal variation in temperature and seasonality, indicating possible adaptation to specific environmental cues (Bradford and Roff, 1993; Mousseau and Roff, 1995). Moreover, *A. socius* shows apparent geographic structure in allele frequencies of the enzyme isocitrate dehydrogenase (*Idh-1*; Figure 2.1), yet this variation has not been analyzed in the context of environmental gradients, such as a latitudinal temperature gradient.

To analyze this structure, I compiled mean annual temperature and precipitation from weather stations near collection sites of *A. socius* from which allele frequencies were estimated, and used regression models to look for significant relationships between allele frequencies and both environmental variables. If the observed structure in *Idh-1* alleles partially resulted from adaptation to temperature, then differences in relative fitness are expected among individuals possessing different alleles when tested in various thermal environments. In a preliminary attempt to examine this possibility, I conducted an exploratory study (*sensu* Jaeger and Halliday, 1998) to assess relative fitness of mating pairs homozygous for either the slow mobility allele (*Idh-1* 1.8) or the fast mobility alleles (2.0 and 2.2; Howard and Furth, 1986) in cool (22°C) and warm (27°C) temperatures; these temperatures were chosen to represent the range of average summer conditions experienced by this species. This exploratory study examines the

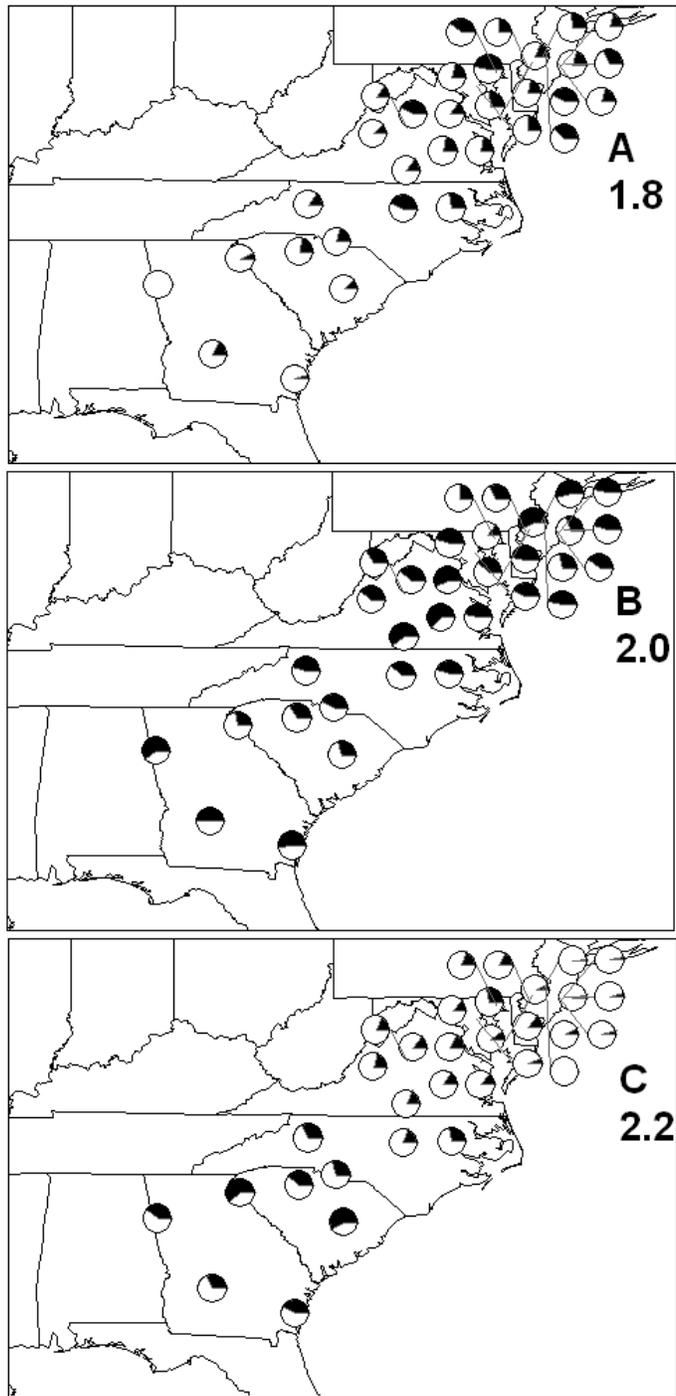


Figure 2.1 Geographic structure of the allozyme isocitrate dehydrogenase (*Idh-1*) in 32 southeastern populations of *Allonemobius socius*. Three alleles unique to *A. socius* are represented: 1.8 (A), 2.0 (B), and 2.2 (C). Data are from Howard (1982), Howard & Waring (1991), and unpublished data from D.J. Howard, J.L. Marshall, and D.L. Huestis; average *n* per location = 38.

hypothesis that a genotype-by-environment interaction (GxE) between different *Idh-1* alleles and temperature may affect fitness, thus providing a partial explanation for the distribution of *Idh-1* alleles while supplying hypotheses for further testing.

Overall, I found that 1) *Idh-1* allele frequencies are significantly related to temperature and moisture gradients in the eastern United States and 2) there is a significant GxE between *Idh-1* genotype and temperature, with trends indicating that individuals homozygous for the slow allele laid more eggs at cool temperatures while individuals homozygous for either fast allele laid more eggs at warm temperatures. These latter results mirror temperature trends of allele frequencies seen in natural populations, suggesting that natural selection may be a plausible explanation for the distribution of *Idh-1* alleles in this cricket species.

## 2.3 Materials and Methods

### *2.3.1 Study system*

Ground crickets in the *Allonemobius fasciatus-socius* complex are an ideal system for studying many aspects of biology. These crickets have a relatively short generation time of approximately 2 months, allowing several generations to be studied in a short period of time. Established protocols allow populations to be easily maintained in a laboratory setting (e.g. Mousseau and Roff, 1989a). In addition, numbers of eggs laid, number of hatchlings produced, and survivorship to adulthood can be quantified, allowing estimation of some components of fitness. This complex also displays apparent geographic variation in allele frequencies of *Idh-1* (Figure 2.1), making them well-suited to the study of allozyme allele-specific fitness.

### 2.3.2 Statistical analysis of allele frequency distributions

Allele frequencies of *Idh-1* and GPS locations for 32 populations of *A. socius* in the southeastern United States were tabulated from Howard (1982), Howard and Waring (1991), and unpublished data from D.J. Howard, J.L. Marshall, and D.L. Huestis. Data from particular locations were included in our analysis if greater than ten individuals were genotyped. This reduced the bias of small sample sizes and resulted in an average of 38 individuals being genotyped per location. Maps of allele frequencies for the three *Idh-1* alleles unique to *A. socius* (from slowest to fastest: 1.8, 2.0, and 2.2) were generated with ESRI's ArcMap 8.2 (2003; Figure 2.1). Relationships of allele frequencies with the positional variables latitude and longitude were examined with linear and nonlinear regression models to assess geographic structure. The best model (i.e., highest  $r^2$  and reduced MSE) was chosen for each allele-variable relationship, in order to demonstrate the kind of relationship allele frequencies have with these positional variables.

Although biological relationships with positional variables such as latitude and longitude can be insightful, they are typically a by-product of underlying environmental variables such as temperature and moisture. Therefore, I analyzed the relationship between allele frequencies and several environmental variables. Specifically, mean annual temperature, mean summer temperature (June-September), and mean annual precipitation (spanning a period of time from 1895-2004) were recorded for each collection site from the NOAA-CIRES Climate Division Plots available online (<http://www.cdc.noaa.gov/usclimate/USclimdivs.html>). Relationships between allele

frequencies and environmental data were examined by plotting allele frequency with mean annual temperature and mean annual precipitation at each locality separately for each of the three alleles. Once again, regression analyses were used to assess significant relationships.

### 2.3.3 Exploratory analysis of fitness effects

The hypothesis that allele frequency distributions are related to temperature was tested experimentally in a preliminary, exploratory study (*sensu* Jaeger and Halliday, 1998). In August 2003, I collected crickets from two locations (Charlestown, MD and Landenberg, PA) approximately 30 km apart at the northeastern part of the range of *A. socius*, where all 3 alleles are present (Figure 2.1). Crickets were transported back to the laboratory at the University of Texas at Arlington and kept in an incubator at 27°C. Juveniles from each location were separated into sex-specific cages as middle instars to prevent mating and raised to adulthood. These laboratory-reared adults were genotyped for *Idh-1* by removing one hind leg, homogenizing the tissue in deionized water, and performing horizontal starch gel electrophoresis using the buffer and staining systems of Howard and Furth (1986) for *Idh* in crickets of the genus *Allonemobius*. Individuals were also genotyped for malate dehydrogenase (*Mdh*) to ensure that they belonged to the *A. fasciatus-socius* complex (Howard and Furth, 1986). Once individuals were genotyped, mating cages were set up with homozygous individuals from each population to produce homozygous offspring for use in fitness trials.

Resulting offspring were maintained in sex-specific cages at 27°C until adulthood; pairs were established two weeks after completing the final molt (to assure

sexual maturity), and were randomly assigned to the cool (22°C) or warm (27°C) treatment. Temperatures were chosen based on the range of mean summer temperatures experienced by populations in the field; note that the faster the mobility of the allele, the higher in frequency that allele is at the warmer summer temperatures (i.e., ~ 27°C), while the reverse is the case at cooler summer temperatures (~ 22°C; Figure 2.2). Pairs were given 7 days to mate and females were permitted to oviposit on folded cheesecloth strips for an additional 7 days. The number of eggs produced in this two-week period were counted and used as a measure of pair fitness. All individuals were frozen at -80°C upon removal from mating cages. Females were dissected to determine if mating had occurred, confirmed by the presence of sperm in the spermatheca. Pairs in which one individual died before the end of the trial or in which females had not mated were excluded.

Given the analyses of geographic structure (see Results), I tested the prediction that individuals with different alleles would perform differently based on temperature treatment. Based on the direction of linear relationships between allele frequencies and average summer temperatures (Figure 2.2) and a preliminary examination of my data, data for 1.8 individuals from both populations were pooled to estimate GxE for the slow allele (S) and data from the 2.0 and 2.2 individuals were pooled to estimate GxE for the fast alleles (F) prior to analysis.

A two-way model I ANOVA was used to test for GxE with genotype (F or S), temperature (22 or 27°C), and their interaction as fixed factors. A Monte Carlo randomization with 1000 replicates was also performed on the data to estimate

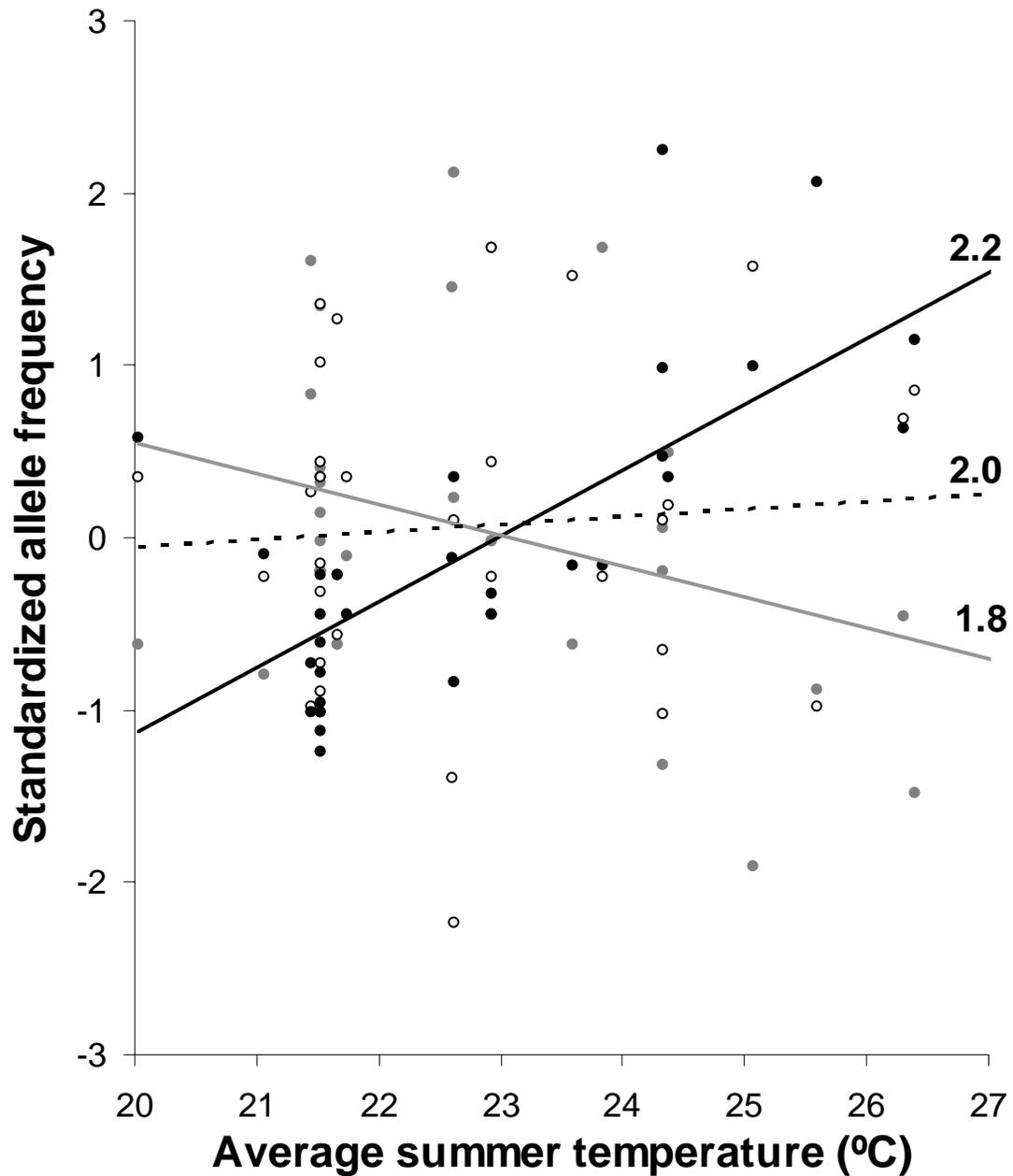


Figure 2.2 Relationship between *Idh-1* allele frequencies and average summer temperature. Allele frequencies were standardized prior to analysis. Summer temperatures were averages from June through September, the period during which *A. socius* are most active.

significance for each of these 3 factors. Analyses were conducted in SAS version 8.02 (SAS Institute, 2001), and all statistics were considered significant at  $\alpha = 0.05$ . The purpose of this exploratory study was to uncover trends and provide hypotheses for future, confirmatory experiments (sensu Jaeger and Halliday, 1998).

## 2.4 Results

### *2.4.1 Geographic and environmental correlates of Idh-1 allele frequencies*

The frequency of the 1.8 allele exhibited a positive linear relationship with latitude ( $F_{1,30} = 11.598$ ,  $P = 0.0019$ ; Figure 2.3A) while the 2.0 ( $F_{1,30} = 0.822$ ,  $P = 0.3718$ ; Figure 2.3B) and 2.2 ( $F_{1,30} = 65.029$ ,  $P < 0.0001$ ; Figure 2.3C) alleles exhibited negative, linear relationships with latitude, although the relationship involving 2.0 was non-significant. The frequency of the 1.8 allele exhibited a negative, linear relationship with longitude ( $F_{1,30} = 14.704$ ,  $P = 0.0006$ ; Figure 2.3D), while the 2.0 ( $F_{1,30} = 0.557$ ,  $P = 0.4613$ ; Figure 2.3E) and 2.2 alleles ( $F_{1,30} = 66.73$ ,  $P < 0.0001$ ; Figure 2.3F) exhibited positive, linear relationships with longitude, although the relationship with 2.0 was not significant. These results indicate that there is significant geographic structure to the distribution of these three *Idh-1* alleles in *A. socius*.

To test the hypothesis that this geographic structure is related to environmental variance associated with latitude and/or longitude, individual regression analyses between allele frequencies and mean annual temperature and precipitation were also conducted. The 1.8 allele showed a negative, curvilinear relationship with mean annual temperature ( $F_{2,29} = 3.805$ ,  $P = 0.0341$ ; Figure 2.4A) while the 2.0 ( $F_{1,30} = 0.415$ ,  $P =$

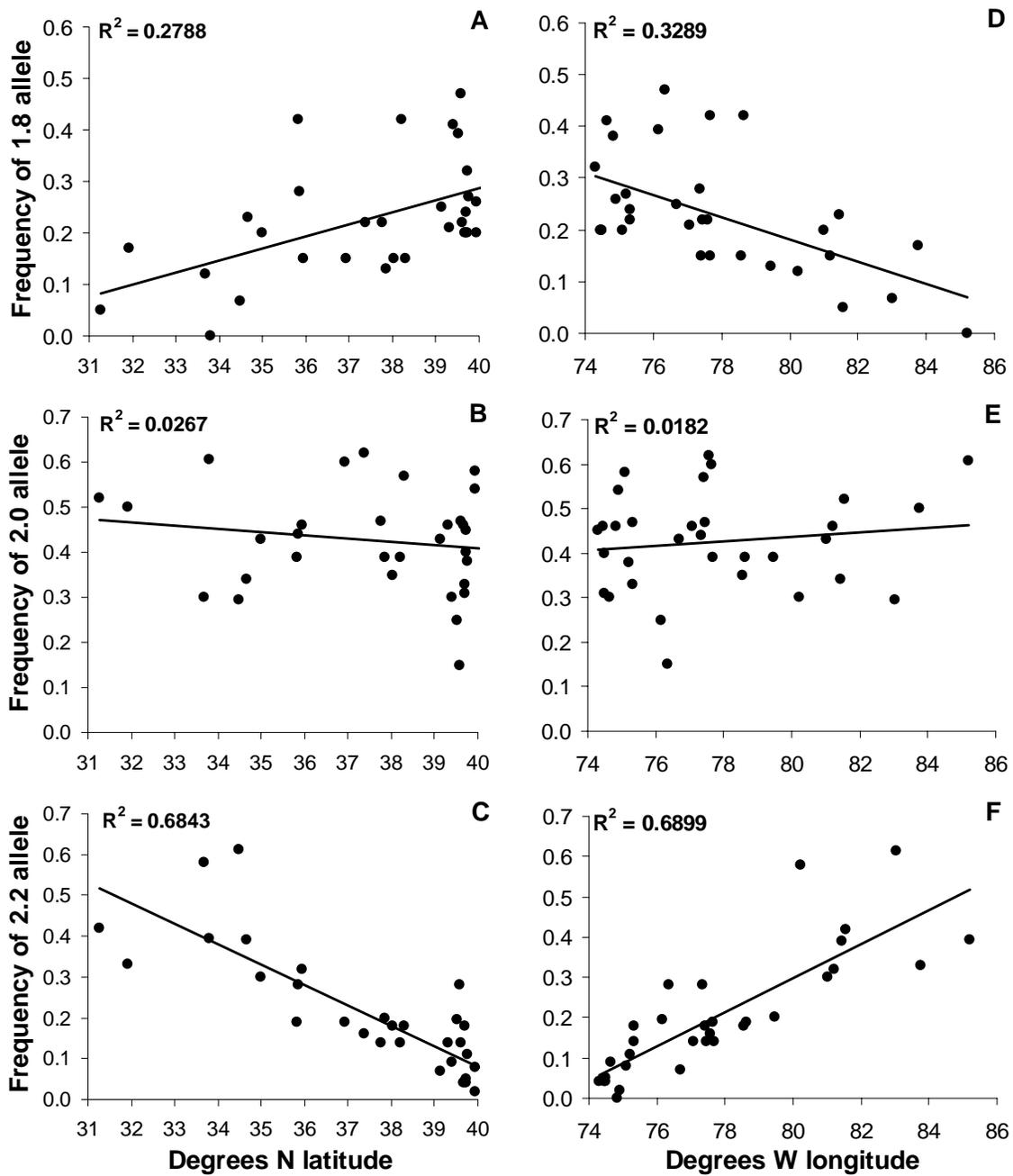


Figure 2.3 Latitudinal and longitudinal structure of *Idh-1* alleles in *Allonemobius socius*. Latitudinal structure of *Idh-1* 1.8 (A), 2.0 (B), and 2.2 (C) alleles. Longitudinal structure of *Idh-1* 1.8 (D), 2.0 (E), and 2.2 (F).

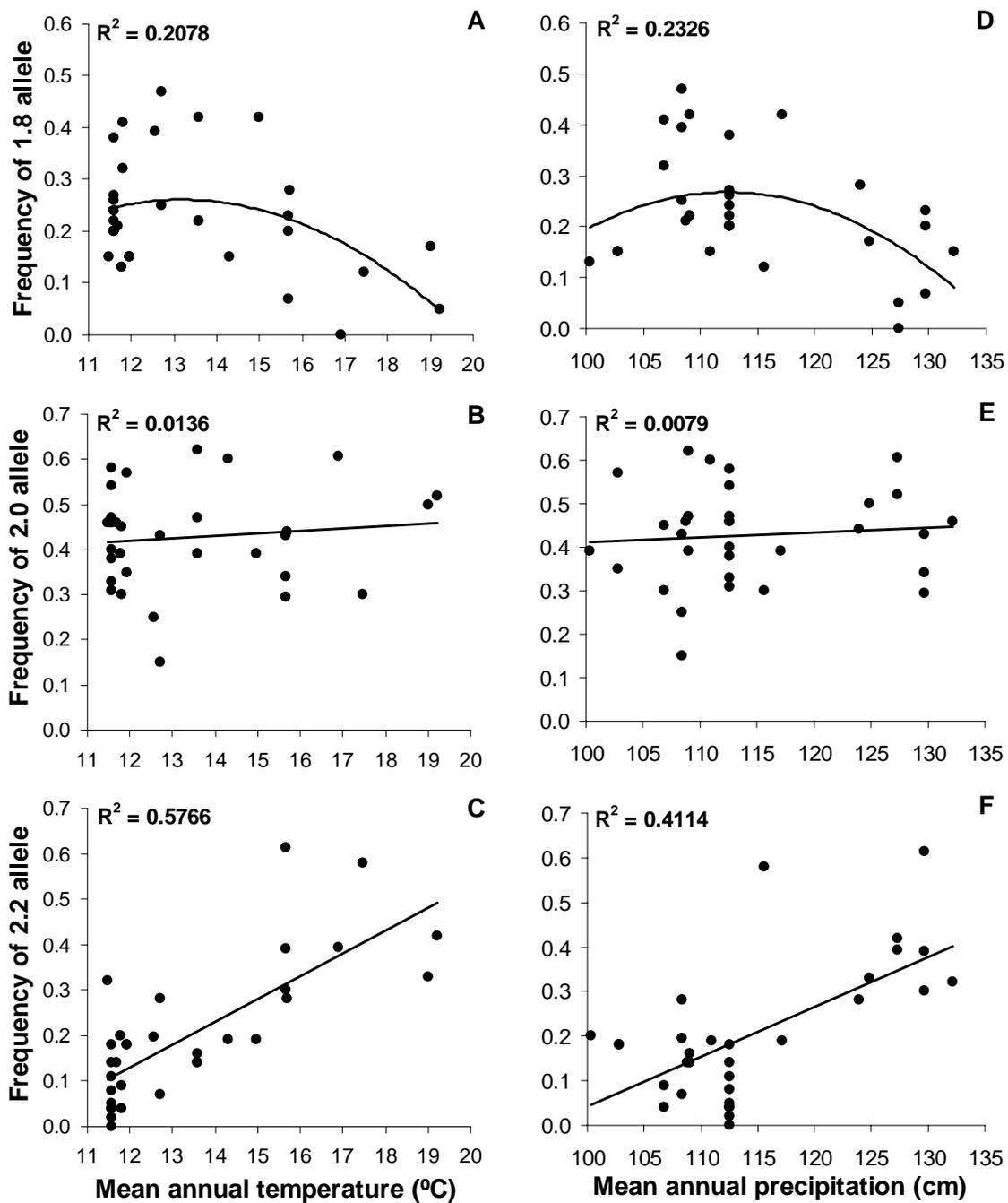


Figure 2.4 Relationships of *Idh-1* allele frequencies with mean annual temperature and mean annual precipitation. Relationship between mean annual temperature and allele frequency for *Idh-1* 1.8 (A), 2.0 (B), and 2.2 (C) is shown, along with the relationship between mean annual precipitation and allele frequency for *Idh-1* 1.8 (D), 2.0 (E), and 2.2 (F).

0.5246; Figure 2.4B) and 2.2 ( $F_{1,30} = 40.861$ ,  $P < 0.0001$ ; Figure 2.4C) alleles showed positive, linear relationships with mean annual temperature (although 2.0 was not significant). Frequencies of the 1.8 allele had a negative, curvilinear relationship with mean annual precipitation ( $F_{2,29} = 4.394$ ,  $P = 0.0215$ ; Figure 2.4D), while frequencies of the 2.0 ( $F_{1,30} = 0.240$ ,  $P = 0.6276$ ; Figure 2.4E) and 2.2 ( $F_{1,30} = 20.966$ ,  $P < 0.0001$ ; Figure 2.4F) alleles has positive, linear relationships with mean annual precipitation, although once again 2.0 was not significant. These results indicate *Idh-1* allele frequencies may depend on local environmental conditions, such as temperature and moisture. Given the effects of temperature, I undertook an exploratory study to test the hypothesis that individuals homozygous for different alleles of *Idh-1* would exhibit differential fitness across a range of temperatures.

#### 2.4.2 Exploratory analysis of *Idh-1* allele effects on fitness

After controlling for insemination (see Methods), 17 pairs of crickets across all genotype and temperature combinations were utilized. A significant interaction between *Idh-1* genotype and temperature was found, indicating a possible GxE interaction (Table 2.1). Females homozygous for the slow allele laid more eggs at 22 than 27°C, while individuals homozygous for the fast alleles laid more eggs at 27 than 22°C (Figure 2.5). The interaction between genotype and temperature was significant based on both standard and Monte Carlo analyses (Table 2.1).

Table 2.1 Full model ANOVA with temperature and *Idh-1* genotype as independent variables and number of eggs laid as dependent variable.  $P_{\text{ran}}$  measured with a Monte Carlo randomization with 1000 replicates.

Source	d.f.	type III SS	MS	<i>F</i>	<i>P</i>	$P_{\text{ran}}$
Genotype	1	4470.453	4470.453	1.735	0.2105	0.3280
Temperature	1	77.808	77.808	0.030	0.8647	0.8760
Genotype x Temperature	1	36936.904	36936.904	14.333	0.0023	< 0.001
Error	13	33500.967	2576.997			

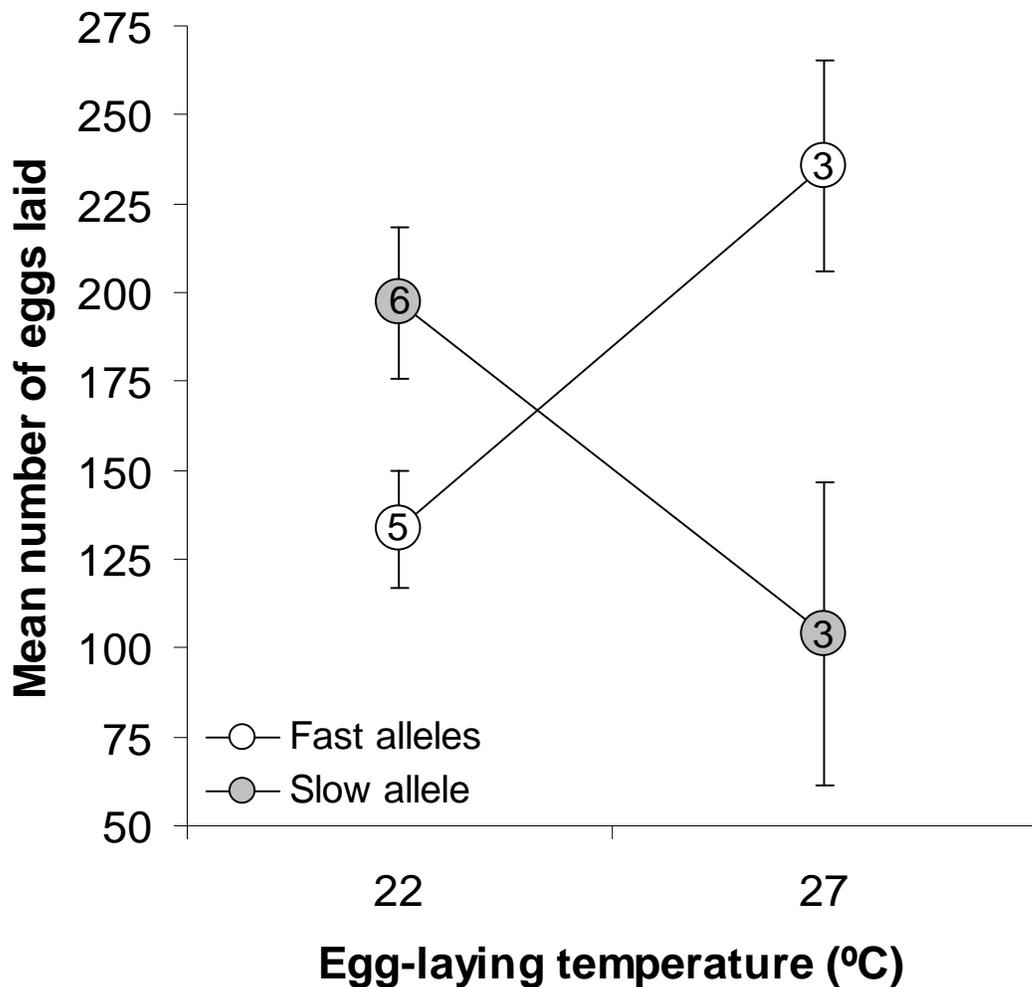


Figure 2.5 Genotype-by-temperature effects of *Idh-1* allele (F and S) at 2 temperatures (22 and 27°C) on the number of eggs laid by *Allonemobius socius*. Means are given  $\pm 1$  standard error. Sample sizes are given inside of data points.

## 2.5 Discussion

### *2.5.1 Geographic and environmental effects on Idh-1 allele frequencies*

I observed that the three *Idh-1* alleles unique to *A. socius* exhibited geographic structure, with the highest frequency of the 1.8 allele being at northern latitudes and eastern longitudes, while the highest frequencies of the 2.2 allele are found at southern latitudes and western longitudes (Figure 2.3). Since latitude and longitude are simply positional variables and in-and-of-themselves not biologically meaningful, I also examined relationships between allele frequencies and both mean annual temperature and mean annual precipitation; as these environmental variables typically covary with positional variables like latitude and longitude. Frequencies of the slow allele (1.8) showed negative relationships with both mean annual temperature and mean annual precipitation, while frequencies of the fast allele (2.2) showed positive relationships with temperature and precipitation (Figure 2.4). Although the relationships between the frequencies of the 2.0 allele and all geographic and environmental variables are weak, the directionality of these relationships parallel that of the 2.2 allele, indicating that the 2.0 allele may act more like 2.2 than 1.8 (Figures 2.2-2.4). Thus, frequencies of the slow allele increases with decreasing temperature and precipitation while frequencies of the fast allele increase with increasing temperature and precipitation (Figure 2.4). These findings led to the hypothesis that the geographic structure of *Idh-1* alleles may have resulted from natural selection via local environmental conditions such as temperature and precipitation.

Interestingly, my results show that the slope of the relationship between allele frequency and geographic/environmental variables changes in accordance with the electrophoretic mobility of the allele. For example, the slope of the line between average annual temperature and allele frequency changes from positive for the fastest allele (2.2), to  $\sim$  zero for the intermediate allele (2.0), to negative for the slowest allele (1.8). This consistent pattern may reflect the increased “fitness” of successively more or less mobile alleles in particular environmental conditions or the operation of strong selection on extreme mobility alleles with the intermediate allele being relatively neutral. At this point, it is not possible to distinguish between these and other alternatives, although they certainly could be addressed with future studies.

Overall, my results are consistent with other studies which have identified latitudinal relationships in allele frequencies for various enzyme loci (e.g. Kreitman and Akashi, 1995; Storz and Nachman, 2003). Clines in allele frequencies may also be correlated with environmental conditions such as temperature, as is the case with alleles of lactate dehydrogenase (*Ldh*) in fish (Powers et al., 1991; Shulte, 2001). Additionally, allele frequencies may be tied to environmental conditions such as stress. For example, a literature survey of dozens of organisms has suggested strong selection on the *Pgi* locus, such that the fast allele is often associated with stressful conditions (Riddoch, 1993). Overall, this correlative approach shows that selection is possibly acting, but does not directly measure fitness of individuals possessing certain alleles under different conditions. Therefore, in our exploratory experiment, we tested the hypothesis that individuals with the slow allele would lay more eggs than individuals with other

alleles at cooler temperatures and that individuals with fast alleles would lay more eggs than individuals with the slow allele at warm temperatures.

### 2.5.2 Exploratory analysis of *Idh-1* allele effects on fitness

In this exploratory study, a significant interaction between *Idh-1* genotype and temperature affected fitness in the cricket *A. socius* (Table 2.1). This significant interaction indicates that individuals possessing certain genotypes may have higher fitness than others at different temperatures. Specifically, as predicted based on geographic distributions, individuals homozygous for the slow *Idh-1* allele laid more eggs at 22 than 27°C, while individuals homozygous for the faster *Idh-1* alleles laid the more eggs at 27 than 22°C (Figure 2.5). These results support the hypothesis that geographic variation in allele frequencies at the *Idh-1* locus may be maintained by selection due to variation in environmental factors such as temperature. However, this is an exploratory analysis and the results should be considered preliminary until confirmed with further experiments. In particular, future studies should not only examine these allele-temperature effects more definitively, but also examine fitness differences of individuals homozygous for different *Idh-1* alleles at varying humidity or moisture levels, since mean annual precipitation also correlates with *Idh-1* allele frequencies (Figure 2.4D-F).

### 2.5.3 Hypotheses for future experiments

Other modes of selection may also be acting to maintain polymorphism in this system and could be tested in future experiments. For example, balancing selection has been hypothesized to maintain polymorphisms of *Pgi* in crickets of the genus *Gryllus*

(Katz and Harrison, 1997; Broughton and Harrison, 2003), and could potentially be acting on this system. The effects of possessing certain allozyme alleles may vary throughout the life-cycle of an individual, leading to the maintenance of multiple alleles within a population. Genotype-specific performance at different life-history stages has not been examined in *Allonemobius*, but could be explored in future studies. Thus, genotype differences may affect performance at other periods during a cricket's life-cycle, such as the juvenile growth period, and could impact adult fitness. Measuring egg and juvenile survival and growth rate of individuals with different allozyme alleles for *Idh-1* could potentially help clarify the processes that shape the geographic distribution of *Idh-1* alleles (Figure 2.1).

Additionally, heterozygotes sometimes have higher fitness than either homozygote, due to heterozygote advantage (Ochando and Ayala, 1999; Hotz and Semlitsch, 2000). These individuals may be adapted to a wide variety of environmental conditions, as they can produce two or more types of enzyme, while homozygotes only produce one. Thus, if one allele performs better under warm conditions, and another under cool conditions, a heterozygote will produce enzymes that function well in a range of conditions, while enzymes produced by a homozygote will function best at one condition. In the case of *Idh*, proteins are formed as dimers, meaning that a heterozygote produces three forms of the protein, in a 1:2:1 ratio (Murphy et al., 1996). Therefore, if one allele performs better in warm conditions, and the other performs better in cooler conditions, a heterozygote may be able to function well over a wider range of temperatures than either homozygote, conferring advantages to the

heterozygote in an unstable habitat where temperatures change often. Given that individuals homozygous for the slow allele (1.8) had higher fitness at cool temperatures and individuals homozygous for faster alleles had higher fitness at warm temperatures, a heterozygote with the 1.8 allele and one of the other alleles (2.0 or 2.2) may have high fitness over a wide range of temperatures, conferring an advantage on such heterozygotes. This hypothesis could be tested by measuring the fitness of individuals with 1.8/2.0 and 1.8/2.2 genotypes across a range of temperatures and comparing these results to those of homozygotes at the same temperatures.

#### *2.5.4 Conclusions*

In conclusion, I demonstrated that the frequency of *Idh-1* alleles in *A. socius* exhibit significant geographic structure, likely related to the environmental variables of temperature and moisture. Moreover, I found a significant genotype-by-temperature interaction that affected fitness, supporting the hypothesis that the observed structure may be due to natural selection imposed by varying environmental conditions. Crickets that were homozygous for the slow *Idh-1* allele laid more eggs at 22°C than crickets with faster alleles, while pairs homozygous for the faster alleles laid more eggs at 27°C (although our findings should be examined further in a confirmatory experiment using a larger number of individuals). Furthermore, tests could be conducted to examine the effects of allozyme allele differences on different phases of the life-cycle, including diapause occurrence and/or growth and survival of juveniles. Additionally, as heterozygotes sometimes have an advantage over either homozygous form, heterozygotes could also be tested to examine the possibility of heterozygote advantage

in this system. Thus, my results indicate that natural selection may be acting on the *Idh-1* locus in *A. socius*, but further tests of this hypothesis should be done to isolate how and when in the life-cycle selection may act.

CHAPTER 3  
INTERACTION BETWEEN MATERNAL EFFECTS AND TEMPERATURE  
AFFECTS DIAPAUSE OCCURRENCE IN THE CRICKET  
ALLONEMOBIUS SOCIUS

3.1 Abstract

The induction of diapause can be adaptive for egg survival during unfavorable conditions, while direct-development can be advantageous under favorable conditions by allowing additional generations to exploit abundant resources. Therefore, the physiological capability of a female to respond to environmental cues indicative of habitat quality by producing eggs of the appropriate developmental phenotype should be under strong selection. Additionally, developing embryos may alter the developmental trajectory initiated by the female in response to changing environmental conditions. In this study, I used a cross-fostering approach to isolate the maternal effects of parental diapause history (not previously studied) and egg-laying temperature from the influence of the incubation environment experienced by the developing embryo on the proportion of diapause eggs produced. I found that an interaction between egg-incubation temperature and parental diapause history strongly affected the proportion of diapause eggs produced and the proportion of eggs that hatched within a 16-18 day incubation period, while egg-laying temperature and all other interactions did not. These novel results indicate that embryos can respond directly to the environmental

conditions they experience during development, but that their ability to do so is influenced by maternal effects such as parental diapause history. The results of this study not only provide evidence, for the first time, of parental diapause history affecting diapause proportions, but also raise additional questions about the mechanism by which environmental information is transmitted from parent to offspring and how offspring are able to respond to conditions experienced during their own development.

### 3.2 Introduction

Traditionally, the phenotype of an organism is thought to result from a combination of its genetic makeup and environmental influences during development (Mousseau and Fox, 1998). For example, adult body size typically has a genetic basis, but varying levels of resource availability may alter the expression of that genotype. Different genotypes often result in a range of phenotypes, but a given genotype can also result in varying phenotypes, depending on the environment in which individuals are raised (Via and Lande, 1985; Schlichting and Pigliucci, 1998). This phenomenon is termed genotype-by-environment interaction (GxE) and these interactions result in a reaction norm between specific genotypes and environmental conditions (Via and Lande, 1985; Stearns, 1992; Schlichting and Pigliucci, 1998; Roff, 2002).

GxE interactions allow individuals to respond to the environment they experience by developing particular phenotypic traits that will be advantageous under those specific environmental conditions. Examples of phenotypic plasticity include changes in tadpole morphology in response to predator presence (LaFiandra and Babbitt, 2004), insect wing development influenced by temperature, photoperiod, or

crowding (Olvido et al., 2003), and sex determination in certain coral reef fish in response to the environment and/or presence of conspecifics (Warner et al., 1975; Hobbs et al., 2004). Egg diapause in response to environmental conditions is an important plastic trait in insects, because the embryo can direct-develop under favorable conditions or diapause under unfavorable conditions. Diapause is thought to be under a combination of environmental and maternal control in many insect species (Mousseau and Dingle, 1991; Olvido et al., 1998; Hockham et al., 2001).

Maternal effects occur when a female alters developmental characteristics of her offspring, often in response to some aspect of the environment she has experienced during her lifetime (Mousseau, 1991; Mousseau and Dingle, 1991; Mousseau and Fox, 1998). As a result, maternal effects allow a female to perceive environmental conditions, pass her experience to her offspring in a non-Mendelian way, and alter some aspect of her offspring's development without changing their genetic makeup, a mechanism that has been termed "adaptive transgenerational phenotypic plasticity" (Mousseau and Fox, 1998, p. 403). Maternal effects are themselves under selection, since the ability to alter offspring phenotype to match the environmental conditions they will experience is advantageous; the strength and accuracy of this mechanism is also subject to selection (Via and Lande, 1985; Kirkpatrick and Lande, 1989; Mousseau and Fox, 1998; Wolf et al., 1998). Environmentally-induced maternal effects have been found in dozens of insect species (Mousseau and Fox, 1998), making insects an ideal model system for studying environmental and maternal effects on development.

Diapause is an important life-history trait in insects that is plastic in response to the environment, and which can occur in the egg, larval, or adult stage of an insect's life-cycle (Mousseau and Dingle, 1991). Egg diapause is characterized by a break in development during which the embryo ceases to develop, and instead enters a suspended developmental stage (Danks, 1987; Olvido et al., 1998); diapause is terminated by environmental conditions favoring development. Egg diapause can be adaptive because it protects the embryo from harsh conditions (cold in winter, or dryness during a drought), but can be broken under more favorable environmental conditions (Danks, 1987; Philippi and Seger, 1989; Mousseau and Dingle, 1991; Mousseau and Fox, 1998; Hockham et al., 2001). This delay in development may be initiated by the female or by the embryo in response to the environment. Environmental cues can be gathered from a range of sources, and in a literature survey by Rossiter (1996), diapause was found to be most often influenced by temperature, photoperiod, or both. Photoperiod is thought to be the most influential factor in diapause initiation, as it provides the most consistent cue to seasonal changes at most latitudes (Mousseau and Dingle, 1991; McWatters and Saunders, 1998; Mousseau and Fox, 1998); temperature and moisture may also be important for many species (Rossiter, 1996). Thus, the initiation and maintenance of egg diapause often depends on environmental influences on the mother, but may also be under embryonic control (Mousseau and Dingle, 1991; Hockham et al., 2001). Additionally, because maternal effects are strongest early in life (Wolf et al., 1998), the occurrence of egg diapause or direct-development represents an ideal phenotype for studying maternal effects.

Ground crickets (*Allonemobius socius* and *A. fasciatus*) have been used as a model system for studying egg diapause, including studies on what environmental factors affect diapause in these species (e.g., Bradford and Roff, 1993; Olvido et al., 1998) and when in embryonic development diapause occurs (Tanaka, 1984, 1986a). However, the majority of studies have focused on photoperiod alone, or altered photoperiod and temperature together when attempting to dissect the nature of diapause initiation via environmental influences on the female. A design altering photoperiod and temperature simultaneously, while providing an accurate representation of conditions in the field, does not allow the researcher to separate out the individual effects of photoperiod and temperature. More importantly, to my knowledge, the effects of parental diapause history on maternal effects have not been evaluated on individuals from the same population, as diapause history is usually examined by using different species or different populations (Tanaka, 1986a; Mousseau and Roff, 1989a). However, in the field, crickets may have gone through diapause or developed from non-diapause eggs, and environmental effects may influence these individuals differently because of their diapause history. Therefore, I tested the influences of parental diapause history as a main-order effect in this study.

When indirect genetic effects are present, as is the case with maternal effects on development, the development of the offspring phenotype will depend on a combination of the genes of the offspring, the environment experienced by the offspring, and those indirect effects contributed by the parents (Wolf et al., 1998), making it difficult to isolate the effects of one factor alone. In this experiment, I sought to isolate the effects

of temperature and parental diapause history on the initiation of egg diapause in the striped ground cricket, *Allonemobius socius*. Using a split-brood design similar to that of Olvido et al. (1998), I simultaneously examined the effects of egg-laying temperature, egg-incubation temperature, and parental diapause history on the proportion of diapause eggs produced by individual pairs of crickets. This design allows for maternal and embryonic influences to be measured independently, and isolates the effects of temperature on diapause initiation. I found that an interaction between parental diapause history and egg-incubation temperature significantly affected the proportion of diapause eggs produced, indicating that diapause is influenced by both maternal and environmental factors in this species.

### 3.3 Materials and Methods

#### *3.3.1 Study system*

Striped ground crickets (*Allonemobius socius*) are a model system within evolutionary biology. This group shows clines in morphological characteristics (Mousseau and Roff, 1995; Roff and Mousseau, 1999; Olvido et al., 2003) and life-history traits (Mousseau and Roff, 1989a; Bradford and Roff, 1993, 1995; Roff and Bradford, 1996) in response to latitudinal variation in temperature and seasonality, indicating adaptation to specific environmental cues (Bradford and Roff, 1993; Mousseau and Roff, 1995). These crickets have a relatively short generation time of approximately 2 months, allowing several generations to be studied in a short period of time. Established protocols (e.g. Mousseau and Roff, 1989a) allow populations to be easily maintained in a laboratory setting, and eggs can be counted and scored for

diapause. Thus, *Allonemobius* are an ideal system for studying the relative contributions of maternal and environmental effects on diapause occurrence.

### 3.3.2 Experimental methods

Adult and juvenile crickets were collected from a wet, grassy field at a roadside picnic area in Charlestown, Maryland in August 2003. Populations in this area are presumably univoltine, as they occur north of the transition zone between univoltine and bivoltine populations of *A. socius* identified by Mousseau and Roff (1989a). All individuals were transported to the laboratory at the University of Texas at Arlington and maintained in an incubator at 27°C with a 14:10 L:D photoperiod. Wild-caught adults were allowed to mate and oviposit immediately, as it was assumed that mating had also occurred in the field prior to collection. Juveniles were separated into sex-specific cages as middle instars to prevent mating, and were screened for malate dehydrogenase (*Mdh*) and isocitrate dehydrogenase (*Idh*), following the methods of Howard and Furth (1986), as adults to ensure that they belonged to the correct species of *Allonemobius* before mating (as several species of *Allonemobius* occur in this region; Howard, 1983; Howard and Furth, 1986). Wild-caught adults were screened for these allozymes after mating, and all were found to possess *A. socius* alleles. Crickets were provided with a dirt-filled Petri dish for oviposition. Male crickets were frozen after allowing one week for mating, and females were given one additional week to oviposit.

The resulting egg dishes were maintained in an incubator at 27°C for several months. Dishes which produced few hatchlings during this time were refrigerated at 4°C to simulate winter conditions, and were transferred back to 27°C after 3 months.

Diapause and non-diapause hatchlings produced were reared separately in small boxes, and separated into same-sex cages as middle instars to prevent mating. Juvenile cages were maintained three times per week, and adults removed once per week.

Pairs were established with individuals of the same diapause history (hereafter called the parental diapause history treatment) approximately one week after the final molt. Wing morphology of each individual (micropterous or macropterous) was noted as pairs were established, as wing morph can affect fecundity in cricket species (Roff et al., 1997; Tanaka and Suzuki, 1998), including *A. socius* (Roff and Bradford, 1996). Mating pairs were randomly assigned to either the hot (32°C) or cold (22°C) treatment (hereafter called the egg-laying temperature treatment), and were left in the assigned treatment for the duration of the experiment; final sample sizes are given in Figure 3.1A (total n = 81). These pairs were provided with rolled cheesecloth strips for oviposition, and cheesecloth strips were removed every 7 days. Eggs were collected each week for 4 weeks, and pairs were frozen at the end of this four-week period.

Immediately after cheesecloth was removed, it was examined for the presence of eggs. If eggs were present, the cheesecloth was cut so that approximately half of the eggs laid were on each resulting piece. One piece was then randomly assigned to either the hot (32°C) or cold (22°C) treatment for incubation, and the other half was placed in the alternate condition (hereafter called the egg-incubation treatment). Cheesecloth strips with eggs were maintained in the assigned treatment for a period of 2 weeks, and hatchlings, if present, were removed and counted. Eggs were counted and scored individually for diapause 16-18 days after being initially collected from the mating pair.

Direct-developing eggs were identified by the presence of eyespots, while eggs that appeared healthy but lacked eyespots were assumed to be in diapause. Rotten eggs, easily identified by the presence of fungus, were counted but not included in the analyses; the total number of healthy eggs examined was 12,614. For each batch, the proportion of diapausing eggs was calculated by dividing the number of healthy eggs lacking eyespots by the total number of eggs observed, including those which produced hatchlings. This design allowed us to test the effects on diapause occurrence from three factors: parental diapause history (diapausing or direct-developing), egg-laying temperature (22 or 32°C), and egg-incubation temperature (22 or 32°C).

### *3.3.3 Statistical methods*

For each female, the total number of eggs incubated in each environment were summed across all 4 weeks, as the proportion of diapausing eggs produced varies little with age in univoltine populations (Mousseau, 1991), resulting in a total number of eggs incubated at both 22°C and 32°C. Mean proportion of diapause eggs at each incubation temperature for each female was calculated by dividing the number of diapausing eggs by the total number of eggs incubated at that temperature. Mean proportions of hatched eggs (in the 2-week incubation period) were calculated using the same method. The average number of eggs produced each week was calculated for each female by dividing the total number of eggs produced by the number of weeks each female remained alive.

The calculated proportions for each female were used in a repeated-measures analysis of variance (ANOVA) using maternal diapause history, egg-laying

temperature, and egg-incubation temperature as fixed factors. Maternal diapause history, laying temperature, and their interaction were compared between females, while incubation temperature and all other interactions were within-female factors. Transforming the proportional data with an arcsine square-root transformation did not affect the results; therefore, the results from the raw data are presented.

Effects of maternal wing morphology, paternal wing morphology, egg-laying temperature, and parental diapause history on the average number of eggs produced per week were analyzed with ANOVA. Counts were square-root transformed prior to analyses. All interactions from these analyses were non-significant and were excluded from the final analysis. Lastly, the number of macropterous and micropterous individuals of each diapause history in the parental generation were compared between the sexes at each diapause history, and between diapause histories using chi-square tests. All statistics were considered significant at  $P < 0.05$ .

### 3.4 Results

Of the 121 replicates originally set up (> 25 per treatment), only 81 resulted in data which could be analyzed (e.g., survived long enough to lay eggs or lay more than a few eggs). From these data, we found that an interaction between maternal diapause history and egg-incubation temperature significantly affected diapause incidence (Table 3.1). Egg-laying temperature was non-significant, although  $P = 0.085$ , and all interaction effects involving egg-laying temperature were not significant (Table 3.1). When incubated at 22°C, eggs laid by females with different diapause histories experienced similar diapause proportions, but eggs incubated at 32°C experienced

Table 3.1 Repeated measures ANOVA on proportion of diapause eggs produced by female *Allonemobius socius* with maternal history, egg-laying temperature, and egg-incubation temperature as fixed factors. Asterisk indicates significant at  $P < 0.05$ .

Source	df	SS	MS	F	P
<i>Between females</i>					
Maternal history	1	0.05479	0.05479	4.07	0.0471*
Laying temperature	1	0.04097	0.04097	3.04	0.0850
History x laying temp.	1	0.00150	0.00150	0.11	0.7391
Error	77	1.03641	0.01346		
<i>Within females</i>					
Incubation temperature	1	0.20901	0.20901	22.38	<0.0001*
Incubation x history	1	0.05683	0.05683	6.08	0.0159*
Incubation x laying temp.	1	0.00290	0.00290	0.31	0.5789
Incubation x history x laying	1	0.00099	0.00099	0.11	0.7458
Error	77	0.71920	0.00934		

different diapause proportions depending on maternal diapause history (Figure 3.1A). Eggs laid by non-diapausing females that were incubated at 32°C had the lowest mean proportion of diapause incidence, while eggs laid by any female at 22°C and incubated at 22°C had the highest proportion of diapause incidence (Figure 3.1A).

The proportion of eggs that hatched in the 2-week incubation period was affected by the same factors as the proportion of diapause eggs produced (Table 3.2). As with the diapause proportion data, the proportion of eggs that hatched when incubated at 22°C is consistently low across all treatments, but the proportion of eggs that hatched at 32°C was much higher for offspring of non-diapause crickets (Figure 3.1B). The highest proportion of hatching eggs were laid by non-diapausing females and incubated at 32°C, while the lowest mean proportion of hatching eggs (essentially 0) were those incubated at 22°C (Figure 3.1B).

No difference between proportion of macropterous individuals were found between males and females that had gone through diapause ( $X^2 = 1.153$ ,  $df = 1$ ,  $P > 0.1$ ) or that were direct-developing ( $X^2 = 0.184$ ,  $df = 1$ ,  $P > 0.9$ ). Therefore, data from both sexes were pooled for comparison of wing morphology between diapause and non-diapause individuals. Direct-developing crickets were about 3 times more likely to be macropterous than diapausing crickets, and this difference was highly significant ( $X^2 = 10.645$ ,  $df = 1$ ,  $P < 0.005$ ).

Maternal wing morphology and parental diapause history significantly affected the average number of eggs laid per week, while egg-laying temperature and paternal wing morphology did not (Table 3.3). The interactions between all variables were non-

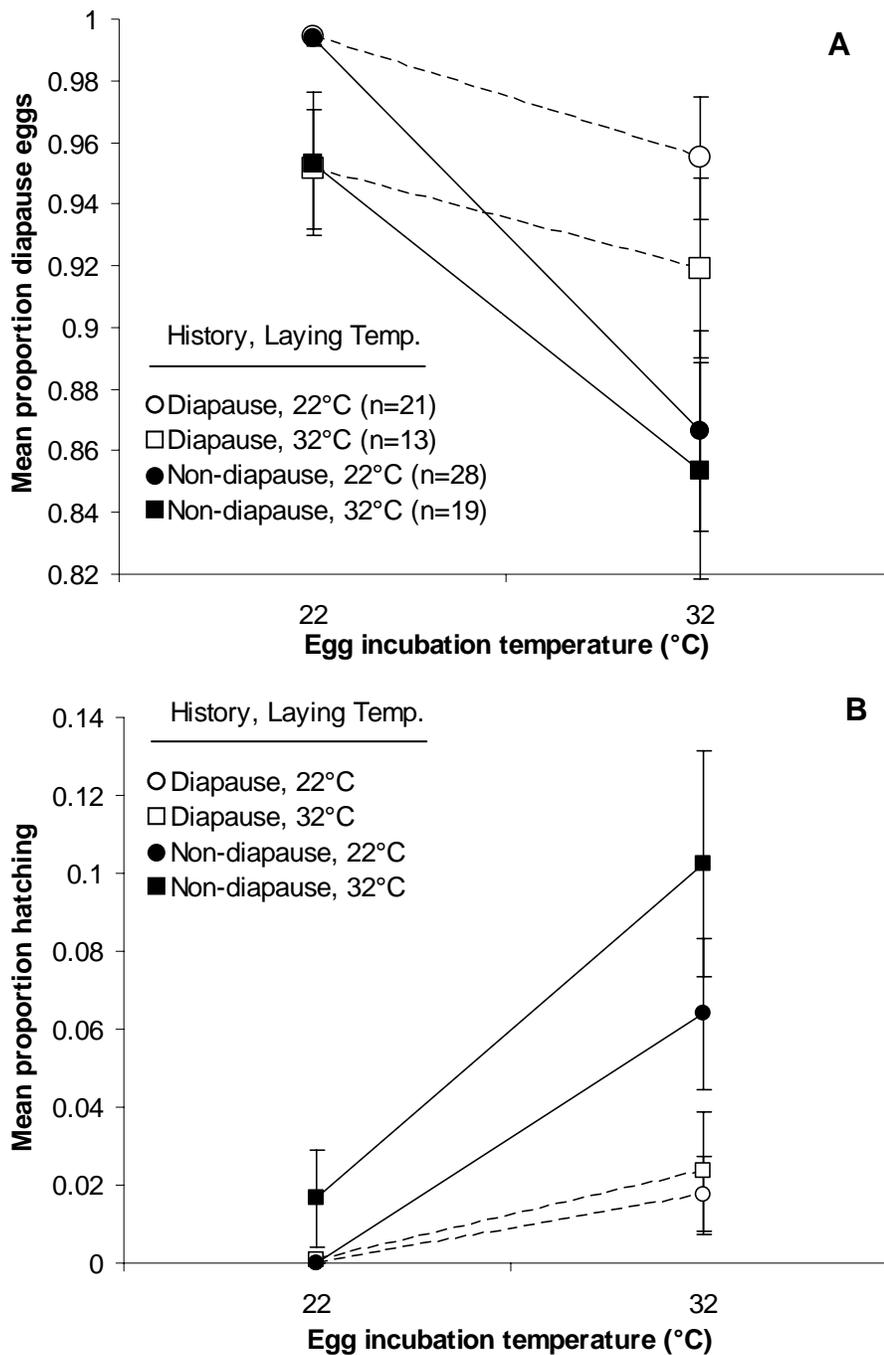


Figure 3.1 Effects of egg-laying temperature, egg-incubation temperature, and parental diapause history on A) proportion of diapause eggs produced and B) proportion of hatching eggs produced by pairs of *Allonemobius socius*. There was a significant interaction between egg-incubation temperature, and parental diapause history (Table 3.1). Means  $\pm$  1 standard error are shown.

Table 3.2 Repeated measures ANOVA on proportion of hatching eggs produced by female *Allonemobius socius* with maternal history, egg-laying temperature, and egg-incubation temperature as fixed factors. Asterisk indicates significant at  $P < 0.05$ .

Source	df	SS	MS	<i>F</i>	<i>P</i>
<i>Between females</i>					
Maternal history	1	0.04689	0.04689	9.13	0.0034*
Laying temperature	1	0.00902	0.00902	1.76	0.1888
History x laying temp.	1	0.00544	0.00544	1.06	0.3063
Error	77	0.39533	0.00513		
<i>Within females</i>					
Incubation temperature	1	0.08472	0.08472	21.47	<0.0001*
Incubation x history	1	0.02825	0.02825	7.16	0.0091*
Incubation x laying temp.	1	0.00173	0.00173	0.44	0.5096
Incubation x history x laying	1	0.00064	0.00064	0.16	0.688
Error	77	0.30376	0.00394		

Table 3.3 ANOVA on average number of eggs produced per week by female *A. socius* with maternal diapause history, parental wing morphology, and egg-laying temperature as fixed factors. Data were square-root transformed prior to analysis. All interactions were non-significant ( $P > 0.05$ ) and were removed from the model. Asterisk indicates significant at  $P < 0.05$ .

Source	df	SS	MS	<i>F</i>	<i>P</i>
Female wing morphology	1	24.07	24.07	5.73	0.0192*
Male wing morphology	1	0.75	0.75	0.18	0.6747
Parental diapause history	1	42.06	42.06	10.01	0.0022*
Egg-laying temperature	1	6.91	6.91	1.64	0.2037
Error	75	315.07	4.20		

significant ( $P > 0.05$ ) and were removed from the final analysis. Micropterous females laid more eggs per week than macropterous females, and non-diapause parents produced more eggs than parents who had gone through diapause, particularly when micropters are compared (Figure 3.2).

### 3.5 Discussion

#### *3.5.1 Environmental and maternal effects on development*

In the present study, I sought to isolate the effects of both maternal and environmental influences on diapause propensity in the striped ground cricket, *Allonemobius socius*. My cross-fostering design allowed for a comparison of three factors: parental diapause history (diapausing or direct-developing), egg-laying temperature (22 or 32°C), and egg incubation temperature (22 or 32°C). I found that an interaction between the maternal effects of parental diapause history and the direct influence of egg-incubation temperature affected diapause proportion in these crickets (Table 3.1). Offspring of parents that had gone through diapause were more likely to enter diapause than offspring of direct-developing parents at warm temperatures (32°C; Figure 3.1A). These results support the hypothesis that maternal background has a strong influence on offspring diapause propensities. This is corroborated by the finding that F<sub>1</sub> hybrids in studies of diapausing and non-diapausing strains most often resemble the diapause history of the female parent (Tanaka, 1986a; Mousseau and Dingle, 1991). Egg incubation temperature also affected the proportion of eggs which entered diapause through its interaction with parental diapause history (Table 3.1; Figure 3.1A), while the temperature the female experienced during egg-laying was non-significant as

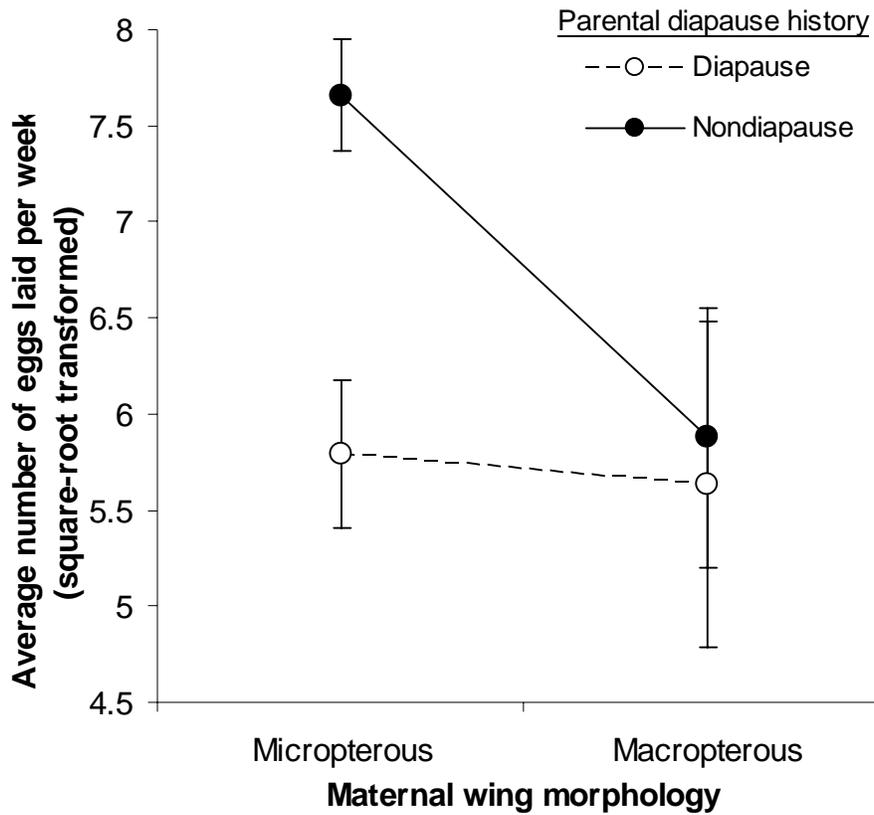


Figure 3.2 Effects of maternal wing morphology and parental diapause history on the average number of eggs produced by pairs of *Allonemobius socius*. Counts were square-root transformed prior to analysis, and transformed data are presented. Means  $\pm 1$  standard error are shown.

a two-tailed test (Table 3.1). However, as it is hypothesized that diapause proportions should be greater at cooler temperatures, these results would be significant if analyzed as a one-tailed test ( $P = 0.0425$ ). In either case, the effects of incubation temperature appear to be much stronger than the effects of egg-laying temperature in determining diapause occurrence in this study, similar to the results of Olvido et al. (1998) who used a similar split-brood design and determined that incubation temperature accounted for 6-fold more of the variance than did egg-laying temperature for a southern population of *A. socius*. The results of both this study and that of Olvido et al. (1998) are opposite that of Tanaka (1992) for *A. fasciatus*, who found that parental temperature was the most influential variable in determining offspring diapause proportions.

The effects of changing egg incubation temperatures are seen in the slopes of the lines in Figure 3.1A; if diapause initiation were solely under maternal control, the slopes would be zero and the proportion of diapause eggs produced would not change when eggs were cross-fostered in differing incubation environments. However, sloping lines indicate that a proportion of the eggs laid in 22°C but switched to 32°C for incubation were able to direct-develop instead of initiating diapause, and that some eggs laid at 32°C but moved to 22°C were able to initiate diapause instead of developing directly (Figure 3.1A). Thus, embryos may modify a maternal signal of diapause induction in response to the conditions they experience during incubation, a result found in other studies of *A. socius* (Tanaka, 1986a; Olvido et al., 1998), *A. fasciatus* (Tanaka, 1986a, 1987, 1992), and at least one other cricket species (*Dianemobius nigrofasciatus*;

Shiga and Numata, 1997). The degree to which embryos may modify a maternal diapause signal may depend on parental diapause history (see below).

A strong interaction effect between parental diapause history and egg incubation temperature indicates that the ability of an embryo to modify its phenotype in response to the environment (by initiating diapause or developing directly) may depend on the diapause history of its parents. The offspring of diapausing parents appear less able to respond to environmental differences than those offspring produced by direct-developing parents (Figure 3.1); thus the maternal effect of diapause initiation may be stronger from females that went through diapause than those that did not, as the slopes of the lines are much shallower for the offspring of diapausing females (Figure 3.1A). Overall, the greatest difference in diapause proportions between the offspring of diapausing and non-diapausing parents was observed in the hot environment, so it appears that the maternal effect (to enter diapause) may be overridden by the embryo under favorable (warm) environmental conditions, and the ability to alter maternal signaling is greater for the offspring of non-diapause females.

The proportion of eggs that hatched within the two-week incubation period followed similar trends to the proportion of diapause eggs produced. As with the proportion of diapausing eggs produced, a significant interaction between parental diapause history and egg incubation temperature influenced the proportion of hatching (rapidly direct-developing) eggs (Table 3.2). Offspring of non-diapause parents were more likely to develop rapidly, and rapid development was more likely to occur at 32°C than at 22°C (Figure 3.1B). The interaction effect again indicates that offspring of non-

diapause parents exhibit a more plastic response to temperature than do offspring of diapausing parents. The concordance of the results observed for the proportions of non-diapausing and hatching individuals indicates that *A. socius* do not experience a “summer diapause” after appendage formation similar to that observed in *A. fasciatus* (Tanaka, 1984), and that eyespots are a reliable indicator of direct-development in *A. socius*.

The result that offspring of non-diapausing parents are more likely to be direct-developing while offspring of diapausing parents are more likely to initiate diapause is opposite of the trends seen in bivoltine field populations. Throughout much of the range of *A. socius*, populations go through two generations per year, whereby the first generation hatches from overwintering, diapausing eggs in the spring, reaches adulthood in early to mid-summer, and lays primarily direct-developing eggs. The direct-developing eggs hatch quickly, develop to adulthood through late summer and early autumn, and lay diapausing eggs, which overwinter and hatch the next spring to form the first generation of the next year (Howard and Furth, 1986; Mousseau and Roff, 1989a). Under this scenario, offspring of diapausing parents (the first generation) will direct-develop to form the second generation, and offspring of these direct-developers will diapause to survive winter. Thus, the effects of parental history in bivoltine populations are likely to be overshadowed by the combined environmental effects of temperature and photoperiod. These results are similar to the findings of Mousseau (1991), who concluded that GxE interactions will mask the effects of increasing age on diapause in field populations. However, the results obtained in this study are reflected in

both univoltine and multivoltine populations of *A. socius*, in which offspring of diapausing individuals typically diapause and direct-development results in continuous generations throughout the year, respectively. Moreover, these results point to the ability of maternal effects and environmental conditions to switch patterns of voltinism, as individuals in univoltine populations could propagate multivoltine populations and vice-versa.

Overall, maternal effects, such as those seen here on the initiation of egg diapause, allow a female to modify the development and phenotype of her offspring in response environmental conditions. For example, females will often vary the phenotypes of their offspring via indirect genetic effects as a bet-hedging strategy, because the environment always varies over time with a certain degree of unpredictability (Philippi and Seger, 1989; Rossiter, 1996). Producing a range of phenotypes is a bet-hedging strategy because at least some individuals along the phenotypic continuum produced will be successful in future, uncertain conditions. The physiological capability to respond to environmental conditions allows for increased accuracy when producing diversified offspring, such that the largest phenotypic class produced is that which will be most advantageous in the future environmental conditions the offspring will experience, and variation around this ideal phenotype allows for environmental stochasticity. Thus, maternal effects which produce high-fitness offspring are highly adaptive, and maternal effects in general are therefore subject to selection (Kirkpatrick and Lande, 1989; Wolf et al., 1998).

### 3.5.2 Diapause history, wing morphology, and fecundity

In this study, we found that direct-developing individuals were 3 times more likely to be macropterous than were individuals that had gone through diapause. These findings are similar to the results obtained by Bégin and Roff (2002), who found that direct-developing *Gryllus veletis* were almost always macropterous while diapausing individuals were 95% micropterous. By contrast, a significant difference was found between the proportion of macropters when males and females were compared in *G. veletis* (Bégin and Roff, 2002), while no differences in macroptery were observed between the sexes in the present study of *A. socius*. In both the present experiment and that of Bégin and Roff (2002), the cricket populations used experience an obligate diapause in the field; thus, the results obtained in both experiments indicate potential phenotypic correlations between diapause and wing dimorphisms not attained in field populations.

Wing morphology and parental diapause history affected fecundity of pairs in this experiment. Micropterous individuals laid, on average, more eggs per week than macropterous individuals (Figure 3.2), a result common to other insect species. As macropterous individuals tend to be larger on average than micropterous *A. socius* (Mousseau and Roff, 1989a), increased fecundity of macropterous individuals would be predicted due to a correlated increase in body size. However, a trade-off between wing morphology and fecundity, particularly early during an individual's reproductive lifespan, has been reported for many cricket species (e.g., Roff et al., 1997; Tanaka and Suzuki, 1998), including *A. socius* (Roff and Bradford, 1996), and is likely the cause for

the results observed in the present study. Non-diapause individuals may face stronger pressures to lay more eggs early in life, as the second generation of a bivoltine population must lay eggs rapidly before the onset of winter.

### 3.5.3 Conclusions

Phenotypic development depends on an individual's genotype, environmental influences, and indirect genetic effects such as maternal effects. In this study, I demonstrated that the induction of egg diapause in the cricket *Allonemobius socius* is influenced by an interaction between egg-incubation environment and parental diapause history, such that the plasticity of an individual to respond to changes in temperature was reduced for the offspring of diapausing individuals relative to the offspring of non-diapausing individuals. These results emphasize the need to further study the mechanism by which diapause is initiated, its genetic basis, and the evolution of this trait, which is critically important for insect life histories.

## CHAPTER 4

### DISCUSSION

I studied environmental, maternal, and genetic factors that affect two key life-history traits in *Allonemobius socius*. Specifically, the number of eggs laid was dependent on a genotype-by-environment interaction between *Idh-1* alleles and temperature, while the proportion of eggs entering diapause was influenced by an interaction between parental diapause history and egg-incubation temperature. Both of these interactions have obvious implications for organismal fitness, which are discussed below.

#### 4.1 *Idh-1* Allele Frequencies in *Allonemobius socius*

##### *4.1.1 Major findings*

In this study, I found that there is strong geographic structure to frequencies of the three alleles of *Idh-1* that are unique to *Allonemobius socius* (Figure 2.1). The 1.8 allele is most prevalent in the north and along the east coast, while the 2.2 allele is found to the south and west; the 2.0 allele is less structured (Figure 2.3). Furthermore, the geographic patterns seen in the 1.8 and 2.2 alleles appear to be related to temperature and moisture gradients that vary with latitude and longitude, respectively (Figure 2.4). These results set up the hypothesis that the *Idh-1* allele frequencies observed in nature may be the result of natural selection on allele-specific performance at varying temperatures. Specifically, individuals homozygous for the 1.8 allele may

perform better at cooler temperatures while individuals homozygous for faster alleles (2.0 and 2.2) may perform better at warmer temperatures.

A preliminary, exploratory analysis supported the hypothesis that individuals homozygous for various types of alleles would perform differently depending on temperature. In this experiment, fitness was measured as the number of eggs laid in a 2-week period and was used as a measure of performance. Individuals homozygous for the slow allele (1.8) laid more eggs at cold temperatures than individuals homozygous for the faster alleles (2.0 and 2.2), while fast-allele individuals laid more eggs at warm temperatures than slow-allele individuals (Figure 2.5). These results supported the hypothesis that an interaction between *Idh-1* allele and temperature affects fitness in this species, and may also support the hypothesis that allele frequency clines seen in natural populations are the result of natural selection.

#### *4.1.2 Areas for future study of GxE*

Additional studies are needed to provide further support for these hypotheses. Sample sizes were small in this preliminary study, and a larger, wide-scale study with larger sample sizes from numerous populations throughout the range of *A. socius* would increase support for my results. Allozyme alleles may affect individuals in other ways, ultimately leading to differences in survival which were not measured in this study. For example, certain alleles may lead to faster growth or greater overwintering ability, potentially resulting in balancing selection. Additionally, heterozygotes may be able to perform well over a wide range of temperatures, conferring increased fitness to 1.8/2.0 and 1.8/2.2 individuals. Heterozygotes were not utilized in this study, but future studies

could compare heterozygotes to homozygotes from the same population across a wider range of temperatures. In summary, I observed a GxE interaction between *Idh-1* genotype and temperature that affected fitness in *A. socius*, but additional studies would be useful in further describing the effects of this interaction.

#### 4.1.3 Areas for future study of *Idh-1* in *Allonemobius*

Allozymes are a somewhat coarse measure of genetic differences, since only nucleotide changes that result in an amino acid substitution that changes the protein's charge will be detected by electrophoresis. Sequencing the DNA of protein-coding genes from individuals with specific allozyme genotypes has revealed that multiple alleles can result in proteins with the same electrophoretic mobility (Katz and Harrison, 1997), indicating that allozymes may underestimate genetic diversity (Lewontin and Hubby, 1966). As a result, the alleles used in this study which are found in populations throughout the range of *A. socius* could potentially be several different alleles with the same electrophoretic mobility. A sequencing study, utilizing individuals homozygous for the three *Idh-1* alleles in *A. socius* from populations throughout its range, would allow further examination of the various alleles at the *Idh-1* locus in this species. Specifically, such a study would allow the researcher to test if alleles with the same electrophoretic mobility have the same amino acid sequence, and what amino acid changes lead to differences in electrophoretic mobility between the alleles.

Additionally, widespread sampling and sequencing of all alleles in the *Allonemobius fasciatus*–*socius* complex would allow for phylogenetic reconstruction of this locus, a determination of what allele is ancestral (potentially also allowing one to

infer which species is ancestral), and what amino acid substitutions have led to changes in electrophoretic mobility. Haplotypes may be shared between closely-related species, as was found in *Pgi* (phosphoglucose isomerase) and other nuclear genes in *Gryllus* (Broughton and Harrison, 2003). One specific question that could be answered by such a study is whether the 1.0 allele, which is shared by *A. socius* and *A. fasciatus* (Howard and Furth, 1986; Howard and Waring, 1991), is a shared allele with both species having identical amino acid sequences, or convergence in electrophoretic mobility caused by different amino acid substitutions. Furthermore, since allozymes have been used in systematic studies of this complex in the past (Howard, 1983, 1986; Howard and Furth, 1986; Howard and Waring, 1991; Britch et al., 2001; Marshall, 2004), the sequencing of these allozyme alleles could either confirm or alter the presently-accepted taxonomy of the group, potentially revealing cryptic species within this complex.

#### 4.2 Maternal and Environmental Effects on Diapause Occurrence

In this study, I used a cross-fostering approach to study the effects of parental diapause history, egg-laying temperature, and egg-incubation temperature on the proportion of diapause eggs produced by *A. socius*. I found that an interaction between parental diapause history and egg-incubation temperature affected the proportion of diapause eggs produced (Table 3.1), such that diapause proportions were lower in the higher egg-incubation temperature and offspring of non-diapausing parents were more affected by the temperature-switching component of the study than were the offspring of diapausing parents (Figure 3.1). These results indicate that some embryos are able to

respond directly to changes in environmental conditions by either entering diapause under cold conditions or not initiating diapause in warm conditions. However, the ability of embryos to modify diapause response depended on the parental diapause history, such that offspring of non-diapausing parents appeared to be more plastic. To my knowledge, the finding that parental diapause history affects offspring diapause response within individuals from the same population is a unique aspect of my study. This result should be explored more thoroughly in future studies with other populations of *Allonemobius* and other insect species.

One long-standing question in the diapause literature concerns the mechanism of diapause induction and how maternal transmission from parent to offspring occurs (Mousseau and Dingle, 1991). Maternal effects arise from a combination of sources, including mate choice, egg size, yolk amount, oviposition site and depth, parental care, and extra-nuclear factors (Kirkpatrick and Lande, 1989; Mousseau and Dingle, 1991; Rossiter, 1996; Mousseau and Fox, 1998). Non-nuclear, cytoplasmic factors have been hypothesized as mechanisms for a broad range of maternal effects early in an organism's development, including mRNAs, proteins, enzymes, and hormones (Rossiter, 1996; Mousseau and Fox, 1998). More specifically, hormones are most often postulated as the mode of transmission for maternal control of diapause in her offspring (Mousseau, 1991; Mousseau and Dingle, 1991, Olvido et al., 1998; Hockham et al., 2001); however, RNA transcripts (Kirkpatrick and Lande, 1989) and DNA methylation (Rossiter, 1996) have also been suggested. One instance of a specific diapause hormone has been found in silkworms, such that the presence of this hormone in eggs leads to

diapause initiation and lack of this hormone causes direct development (Mousseau and Dingle, 1991). This finding has led to the postulation of the occurrence of some unknown diapause hormone in many studies on insect diapause, although the true mechanism is not known in most cases.

At the present time, the widespread availability of genomics tools, such as microarrays, mRNA differential display, and 2-D protein gel electrophoresis, will allow researchers to study the genetic basis of a wide variety of traits (e.g. Liao and Freedman, 2002; Stein and Liang, 2002). Use of these technologies would potentially allow for the identification of a diapause hormone or mRNA transcript transferred from a female to her eggs during oogenesis, providing insights into the actual molecular mechanism of diapause occurrence (Hirai et al., 1998). Follow-up studies utilizing RNA interference (RNAi) technology would then potentially be able to confirm the hypothesized mechanism, and allow for even more elegant studies on the evolution of the insect diapause pathway. Lastly, sequencing of any found transcripts would allow researchers to test the hypothesis of Mousseau and Dingle (1991) that insect diapause hormones evolved from other developmental hormones, by allowing comparison between the transcripts found and other developmental hormones already sequenced.

#### 4.3 Conclusions

Fitness ultimately depends on the ability of an individual to survive to reproductive age, mate, produce offspring, and have at least some offspring survive and reproduce. In this study, the number of eggs produced, an obvious measure of fitness, depended on an interaction between genotype and temperature, in accordance with

predictions derived from geographic patterns. However, once eggs are laid, they must hatch in order to provide fitness benefits for the mother. In insect species with seasonal life cycles, egg diapause is an important life-history stage that allows individuals to survive inclement conditions. Since egg diapause is at least in part under maternal control, the ability of a female to produce offspring of the “best” phenotype for a given set of environmental conditions should be highly adaptive. Therefore, maternal effects genes should be under selection. The genetic basis of diapause initiation and other maternal effects are still not known in many instances, but would provide an interesting area for future research. In conclusion, I found that complex interactions between an individual’s genetic background, life history, and temperature affected two factors affecting organismal fitness in *Allonemobius socius*; the exact mechanisms of these interactions are still not fully known, but my results provide the basis for future research in this area.

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## BIOGRAPHICAL INFORMATION

Diana L. Huestis received a Bachelor of Science with High Honors from Eckerd College in St. Petersburg, Florida in May 2003, where she majored in Marine Science with a concentration in Biology and a minor in Chemistry. After working with salamanders during a summer NSF-REU internship at Furman University in 2002, she pursued a senior thesis project at Eckerd College on life-history traits of salamanders of the genus *Eurycea*. Diana was chosen as a Ford Foundation Apprentice Scholar and participated in their 2-year program which aims to prepare undergraduates for careers in academia. She also participated for 4 years on a long-term study of freshwater turtle populations, was project coordinator for 2 years, and co-authored a manuscript on the results of this study while at Eckerd College.

Diana began the Master of Science program at The University of Texas at Arlington in June 2003, and has continued pursuing her interest in life-history evolution during her thesis research. To date, she has one publication in *Southeastern Naturalist* and has submitted manuscripts to *Oikos*, *Journal of Herpetology*, *Oecologia*, *Ecological Entomology*, and *Molecular Ecology*.

In May 2005, Diana received a 3-year doctoral fellowship through the EPA S.T.A.R. program to study the genetic basis of diapause occurrence in *Allonemobius* and plans to conduct this research jointly through UTA and Indiana University.