

Macro— and Microevolutionary Insights into the Evolution of Foraging Behavior and the Life History

By

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ABSTRACT

EVOLUTION can be studied from two different approaches known as macroevolution and microevolution. While the former focuses on the long-term fate of species or higher-rank taxa through geologic time, the latter deals with the processes that lead to evolutionary change and reproductive isolation within and among populations. Despite their differences, evolution at both of these levels relies on the same mechanisms of evolutionary change: genetic drift, migration, mutation, recombination, and natural selection. A major goal of this dissertation is to connect the knowledge of the mechanisms of evolution with the patterns observed across the tree of life, and those observed within and among populations. To accomplish such a task, I use phylogenetic comparative methods to generate hypotheses and draw broad conclusions about the evolution of foraging behavior and the life history among wide ranges of taxa. I then test those hypotheses within and among populations of *Drosophila melanogaster*. Specifically, I examine the frequency of alleles with underlying effects on the foraging behavior of individuals evolving under different selective pressures, which enables me to make strong inferences concerning phenotypic and genetic responses to selection. Although major progress has been made in past years, long-standing predictions of a relationship between foraging behavior and life-history traits remain unsupported. This work tackles this issue and suggests alternative mechanisms to better understand the evolution of behavior and the life history.

To my mom, for her love, encouragement, and understanding.

ACKNOWLEDGEMENTS

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I would also extend my thanks to my friends, Dr. Zack Graham, Dr. Lauren Kneel, and Dr. Jake Youngblood. Without their support, help, and cooperation, I would not have been able to join the lab. A big shoutout to Zack for his friendship, regular phone calls, and the good times we spent together.

I am grateful to Jennifer Vazquez and Desiree Bogen for allowing me to drink large amounts of coffee at the Center for Evolution and Medicine. Coffee was definitely my main fuel for work; the stain you see on this page indicates how bad it got sometimes!

Finally, I want to thank my family for motivating me and boosting my morale in difficult times. This journey has taken me to places far away from my mom for almost 10 years. However, she has not let a single day go by without calling me. Her love and support could not be more gratifying for me. I thank Camila Tabares for her patience and encouragement; she always offered me a hand when I needed it the most. Thanks to all of you who believe in me!



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PREFACE

THIS DISSERTATION would not have been written without the support of my friends Zack, Lauren, and Jake. When I first came to the lab, Mike was transitioning from the School of Life Sciences, where he used to teach, to a fully administrative position at EdPlus. Given his new job, he thought there was going to be no time for him to mentor any other student. But I was lucky enough to gain the trust of my friends, who did their best to convince Mike to take me as a PhD student. I am so grateful to them and hope I can do something as kind in return at some point. I am also grateful to Mike for taking such a risk. Although he was swamped with work at the time, he always made time for me. Meeting weekly with him even though he had a tight schedule was never a problem. We could even find time to go out for dinner or a couple of beers here and there. By the way, the body of work you are witnessing in this dissertation derived from a conversation I had with Mike and John over beers. It was one of those nights at a bar where they decided to challenge me to come up with novel ideas for my research. I took the challenge and proposed what you are going to see in the following 4 chapters.

I would like to point out that the first 3 chapters of this dissertation are published in high-impact scientific journals. The last chapter will be submitted for publication shortly. If you happen to read this document on your computer, I encourage you to click on the following references to get the papers:

- Padilla Perez, D. J., & Angilletta Jr, M. J. (2022). Macroclimatic and maternal effects on the evolution of reproductive traits in lizards. *Ecology and Evolution*, 12(5), e8885.
- Padilla Perez, D. J., DeNardo, D. F., & Angilletta Jr, M. J. (2022). The correlated evolution of foraging mode and reproductive effort in lizards. *Proceedings of the Royal Society B*, 289(1976), 20220180.
- Padilla Perez, D. J. (2024). Geographic and seasonal variation of the for gene reveal signatures of local adaptation in *Drosophila melanogaster*. *Journal of Evolutionary Biology*, 37(2), 201-211.



The foraging behaviors of organisms lie along a continuum, ranging from the energetically-demanding strategy of foraging actively to the more energetically-conservative strategy of ambushing a prey. The illustration above shows good examples of an active forager, such as the Hummingbird on the left, and an ambush predator, such as the Kingfisher in the background.

CHAPTER 1

MACROCLIMATIC AND MATERNAL EFFECTS ON THE EVOLUTION OF REPRODUCTIVE TRAITS IN LIZARDS

1.1 Introduction

Life-history theory consists of models designed to explain how environmental factors shape the evolution of survival, growth, and reproduction (Roff 2002; Stearns 1992). Much of this theory focuses on the covariation of body size and reproductive traits, given a tradeoff between the size and number of offspring (C. Smith and Fretwell 1974). The optimal covariation depends on ways in which body size affects the availability of energy, the constraints on reproduction, and the survival of offspring (De Jong and Van Noordwijk 1992; Van Noordwijk and De Jong 1986; Parker and Begon 1986). Often, larger females have more resources to spare, can carry more offspring during gestation, pass larger offspring during birth, or provide better care after birth. These factors influence the constraints on or the benefit of specific reproductive tactics. For example, when the abdominal cavity and pelvic opening constrain offspring size, larger females can produce larger offspring. Conversely, a small female may be unable to produce an offspring of the optimal size when this size exceeds the physical constraint Vitt and Congdon (1978). This constraint results in a positive relationship between maternal size and offspring size (Oufiero, Smith, and Angilletta 2007), in which each female produces the largest egg possible for its body size. Natural selection can produce a similar relationship when larger females have greater surplus energy (Parker and Begon 1986). Depending on both its foraging efficiency and the availability of resources, a larger female should use its additional energy to produce larger clutches. However, given a tradeoff between offspring size and offspring number, the investment in more offspring may be balanced by a decrease in the size of offspring. Similarly, when increasing the number of offspring increases competition among the resulting siblings, a female should use additional energy to produce larger offspring instead of more offspring. Interestingly, a mixed strategy can be optimal when intraspecific competition and offspring size both exert important influences on offspring survival; in such cases, larger females should lay larger offspring and larger clutches (Parker and Begon 1986).

Because growth and body size are affected by environmental conditions, the covariation among abiotic factors should shape the evolution of life-history traits (Figure 1.1). Temperature is a well-known factor that directly and indirectly affects the evolution of the life history. The direct effect occurs when environmental

conditions determine the relationship between the size and performance of offspring. Warmer environments often promote a high relative growth rate, potentially reducing the minimal size of offspring. In such cases, a female can produce more, smaller eggs as the optimal reproductive tactic. The indirect effect occurs because temperature greatly affects the size at which an organism reproduces (Atkinson 1994, 1995; Atkinson and Sibly 1997). When exposed to a warmer environment, an organism may grow faster but mature younger at a smaller size, a pattern of phenotypic plasticity observed among some ectotherms (Ashton 2002; Berrigan and Charnov 1994; Sibly and Atkinson 1994) and many endotherms (Ashton, Tracy, and Queiroz 2000; Bergmann 1848). If a warmer environment causes an organism to reproduce when younger and smaller, the smaller parent should make fewer or smaller offspring. In addition, variation in body size may also depends on precipitation, which is a good predictor of primary production (Yom-Tov and Geffen 2006; McNab 2010). Generally, abundant precipitation and radiation increase the rate of photosynthesis (Cramer et al. 1999), which provides the energy required for growth and reproduction of organisms at higher trophic levels. If more productive areas enable organisms to consume more prey, one should expect these organisms to mature younger at smaller size; this indirect effect on maternal size should affect the size and number of offspring of the mother, as discussed earlier.

Although a covariation among environmental and maternal factors is expected to drive the evolution of the life history, most studies have focused on temperature as the main selective pressure. This bias has resulted in a wealth of conflicting results in the current literature as several comparative and species-specific studies have found no consistent trend. For instance, from an analysis of over 100 studies, Atkinson (1994) concluded that in more than 80% of the cases, decreased temperature leads to maturation later at a larger size. However, the variation among populations in natural environments paints a more complex picture. Given that environmental temperature generally increases with decreasing latitude, we should expect to observe a positive relationship between latitude and offspring size. Interestingly, a meta-analysis by Marshall, Pettersen, and Cameron (2018) revealed no general relationship between latitude and offspring size; relationships ranged from strongly negative to strongly positive. Moreover, the variation among natural populations likely reflects a combination of phenotypic plasticity and genetic divergence. A recent experiment by Kouyoumdjian et al. (2019) illustrates this point. They compared the reproductive traits of lizards from low or high elevation to that of lizards transplanted from low elevation to high elevation. Lizards from high elevation produced larger eggs than did lizards from low elevation; however, lizards transplanted to high elevation produced eggs of intermediate size. Thus, the difference in egg size between populations might partly stem from genetic factors, as well as environmental conditions during early development. Moreover, body size has diverse effects on reproductive traits, when analyzed independently

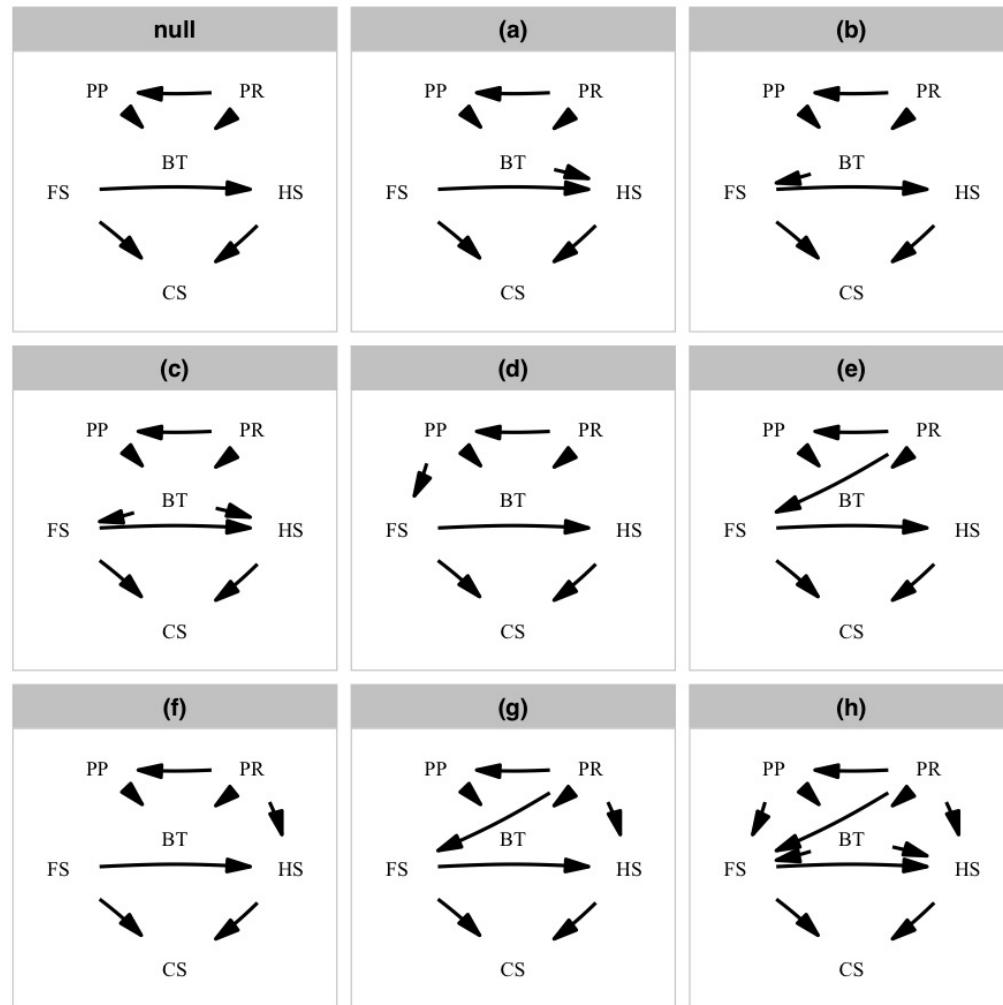


Figure 1.1: Path models depicting relationships among body temperature and life-history traits derived from optimality models. Abbreviations in the path diagrams are as follows: BT = body temperature; CS = clutch size; FS = maternal length; HS = hatchling/neonate length; PP = primary production; PR = annual precipitation.

of temperature. Larger females increase annual biomass production (Shai Meiri, Brown, and Sibly 2012), produce more eggs (Le Gouvello, Nel, and Cloete 2020), or both larger and more eggs (Warner and Shine 2008). If these diverse patterns reflect diverse processes among taxa, a model focused on a single mechanism would be unable to explain variation in reproductive traits. Therefore, a unified approach to examine the combined effects of different environmental and maternal factors on the broad-scale variation in reproductive traits might provide the best insight.

Phylogenetic path analysis is an effective way to test hypotheses about multiple causes of evolutionary patterns (Gonzalez-Voyer and Hardenberg 2014; Hardenberg and Gonzalez-Voyer 2013). Previously, Angilletta, Oufiero, and Leache (2006) combined path modeling, phylogenetic analysis, and information theory to infer the most likely relationships among environmental temperature, body size, and reproductive traits for 19 populations of sceloporine lizards. They found that temperature likely affects the number and size of offspring indirectly, via its effect on maternal body size. Since then, other researchers compiled published data for body sizes and reproductive traits of several species of lizards (Shai Meiri 2008, 2018; Shai Meiri, Brown, and Sibly 2012; S. Meiri, Feldman, and Kratochvil 2015; Warne and Charnov 2008). Here, I use these data to evaluate competing hypotheses about the evolution of reproductive traits through phylogenetic path analyses (Figure 1.1). In doing so, I found that annual precipitation is the major driver of the life history. By using phylogenetic path analysis, I suggest a more general mechanism underlying the evolution of life-history traits of lizards.

1.2 Materials and Methods

Data source

I used published estimates of life histories for 486 species of lizards, belonging to 34 families. I used the mean snout-vent length for hatchlings or neonates (mm), and adult females, as a measure of body size among species. Similarly, I averaged the lowest and highest reported means of clutch or litter sizes for each species (see Shai Meiri (2018) for details). I used the length of hatchlings as an estimate of offspring size, rather than the mass of eggs, because the latter might reflect variation in water content rather than energy content in species of lizards that lay poorly calcified eggs (Deeming 2004; S. Meiri, Feldman, and Kratochvil 2015). Moreover, hatchling size can be estimated for viviparous species as well as oviparous species.

Because the range of nearly all reptiles have been recently mapped (Roll et al. 2017), I could obtain data on annual air temperature and annual precipitation across the range of each species. These climatic

variables derived from the monthly values of temperature and rainfall for global land areas at a resolution of 30 s (Fick and Hijmans 2017). Similarly, I obtained data on log-transformed values of net primary production—the net amount of solar energy converted to plant organic matter through photosynthesis—measured in units of elemental carbon. This measure represents the primary source of trophic energy for the world's ecosystems (Imhoff et al. 2004). I used polygonal range maps—representing species extent of occurrence—to run a zonal statistical algorithm in the software QGIS version 3.22.2-Białowieża; this algorithm enabled me to extract the means of climatic data across pixels of a map for each species. In addition to extracting climatic data, I also gathered published records of body temperature measured in the field for 320 species. These data consist of an average of the minimal and maximal temperatures reported in the literature (Shai Meiri 2018).

Statistical analyses

I tested my hypotheses (see Figure 1.1) by performing phylogenetic path analysis of data of body temperature, and environmental temperature. Examining the explanatory power of competing models involving different relationships among variables enabled us to identify the most plausible hypothesis given the available data, and thus infer the relative importance of mechanisms described by optimality models. These analyses were performed with the *phylopath* library of the Statistical Software R version 4.0.5 (Bijl 2018; Team 2013), and a recent time-calibrated phylogeny for squamate taxa (Zheng and Wiens 2016). I calculated each model's goodness of fit with the Fisher's C statistic (Bill Shipley 2000), obtained as follows:

$$C = -2 \sum_{i=1}^k \ln(p_i)$$

where k is the number of conditional independencies in the minimal set and p_i is the null probability associated with each of the predicted independence claims tested. A conditional independency specifies the list of pairs of variables that are statistically independent conditioning on a set of other variables in the causal model. For example, a conditional independency that supports one of our hypotheses relates body temperature (BT), maternal size (FS) and clutch size (CS; see Figure 1.1b). In this example, clutch size is d-separated from body temperature, which means that there is not an arrow linking these variables (or “vertices”) in the directed acyclic graphic. Maternal size is considered the causal “parent” of both body temperature and clutch size, because it is the variable directly linked with both vertices (BT and CS). It is possible now to translate these d-separation statements to statistical linear models in which I test the independence of clutch size and temperature, conditioning on their parent, maternal size. The C

statistic is a maximum likelihood estimate that follows a x^2 distribution with degrees of freedom $df = 2k$. Therefore, it provides a convenient statistic for testing the goodness of fit of the whole path model (B. Shipley 2013). The path model does not provide a good fit to the data if the p value of the C statistic is below the alpha value (0.05). I compared models using a modified version of the Akaike Information Criterion (AIC) known as the C statistic Information Criterion (CIC), proposed by (Hardenberg and Gonzalez-Voyer 2013). Here, I calculated the CIC_c , which is the equivalent of CIC with a correction for small sample sizes, as follows:

$$CIC_c = C + 2q \frac{n}{(n - 1 - q)}$$

where C is the maximum likelihood of the particular model, q is the number of parameters estimated in the path model, and n is the sample size (number of species). The most likely model has the lowest CIC_c value (Burnham and Anderson 2002).

The data analyses were entirely based on full model averaging, by which one calculates a likelihood-weighted average of parameters among all models ranked. Model averaging causes the path coefficients that do not occur in all models to shrink toward zero (Bijl 2018). I reported the conditional independencies that supported the averaged model. I also reported the p-values of each independence statements, and an estimate of the Pagel's λ to account for phylogenetic non-independence of each statement. The parameter λ can vary between 0 and 1; a value of 0 for a conditional independency indicates that the relationship between the life histories involved is not constrained by the phylogeny, whereas a value of 1 indicates the converse.

1.3 Results

Phylogenetic path analyses revealed that annual precipitation directly and indirectly affected the evolution of the reproductive traits in lizards (Figure 1.2). The direct effect stems from its negative relationship with hatchling size, while the indirect effect is mediated by its effect on maternal size. These analyses also indicate that body temperature had little or no effect on the evolution of reproductive tactics, either directly or indirectly. I drew this conclusion from the coefficients derived from full model averaging, which indicated negligible effects of body temperature on maternal size and hatchling size (Figure 1.2a). Importantly, I found a similar pattern when using the mean environmental temperatures across the species range as an independent variable in our analysis (Figure 1.2b).

The analyses also revealed that maternal size greatly determined the evolution of hatchling size and

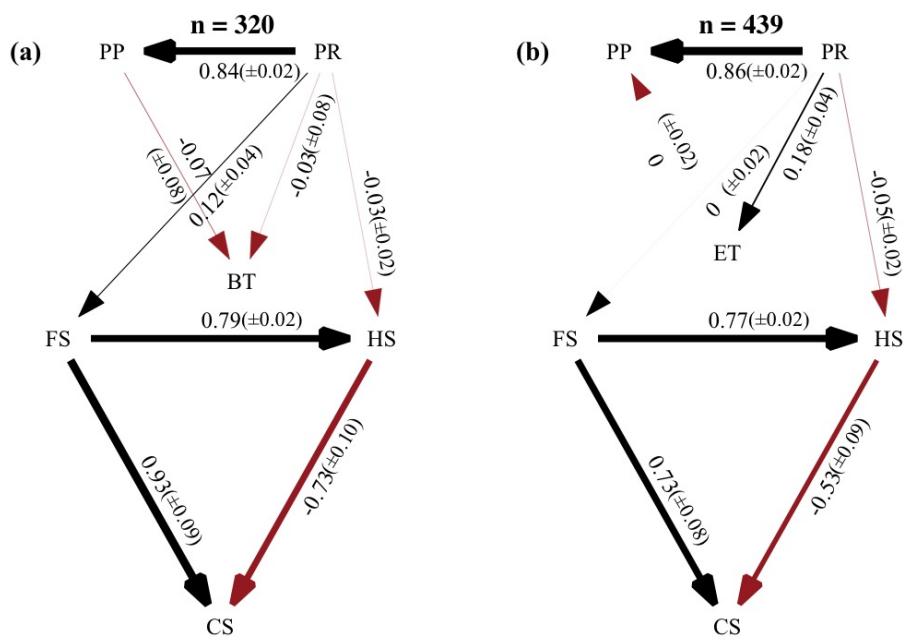


Figure 1.2: Best-fit models of the evolution of reproductive traits considering body temperature (a) and environmental temperature (b) as independent variable. Thicker arrows indicate stronger effects. Values in parentheses denote the standard errors of the path coefficients.

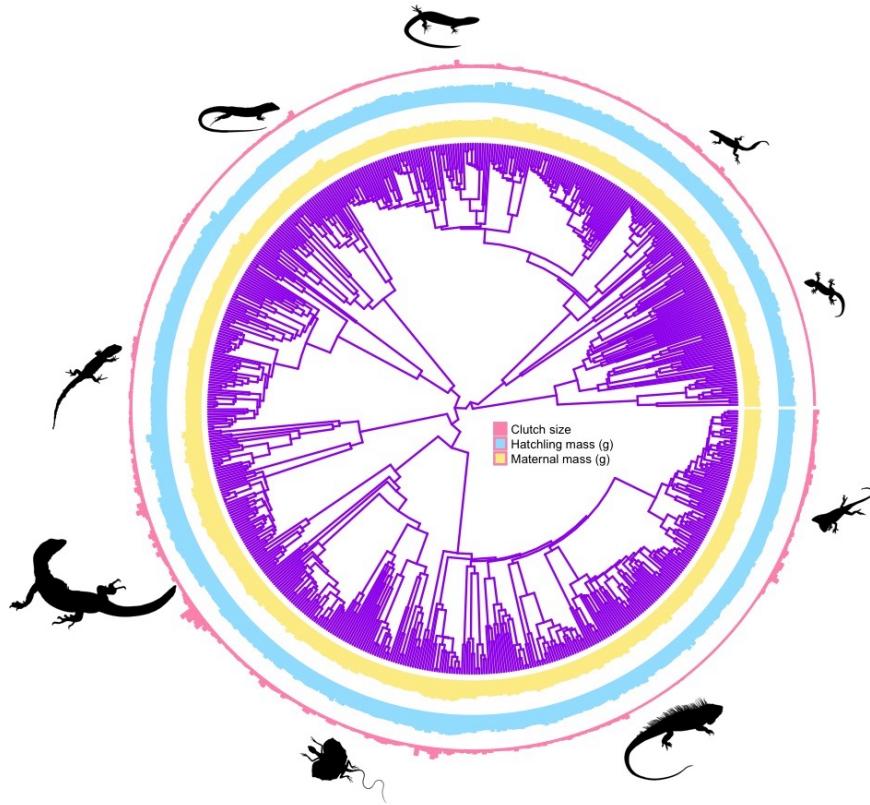


Figure 1.3: Evolution of the reproductive effort among 669 species of lizards. The dataset used to perform path analyses was reduced to 486 species because I could not extract data of climatic variables for all species present in the phylogeny.

clutch size. Specifically, larger females simultaneously produce larger offspring and more offspring (Figure 1.2). By accounting for maternal size in our phylogenetic path analysis, I observed the expected negative relationship between hatchling size and clutch size. Likely, larger females produce larger clutches of larger offspring by having more surplus energy to allocate to reproduction (Figure 1.3).

1.4 Discussion

A model describing a direct and an indirect effect of precipitation on the evolution of reproductive traits was strongly supported (see Figure 1.1g). On one hand, I found a negative relationship between precipitation and hatchling size. On the other hand, I found a positive effect of precipitation on maternal size. Both relationships might reflect selective pressures on body size associated with food availability. Greater precipitation often results in greater primary production, which translates to greater food abundance for lizards (Yom-Tov and Geffen 2006; McNab 2010). In a more productive environment, a juvenile lizard

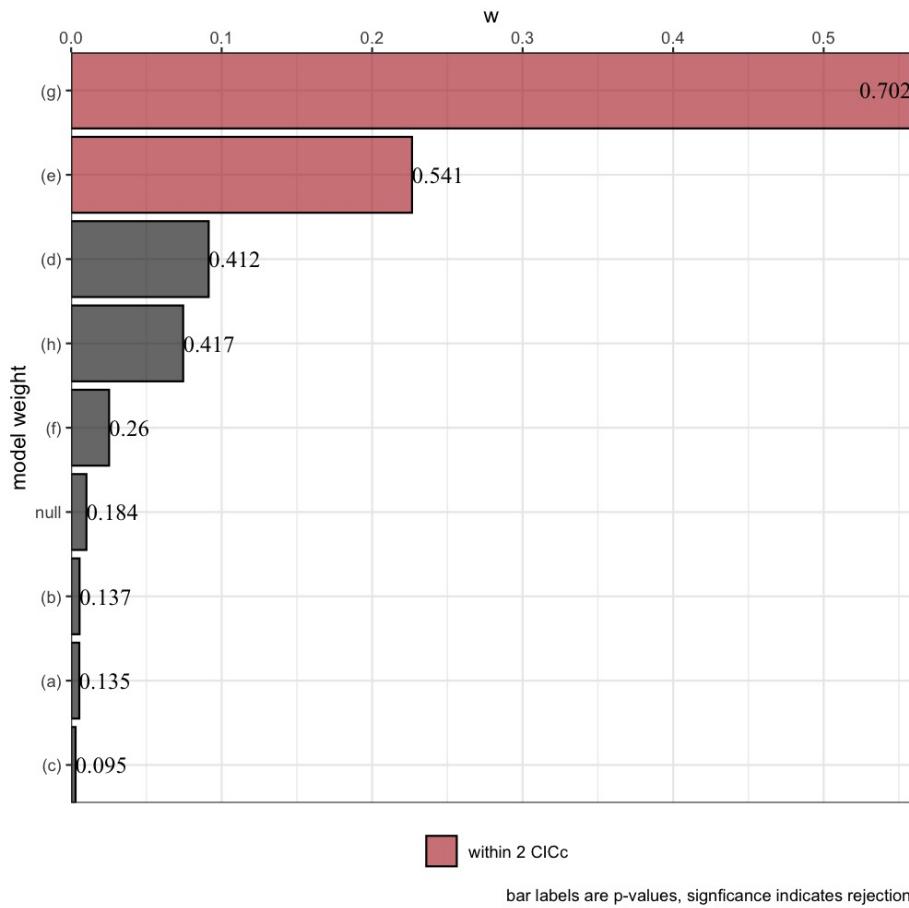


Figure 1.4: Relative importance of the causal models describing the evolution of reproductive traits in lizards.

could mature younger at smaller size. Additionally, given the tradeoff between the size and number of offspring, a mature lizard should produce more, smaller offspring in an environment that favors growth. Although the averaged model indicates no strong direct effect of primary production on maternal size (Figure 1.2a), our analysis revealed that a model including this path should also be considered important based on the ΔCIC_c (Figure 1.4). Alternatively, I consider other mechanisms by which precipitation can directly influence body size. The water-conservation hypothesis provides one such mechanism. This hypothesis predicts stronger selection for large size in dry environments, given that the size-specific rate of water loss decreases with increasing size (Nevo 1973; Olalla-Tarraga et al. 2009; Gouveia and Correia 2016; Pincheira-Donoso, Hodgson, and Tregenza 2008).

The absence of a thermal effect on the evolution of reproductive tactics might partially be explained by thermoregulation. Many species of lizards use behavior and physiology to buffer variation in environmental temperature (Raymond Huey, Hertz, and Sinervo 2003). This buffering ability reduces selective pressures

on thermal physiology, slowing evolutionary divergence over space and time (Bogert 1949; Buckley, Ehrenberger, and Angilletta 2015; Raymond Huey, Hertz, and Sinervo 2003). The conservation of thermal tolerance within species has been widely recognized (Buckley, Ehrenberger, and Angilletta 2015; Van Damme, Bauwens, and Verheyen 1990), but this phenomenon is also evident among some species of lizards (Cruz et al. 2009; Sunday, Bates, and Dulvy 2011). Body temperature constrains life histories because it exerts an important influence on the acquisition of energy required for growth and reproduction. Normally, the detection, capture, ingestion, digestion, and assimilation of food are processes that determine the acquisition of energy. These processes perform well over an optimal range of body temperatures and perform poorly at temperatures outside this optimum (Raymond Huey 1982). Whether a lizard's optimal temperature for performance remains similar in space and time depends on the effectiveness of thermoregulation. A thermoregulating lizard might bask in open areas more often at high altitudes or latitudes, but it might use shaded areas more often at low altitudes or latitudes (Adolph 1990; Rand 1964). Consequently, the optimal body temperatures of species from different altitudes or latitudes might not change drastically (Raymond Huey, Hertz, and Sinervo 2003). If this behavior enabled some lizards to maintain their body temperature close to the thermal optimum regardless of the climate, thermoregulation would have slowed the divergence of life-history traits among species. Because nocturnal lizards have fewer opportunities to behaviorally thermoregulate than diurnal lizards do, I expect temperature to have a greater effect on the reproductive tactics of these lizards.

Additionally, local adaptation of thermal physiology among species might reduce the impact of environmental temperature on the life history of lizards. If the preferred body temperatures of species have diverged to track the environmental temperatures of their habitats, natural selection would favor a subsequent shift in the thermal optima for performance (Raymond Huey and Bennett 1987). This coadaptation of thermoregulatory behavior and thermal physiology would enable each species to perform best at the body temperature commonly experienced in its environment. In lizards, Bauwens et al. (1995) found a significant correlation between the preferred temperature and the thermal optima for sprint speed among 13 species of lacertids. Similarly, Raymond Huey and Kingsolver (1993) found that thermal optima for sprinting correlated strongly with field body temperature among 19 species of iguanid lizards. Thus, the coadaptation of thermoregulatory behavior and thermal physiology seems a likely factor contributing to the patterns of interspecific variation that I observed in general among lizards.

The evidence that females produce larger clutches of larger offspring accords with certain optimality models in which natural selection maximizes the reproductive success of the parent (Roff 2002). This positive relationship between maternal size and clutch size, or fecundity, mirrors those observed within

most species (Parker 1970; Ridley and Thompson 1979; Roff 2002). Presumably, larger females lay larger clutches because they have more surplus energy (Parker and Begon 1986). However, I also found that larger mothers produce larger offspring. For this strategy to be optimal, the size of the mother must affect the relationship between the size and fitness of offspring (C. Smith and Fretwell 1974). Several hypotheses have been proposed to explain why larger mothers should produce larger offspring (Marshall et al. 2010; Sakai and Harada 2001). Following Taylor and Williams (1984), Sargent, Taylor, and Gross (1987) showed that mothers should produce larger offspring when offspring size promotes growth and reduces mortality. They interpreted this result to mean that larger females should produce larger eggs whenever they provide better parental care, which increases growth or reduces mortality. In support of this model, seed beetles produced larger offspring when they were able to lay their eggs on better-defended seeds (Fox, Thakar, and Mousseau 1997). But do larger species of lizards provide better parental care for their offspring? Possibly, larger mothers deter more predators and provide more food for their offspring. Consistent with this idea, a large species of skink, *Eutrophis longicaudata*, better deterred snakes from eating their eggs than did a small species, *Sphenomorphus incognitus* (Huang 2006; Tseng and Huang 2012). Still, more evidence is needed to determine whether parental care, other than energy provisioning, plays an important role in the growth and survival of juveniles, particularly for oviparous lizards that do not interact with their offspring after laying eggs in a nest. Alternatively, larger mothers might produce larger offspring because of competition among siblings; this strategy simultaneously reduces the intensity of competition (a smaller clutch) and increases the competitiveness of offspring (larger offspring). Both of these models could explain the positive relationship between maternal size and offspring size that I observed among species of lizards.

These models of optimal reproductive allocation depend on a fundamental tradeoff between the number and size of offspring (Roff 2002; C. Smith and Fretwell 1974; Warne and Charnov 2008). I found strong evidence for this tradeoff among species. One's ability to detect a tradeoff between the size and number of offspring depends on the variation in energy supply among mothers (Van Noordwijk and De Jong 1986). Controlling for body size should reveal the expected tradeoff between the size and number of offspring. However, some families of lizards provide evidence of exception for this tradeoff, such as chamaeleonidae and varanidae. For example, Diaz-Paniagua et al. (2002) showed that egg characteristics (length, width, and mass) remained constant despite variation observed in clutch size and maternal body length and mass of chameleons (Figure 1.3). This finding supports the idea of an optimal egg size in chameleons (C. Smith and Fretwell 1974). According to the optimal egg size theory, clutch size, rather than egg and offspring size, varies with fluctuating resource availability. If offspring size does not dependent on clutch size or

maternal size, such that large females produce the same size of eggs as small females do, the expected offspring size and offspring number tradeoff might be muted. These exceptions show the importance of analyses such as mine, which can help determine whether body size drives resource availability through comparative evidence.

This study indicates that precipitation is the most likely driver of the evolution of reproductive traits in lizards. Although biologists have intensely debated the mechanisms by which temperature affects body size (Angilletta 2009; Atkinson and Sibly 1997; Partridge and French 1996), I found no consistent relationship between temperature and body size among species of lizards. Interestingly, we now know that lizards with larger amounts of reproductive resources produce both larger hatchlings and larger clutches. Although I do not know the selective pressures responsible for this pattern, theoretical models tell us that parental care or intraspecific competition could play a role (Parker and Begon 1986). In lizards, I lack experimental evidence that larger females provide better parental care. I also do not know the relationship between offspring size and competitive ability for most species. Therefore, empirical evidence of this nature would clarify whether these mechanisms play any role in the evolution of life histories in lizards. For longer-lived species such as lizards, experimental studies of life-history evolution have lagged behind comparative studies. Still, phylogenetic path analysis has enabled me to disentangle some evolutionary relationships among life histories. This analysis sharpens our focus on mechanisms underlying major evolutionary patterns, stimulating the development of a more general life-history theory.

CHAPTER 2

THE CORRELATED EVOLUTION OF FORAGING MODE AND REPRODUCTIVE EFFORT IN LIZARDS

2.1 Introduction

The foraging behaviors of vertebrates lie along a continuum, ranging from the energetically demanding strategy of searching for prey to the energetically conservative strategy of ambushing prey (Pianka 1966; Perry 1999). During the past 50 years, ecologists have developed a set of hypotheses about how an organism's foraging mode relates to its life history (Vitt and Congdon 1978; Vitt and Price 1982; Webb, Brook, and Shine 2003; Dunham, Miles, and Reznick 1988). These relationships stem from two major assumptions (Figure 2.1). First, a widely-foraging lizard spends more energy while foraging than a sit-and-wait lizard, but might also consume enough food during an activity season to have more surplus energy (Raymound Huey and Pianka 1981; Roger Anderson and Karasov 1988; R. Anderson and Karasov 1981; Nagy, Huey, and Bennett 1984; Bury 2021; Stuginski et al. 2018). However, a sit-and-wait lizard might have a more diverse diet, because it encounters prey less frequently than a widely-foraging lizard does (Glaudas et al. 2019). Second, a widely-foraging lizard could suffer a greater risk of predation when movements are conspicuous to predators (Cooper and Perez-Mellado 2004). Risk of predation might also affect the evolution of reproductive output, because carrying a greater mass of offspring reduces a female's ability to evade a predator (Shine 1980). Both a greater energy supply and a greater mortality risk would select for a genotype that matures at an earlier age and allocates more energy to reproduction, manifested as more or larger offspring (Bonnet et al. 2001; Silva et al. 2020). Finally, the load associated with food consumption may compromise the speed of an animal, increasing its vulnerability to predators (Cooper 2000; Werner and Anholt 1993). In this model, a suite of traits associated with foraging mode would coadapt to the spatiotemporal distributions of prey and predators.

Despite the wealth of conjecture, these hypothetical relationships among foraging mode and other traits have been assessed in only a handful of cases. Ideally, one would isolate each relationship and conduct experiments to quantify the evolution of traits in a controlled environment (R. Huey and Bennett 1986). However, such data are difficult to gather for many species and will probably remain rare. For instance, limited circumstantial evidence exists for the putative relationship between foraging mode and predation risk. Stomach contents of vipers suggested that widely-foraging species of lizards are more vulnerable

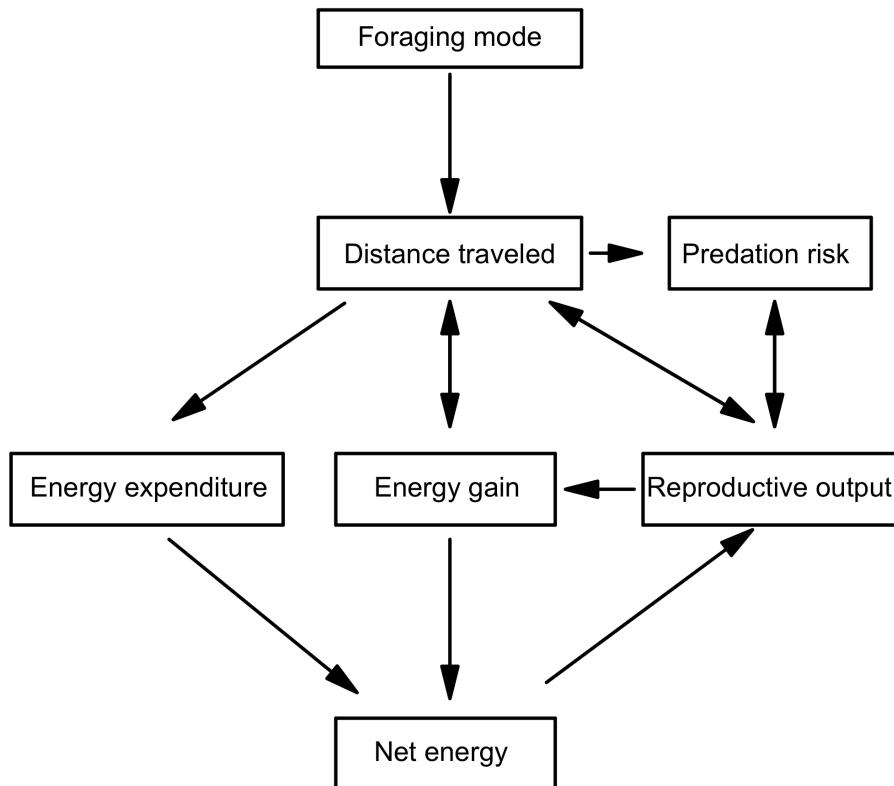


Figure 2.1: A conceptual model depicting putative relationships among foraging behavior, energetics, predation risk, and reproductive effort. The predicted relationships were derived from theoretical models of life-history evolution.

to predators than sedentary species (Raymound Huey and Pianka 1981), but a number of confounding factors can explain this observation as well as foraging mode can. Alternatively, researchers have evaluated the relationship between mortality and life-history traits with no emphasis on foraging behavior. For example, experimental evolution with guppies and fruit flies revealed that genotypes that evolved in risky environments developed more rapidly, matured at a smaller size, and reproduced earlier in life than did genotypes that evolved in safe environments (Stearns 1992; D. Reznick et al. 2001). From these results, I expect that a greater risk of predation among widely-foraging species selects for greater reproductive output; however, given the scarce evidence, I cannot conclude whether foraging mode generally affects predation risk.

Although experimental data are lacking, comparative methods have been used to explore how foraging mode and life-history traits have evolved. These interspecific analyses have focused mostly on the reproductive effort of lizards. The earliest analysis of 22 species revealed an interesting pattern: sit-and-wait species had a greater reproductive effort than widely-foraging species (Vitt and Price 1982). The authors suggested that widely-foraging species might have to carry fewer or smaller offspring, because moving long distances with a voluminous clutch compromises speed, which in turn decreases the chance of escaping a predator. A subsequent analysis of data for 50 species of lizards supports this result by testing a model in which predation risk increased with increasing reproductive effort (Vitt and Price 1982). Roff (2002) extended support for this model in a comparative analysis of 130 species of lizards. However, none of these early analyses controlled for potential phylogenetic correlations that might generate spurious relationships between foraging mode and reproductive effort (Felsenstein 1985), especially because foraging mode varies more among families of lizards than within them. A more recent analysis, using phylogenetic comparative methods, failed to detect a significant relationship between foraging mode and reproductive effort (Mesquita et al. 2016). Thus, the long-standing prediction of a relationship between foraging mode and life-history traits is currently unsupported.

I present the first evidence that the evolution of widely-foraging in lizards was associated with the evolution of greater reproductive output, specifically among large-bodied species of lizards. This evidence comes from a comparative analysis of foraging mode and reproductive output comprising 485 species of lizards from 32 families. In this analysis, I inferred the evolutionary history of foraging modes, complementing past reconstructions of ancestral states (Miles, Losos, and Irschick 2007). In contrast to previous analyses, this study partially supports the prediction of theoretical models of the optimal reproductive output, paving the way for ecologists to test mechanistic hypotheses at the intraspecific level.

2.2 Materials and Methods

I used published estimates of life history and foraging behavior for 485 species of lizards grouped in 32 families, excluding amphisbaenians and snakes. These data represent a subset of those assembled by ecologists from primary and secondary literatures (Shai Meiri 2018). I defined reproductive effort as the mean product of offspring mass and offspring number. Accordingly, I computed the product of the mean scaled mass index for hatchlings or neonates, $\hat{M}(g)$, and the mean clutch or litter size for each species of lizards. This product was interpreted in terms of reproductive effort after adjusting for the mass of the parent (i.e., the mean $\hat{M}(g)$ of adult females). The scaled mass index standardizes body mass at a given fixed value of a linear body measurement, based on the scaling relationship between mass and length (Peig and Green 2009). I first estimated hatchling mass and maternal mass from values of snout-vent-length (SVL) using published allometric equations (Feldman et al. 2016; S. Meiri, Feldman, and Kratochvil 2015; Shai Meiri 2018). Then, I computed \hat{M} as follows:

$$\hat{M} = M_i \left(\frac{L_0}{L_i} \right)^b_{SMA}$$

where M_i and L_i are the raw body mass and the linear body measurement of individual i , respectively; b_{SMA} is the scaling exponent estimated by the standardized major-axis regression of $\ln(M)$ on $\ln(L)$; L_0 is an arbitrary value of L (e.g., the arithmetic mean of SVL values for the study species); and \hat{M}_i is the predicted body mass for individual i when the linear body measure is standardized to L_0 . The scaled mass index performs best as a predictor of variation of energy stores, such as fat as well as other body components (Peig and Green 2009). Given that I could not find clutch frequencies and ages of first reproduction for all of the widely-foraging and sit-and-wait species in this study, my analysis focused on reproductive effort in a single event rather than the lifetime reproductive effort, which corresponds to the total production of offspring over the lifetime of a female.

Among species of lizards, widely foraging females and sit-and-wait females have been compared mostly in terms of their relative clutch mass. Relative clutch mass accounts for variation in energy availability among individuals by computing the ratio of reproductive mass to maternal mass, where mass is a proxy for energy. Although relative clutch mass is commonly used as measure of reproductive effort, regressing this index on female body mass for further analyses might be problematic, because ratios of random numbers regressed against their denominator will yield spurious correlations (Atchley, Gaskins, and Anderson 1976; Packard and Boardman 1988). By regressing the mean product of offspring mass and offspring number on maternal mass, the slope of the linear relationship can be interpreted as reproductive effort—proportion

of mass allocated to reproduction at a given maternal mass—which enabled me to avoid statistical issues associated with the analysis of ratios.

I defined the foraging mode of a species based on whether it has been reported as ambushing prey (sit-and-wait forager), searching for prey (widely foraging), or using a mixed strategy (mixed). Although this categorization seems somewhat artificial, most species of lizards clearly belong to one of these categories (Perry 1999; Raymond Huey and Pianka 1981). I focused our analyses only on carnivores, because herbivores do not fit into the classical paradigm of foraging modes (Pianka and Vitt 2003).

Statistical analyses

To determine the appropriate model of evolution, I used a set of continuous-time, discrete-state Markov-chain models to sample the character histories from their posterior probability distribution (Huelsenbeck, Nielsen, and Bollback 2003), and a time-calibrated phylogeny of squamate reptiles (Zheng and Wiens 2016). I rooted the tree with the tuatara (*Sphenodon punctatus*) as the outgroup for our study taxa. Based on previous analyses, I coded this outgroup as a sit-and-wait species (Pianka and Vitt 2003; Vitt et al. 2003). However, I also performed the analysis with no outgroup to evaluate the consistency of our estimates. Additionally, I estimated phylogenetic signal (character dispersion on a phylogeny) using Fritz and Purvis' *D test*, available through the function *phylo.d* in the "caper" package of R, version 1.0.1 (Team 2013; Fritz and Purvis 2010; Orme et al. 2013).

I used Phylogenetic Generalized Least Squares (PGLS) to model the relationship among maternal mass, foraging mode, and reproductive output. PGLS models enabled me to account for non-independence of the data (Felsenstein 1985; Harvey and Pagel 1991). To do so, I used the *gls* function from the "nlme" package of R, version 3.1.153 (Team 2013; Pinheiro et al. 2007). I included maternal mass and foraging mode as independent variables and the mean product of hatchling mass and clutch size as the dependent variable. Then I fitted multiple models assuming that the species trait values evolved via a Brownian motion model, an Ornstein-Uhlenbeck motion model, and a Pagel's lambda model using the R package "ape" version 5.6.2. To evaluate the models' goodness of fit, I used information-theoretic criteria such as AIC_c . I ranked candidate models accordingly and selected the most likely one (lowest value of AIC_c) for inferences. To control for variation in maternal size among species, I examined models in which maternal mass was standardized (\hat{M}). This analysis enabled me to deal with outliers, thus increasing the robustness of my conclusions.

Table 2.1: Analysis of Deviance Table (Type III tests) for the most likely model, based on the ranking of AIC_c for potential candidate models.

	Df	Chisq	Pr(>Chisq)
(Intercept)	1	0.005	0.941
\hat{M}_{females}	1	4.760	0.029
foraging.mode	2	10.372	0.005
$\hat{M}_{\text{females}}:\text{foraging.mode}$	2	18.104	0.000

A colon mark (:) in a model represents an interaction term.

2.3 Results

The evolutionary transitions in foraging mode among species were best described by a model in which rates of evolution were equal among foraging modes, referred to as the equal-rates model (ER). According to the posterior probabilities from stochastic mapping, sit-and-wait foraging is the most likely ancestral state of all lacertilians (Figure 2.2). I drew this conclusion from the ancestral state reconstruction analysis including the outgroup (i.e. the tuatara) and with no outgroup. Sit-and-wait foraging evolved in two major clades of lizards; near the root of the tree, I found strong evidence suggesting that the ancestor of the basal gekkotans was likely a sit-and-wait predator. Likewise, the ancestor of the more derived iguanians was likely a sit-and-wait predator. During the Jurassic and middle Cretaceous, major transitions from sit-and-wait foraging to widely foraging seemed to have occurred in the ancestors of Scincoidea, Lacertoidea, and Anguimorpha. However, a major reverse transition from widely foraging to sit-and-wait foraging has also occurred during the Jurassic, in the ancestor of iguanians. The D test for phylogenetic signal indicated that foraging mode is phylogenetically conserved among lizards ($D = -0.14$, $p[D < 1] = 0$, $p[D > 0] = 0.80$). Accordingly, closely related species tend to exhibit the same foraging mode, but this similarity decreases as phylogenetic distance increases.

The evolution of reproductive effort in lizards was driven by an interaction between maternal mass and foraging mode (Table 2.1). The information-theoretic approach revealed that a model including this interaction was strongly supported. Widely foraging was associated with the evolution of greater reproductive effort than was sit-and-wait foraging, specifically in large-bodied species of lizards (Figure 2.3a). Yet, this difference seemed small when hatchling mass and maternal mass were standardized (Figure 2.3b). A model in which mass was standardized, \hat{M} , suggested that species adopting a mixed-foraging strategy evolved the greatest reproductive effort (Figure 2.3b).

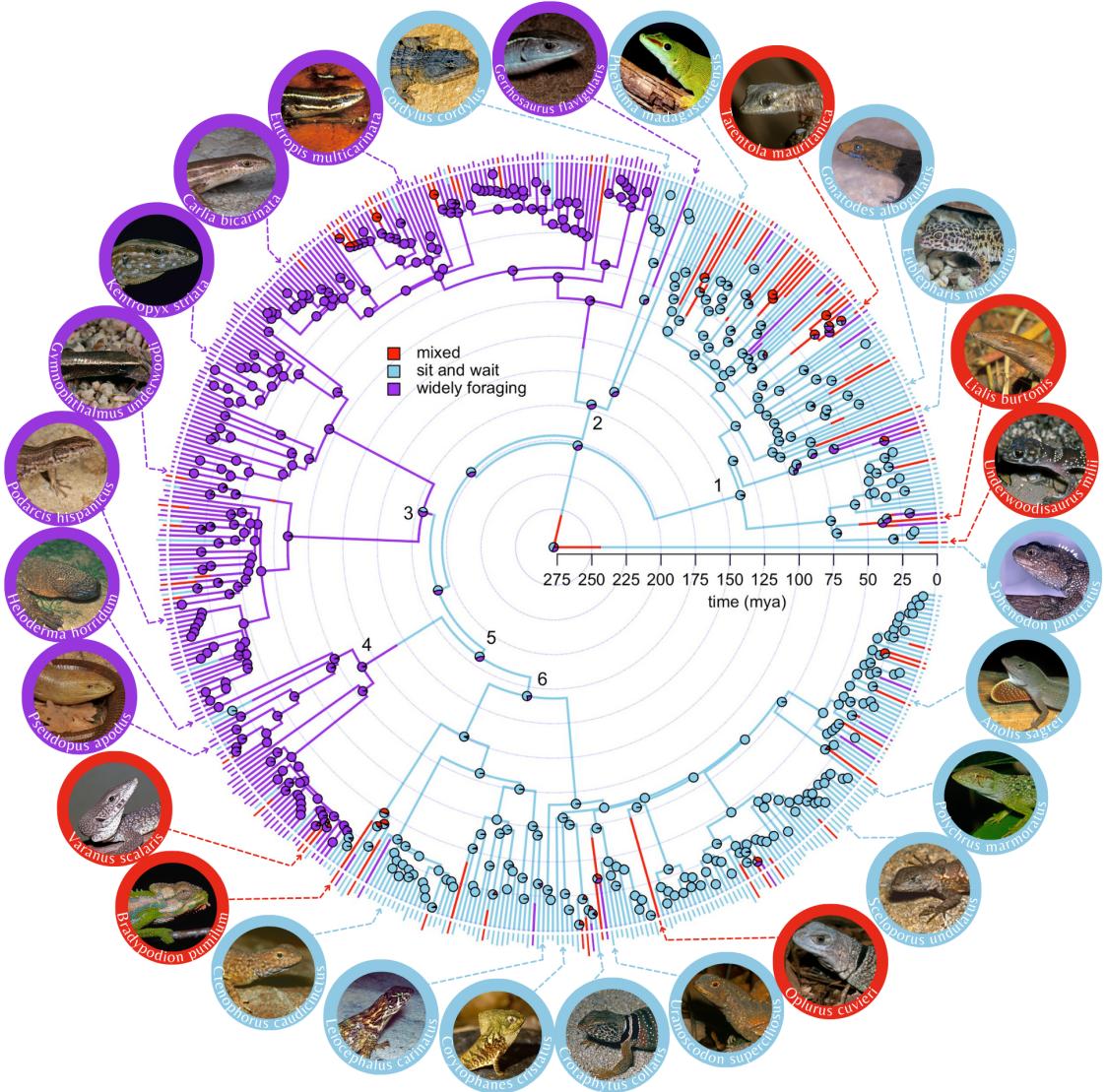


Figure 2.2: Random sample of stochastic character maps depicting the evolution of foraging mode in 485 species of lizards. Bars at the tips of the phylogeny represent log-transformed values of reproductive effort for all lizards, but not the outgroup, *Sphenodon punctatus*. Pie charts on internal nodes represent posterior probability estimates resulting from 1,000 simulations of the character histories. Major clades are enumerated as follows: 1) Gekkota, 2) Scincoidea, 3) Lacertoidea, 4) Anguimorpha, 5) Toxicofera, and 6) Iguania. Lizard photos by Mark O’Shea.

2.4 Discussion

My analysis provides evidence that species of widely-foraging lizards have evolved greater reproductive output, but only in heavier species (see Figure 2.3b). Presumably, this pattern stems from the ability of widely-foraging species to harvest and assimilate more resources than sit-and-wait species can. In the field, a widely-foraging lizard spends 32% more daily energy than a sit-and-wait lizard, but this extra energy expenditure is probably paid off with greater daily food consumption (Brown and Nagy 2007).

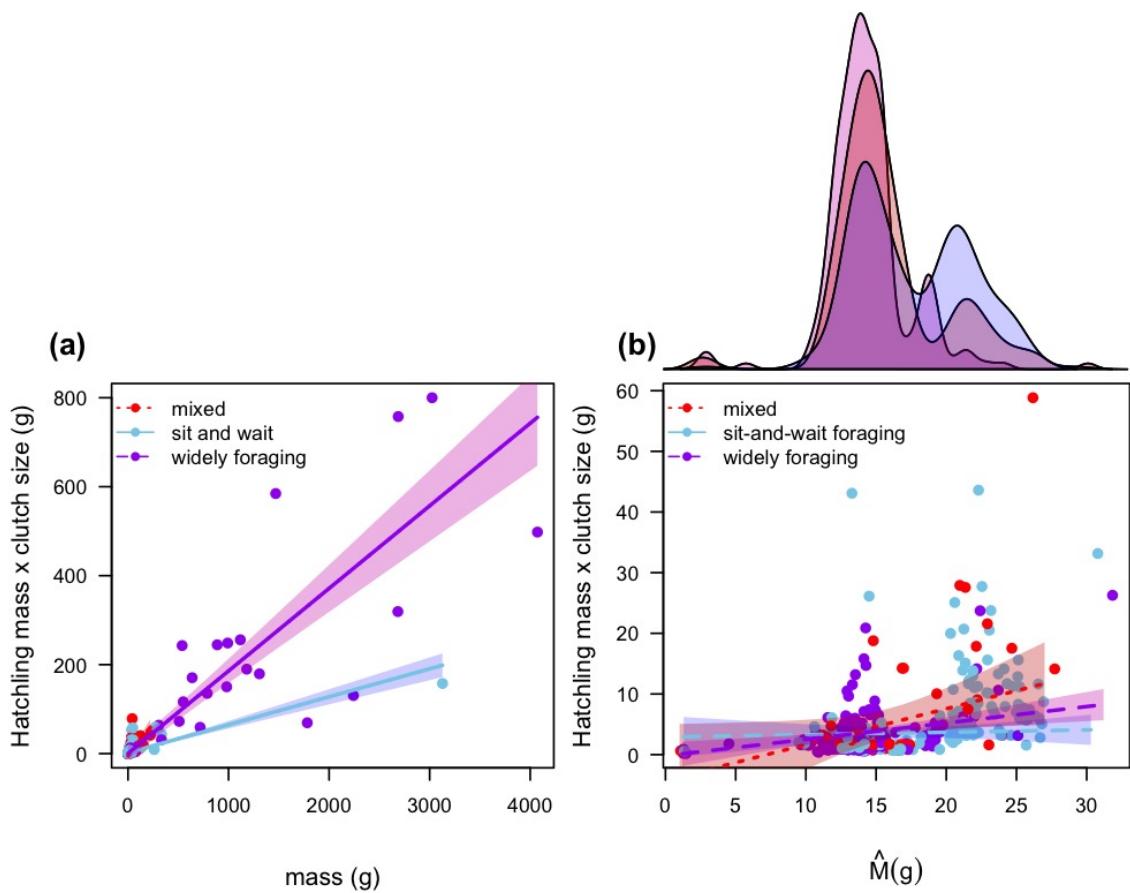


Figure 2.3: Effects of maternal mass and foraging mode on the evolution of reproductive effort of lizards, as determined by phylogenetic generalized least squares analysis. (a) Difference in reproductive effort among foraging modes assuming body mass as a proxy for energy. (b) Difference in reproductive effort among foraging modes assuming the scaled mass index as a proxy for energy.

Food consumption may play an important role in determining the reproductive output of lizards in three ways: 1) promoting follicular growth during the reproductive season; 2) increasing energy stores to initiate reproduction, and 3) reducing age at first reproduction. For example, female vipers that had good body condition early in vitellogenesis produced large litters (Bonnet et al. 2001). Similarly, vipers that gained more mass during follicular growth produced larger offspring. Early reproduction gives offspring sufficient time to mature in the same year that they hatched, which enables them to participate as adults in the subsequent breeding season (Hahn and Tinkle 1965). Therefore, a widely-foraging species might gain more resources before and during each reproductive event than a sit-and-wait species would, potentially boosting their reproductive output.

In many species of animals and plants, the reproductive output increases with increasing body size (Barneche et al. 2018; Marshall, Barneche, and White 2022; Primack 1979; Tinkle 1967; Bownds, Wilson, and Marshall 2010; Marshall, Pettersen, and Cameron 2018). This observation indicates that increased size at maturity might evolve according to a reproductive benefit of larger size, balanced by the risk of mortality associated with delayed maturation. Interestingly, the effect of maternal size on the reproductive output of lizards depended on foraging mode. A model of the evolution of optimal reproduction predicts this pattern (Parker and Begon 1986). Assuming same daily and seasonal activity durations among species, this model suggests that the total energy accumulated for reproduction (m) depends on the time spent foraging (t) and maternal size. Thus, the ratio of the energy accumulated for reproduction to the time spent foraging ($\frac{m}{t}$) corresponds to the foraging efficiency of an organism. If two organisms have the same foraging efficiency, the smaller one would reach its maximal capacity to accumulate resources at a lower value of m , producing fewer or smaller offspring (Figure 2.4a). However, if a widely-foraging species has a smaller body size but a higher foraging efficiency, it might invest more energy in reproduction than would a large sit-and-wait species (Figure 2.4b). The same outcome should be observed if a widely-foraging species is larger and more efficient than a sit-and-wait species (Figure 2.4c). Furthermore, if a widely-foraging lizard is more efficient at foraging, it might forage for less time and reproduce more frequently than would a sit-and-wait lizard. Therefore, widely-foraging lizards could not only produce larger or more offspring in a single reproductive event, but they could also increase the number of such events throughout life.

Because foraging usually increases the risk of predation, individuals that require less time to accumulate resources should incur a lower chance of death. Schoener (1971) hypothesized that the energy accumulated from foraging increases monotonically toward an asymptote. In such cases, foraging for twice as long would not result in twice the energetic return (Figure 2.4b). However, the rate of energy gain likely depends on foraging mode, as well as the abundance and distribution of prey. Consistent with this idea,

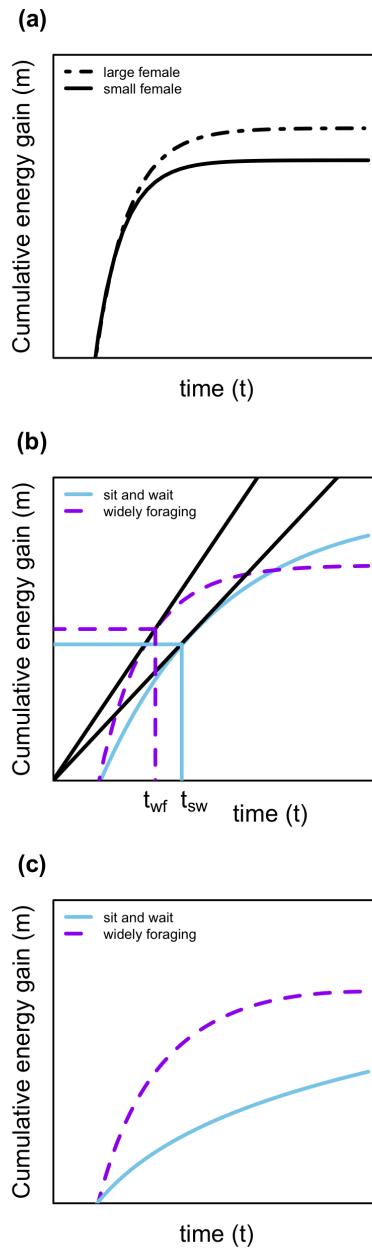


Figure 2.4: A theoretical model relating the cumulative energy gain for reproduction, m , as a function of time spent foraging, t , and maternal size. (a) Larger females reach their maximum capacity at a higher value of m than small females. (b) Widely-foraging females that are smaller but more efficient foragers may produce a greater reproductive output than larger sit-and-wait females. (c) Widely-foraging females may also produce a greater reproductive output than sit-and-wait females if they are both larger and more efficient foragers. t_{wf} and t_{sw} in (b) represent the optimal foraging time of widely-foraging females and sit-and-wait females, respectively.

widely-foraging lizards grew to a larger body size while foraging for less time than sit-and-wait foragers did (Nagy, Huey, and Bennett 1984). The ability of widely-foraging lizards to forage more efficiently should reduce their risk of predation. Rapid growth by widely-foraging lizards might also cause them to outgrow the gape limitations of predators (Lynch 1980; David Reznick and Endler 1982). Therefore, if body size is critical to outperform predators and outcompete conspecifics, the optimal pattern may be to grow to the maximal size that leads to the greatest reproductive output.

The foraging-mode paradigm is focused on dichotomous variation, yet plastic variation in foraging mode (Butler 2005) may drive the evolution of the reproductive traits in some species. Recent studies have revealed that the foraging mode of an organism depends on the ecological context, such as the presence of predators, the abundance of prey, or the surrounding habitat (Greeff and Whiting 2000; Hawlena and Perez-Mellado 2009; Wasiolka et al. 2009; Donihue 2016). Such plasticity of behavior could precede rapid evolutionary change and local adaptation of the life history (Richardson et al. 2014). A mixed foraging strategy was represented by species in the superfamilies Anguimorpha, Gekkota, Iguania, and Scincoidae (Figure 2.2). Because organisms adopting a mixed foraging strategy are possibly exposed to a wide range of environments with different selective pressures, these species might actively select habitats that maximize their reproductive output, indirectly resulting in local adaptation (Richardson et al. 2014). My analysis, which provides some evidence that a mixed foraging strategy likely leads to the greatest reproductive output, should encourage others to address the questions raised by our observations. Additionally, future analyses might consider temporal patterns of foraging associated with seasonal environments. For example, widely-foraging lizards in the Kalahari Desert consume more food during the summer and stop eating during winter, as they hibernate. By contrast, other species of lizards ambush prey during both seasons (Raymond Huey, Miles, and Pianka 2021). Evidence of this nature is still rare, revealing the need for long-term studies of the energetics of foraging modes within and among species.

Expanding my analysis to include additional life-history variables could resolve or even alter the observed patterns. For instance, in areas with long growing seasons, production of multiple clutches offsets the constraint of optimal egg size on clutch size, leading to a greater lifetime reproductive output than a single clutch of either a few large eggs or many small eggs (Gibbons 1982). Similarly, age of first reproduction is a critical component of the life history. In risky environments, for example, a species of lizard should evolve a reduced age of first reproduction and greater reproductive output (Stearns 1992). Similarly, direct comparisons of the mortality rates of widely-foraging species versus sit-and-wait species are required to know whether foraging mode affects a lizard's vulnerability to predation. Although this evidence would

be difficult to obtain for hundreds of species, detailed studies of a strategic sample of species would complement the broad comparative analysis that we conducted. Ultimately, a combination of comparative and experimental analyses will be needed to develop a general perspective of how foraging mode shapes life-history evolution.

This study presents the first evidence that the early shift in foraging mode—from sit-and-wait foraging to widely foraging—in the evolutionary history of lacertilians was likely accompanied by the evolution of a greater reproductive output, specifically in large-bodied species of lizards (Figure 2.2). Yet, much variation in reproductive output exists among species with the same foraging mode, such that ecologists must consider other factors that influence reproduction. Importantly, this study captured the effects of foraging plasticity on the reproductive output of lizards. For instance, lizards that adopt a mixed-foraging strategy produced the greatest reproductive output. Hopefully, my findings encourages others to investigate how foraging efficiency and predation risk associated with foraging modes influence the evolution of reproductive traits. Finally, the analyses revealed that the scatter in the data was very large, preventing me from drawing robust conclusions. To deal with this issue I removed the mixed category and made the species behave randomly as widely foragers or sit-and-wait foragers. That way, I could recalculate the significance of the interaction to determine whether the results hold. Doing this multiple times (>1000) enabled me to calculate the proportion of time that widely-foraging species had greater reproductive output than sit-and-wait species, as indicated by the original results (Figure 2.5). Such new analysis suggested that 57.4% of the time the p-value of the interaction was significant, which supports my previous observations.

Figure 2.5: Animated visualization of the effects of maternal mass and foraging mode on the evolution of reproductive effort among lizards. *For the best visualization, readers should view this illustration in Adobe Reader 9 or a later version: <http://www.adobe.com/products/reader/>.*

CHAPTER 3

GEOGRAPHIC AND SEASONAL VARIATION OF THE *FOR* GENE REVEAL SIGNATURES OF LOCAL ADAPTATION IN *DROSOPHILA MELANOGASTER*

3.1 Introduction

Species distributed over broad geographic ranges face drastic variation in environmental conditions. Such species provide an opportunity to understand the mechanisms underlying evolutionary responses to environmental variation. *Drosophila melanogaster* represents a good example of a species with a widespread distribution range, being found on every continent and most islands (Markow and O'Grady 2005). Since the early 1900s until the present days, this species has been a central model to understand the genetic basis of adaptation (Flatt 2020). Identifying genes underlying adaptation of populations is a key issue in evolutionary biology (Tenallon and Tiffin 2008). For instance, the foraging gene (*for*) is best known for underlying different foraging strategies adopted by *D. melanogaster* in response to environmental variation (Anreiter and Sokolowski 2019). Detailed characterization of this gene in laboratory experiments revealed the existence of two phenotypic variants: rover and sitter (Sokolowski 1980). In the presence of nutritive yeast, larval rovers travel longer distances and eat less than sitters do. This difference is not evident in the presence of a non-nutritive agar medium (Kaun et al. 2007), suggesting a gene-by-environment interaction (Burns et al. 2012). Similarly, Pereira and Sokolowski (1993) showed that adult rovers walked significantly farther from the food source after eating than did sitters, but there were no differences in walking behavior in the absence of food. This behavioral variation should also be observed in the wild, because food availability varies temporally and spatially across the range of *D. melanogaster*. Because these behavioral differences between rovers and sitters arise from genetic variation at the *for* gene, one should expect the frequency of alleles of this gene to vary over space and time.

Extensive geographic and temporal variation in various genetic markers has been identified in *D. melanogaster* (Hoffmann and Weeks 2007; Kapun et al. 2020; Sezgin et al. 2004). In some cases, clinal genetic variants have been directly linked to phenotypic variants (Lee et al. 2013; Paaby et al. 2014, 2010; Schmidt et al. 2008). For example, associations between the *Adh* allozyme locus and development, as well as the *hsp70* gene and heat knockdown resistance have been discovered from a mid-latitude population along the east coast of Australia. There is also good evidence indicating that *D. melanogaster*

evolved Bergmann's clines on more than one continent. When raised in a common environment, both South American and Australian flies from high latitudes developed faster and matured at larger sizes than their counterparts from low latitudes (James, Azevedo, and Partridge 1997). Similarly, seasonal stresses at high latitudes, such as overwinter survival and ephemeral food resources, impose strong selection for increased body size and somatic maintenance, whereas warm climates with ample feeding opportunities favor reduced size and decreased maintenance (James, Azevedo, and Partridge 1997; Paaby and Schmidt 2009). Because the foraging behavior of organisms influences their ability to gather and assimilate food to fuel their somatic maintenance, one may expect the allele frequency of the *for* gene to differ in genotypes living at high latitudes and over stressful time (e.g., winter), relative to those living at lower latitudes and less stressful conditions.

Although recent studies have provided good insights on the maintenance of the *for* gene polymorphism in the laboratory, the significance and dynamics of such variation are yet to be investigated in nature. Studies based on experimental evolution suggest that density-dependent selection during the larval stage of *Drosophila*, rather than the adult stage, can maintain genetic variation in foraging behavior. For instance, a high larval density favors the rover variant and low larval density favors the sitter variant (Sokolowski, Pereira, and Hughes 1997). Similarly, negative frequency-dependent selection under low food condition promotes the increase in frequency of one variant when it is rare relative to the other variant (Fitzpatrick et al. 2007). In addition, Anreiter, Kramer, and Sokolowski (2017) showed evidence of an epigenetic regulator of the *for* gene (the *G9a* methyltransferase) responsible for rover-sitter differences in adult foraging behavior. Specifically, they showed that rovers bearing *G9a* alleles, had significantly greater foraging success than sitters, but this rover–sitter difference disappeared when they carried *G9a* null alleles. In sum, researchers have now detailed advances on the mechanisms that produce variation in the *Drosophila for* gene under controlled conditions. Yet, our understanding of the environmental drivers of the genetic variation that influences the foraging behavior of flies in nature remains unknown. A good approach to fill this gap is referred to as genotype-environment association analysis (Joost et al. 2007; Rellstab et al. 2015), a statistical tool used in the fields of landscape genetics and population genomics to identify the environmental factors that have shaped present-day genetic variation, and the gene variants that drive local adaptation.

Here, I present results of the first genotype-environment association analysis that quantifies variation at the *for* gene among samples of *D. melanogaster* structured across space and time. These samples consist of published genomes of adult flies collected worldwide, and at least twice per site of collection (during spring and fall). Thus, not only could I test whether the allele frequency of the *for* gene changes geographically,

but also in response to seasonality. In natural populations, quantitative traits that exhibit continuous geographic variation are often associated with specific ecological variables reflecting selective pressures acting on individual phenotypes. Though, evidence for local adaptation to continuous environments can be efficiently detected if there is highly significant association with the environmental variables at some loci compared with the background genomic variation (Kelley et al. 2006). Accordingly, a screening procedure to detect locus-specific signatures of positive selection that accounts for chromosome-wide patterns of polymorphism should then provide robust insights. This study provides an analytical framework that compares the allelic variation at the *for* gene relative to the variation of the whole chromosome 2L. In doing so, I found some degree of genetic differentiation among samples within America and Europe based on a genetic admixture analysis, suggesting a total of 9 ancestral populations ($k = 9$) with unique patterns of allelic variation at the *for* gene. Interestingly, I found a lower degree of genetic differentiation among populations through the screening of the whole chromosome 2L ($k = 8$). The results suggested that seasonality strongly contributed to the genetic differentiation of populations, but its effect depends on the site of collection.

3.2 Materials and Methods

Data source

The data used in this study represent a subset of a dataset assembled by the Drosophila Genome Nexus project (DGN), the European Drosophila Population Genomics Consortium (DrosEU), and the Real Time Evolution Consortium (DrosRTEC). This dataset is known as the Drosophila Evolution over Space and Time (DEST), and it is coupled with environmental metadata (see https://github.com/DEST-bio/DEST_freeze1/blob/main/populationInfo/samps_10Nov2020.csv). It constitutes the most comprehensive spatiotemporal sampling of *D. melanogaster* populations to date, including estimates of genome-wide allele frequencies for more than 270 populations. Collectively, the data are based on sequencing of more than 13,000 flies collected around the world over multiple years (Kapun et al. 2021). Importantly, the analyses of this study relied on pooled samples stored in the Genomic Data Structure (GDS) file named “dest.all.PoolSNP.001.50.10Nov2020.ann.gds”, available at <https://dest.bio/data-files/ SNP-tables>. Pooled samples from this file consist of 33-40 wild-caught males, which are more easily distinguishable morphologically from similar species than females are. Detailed information on quality control, mapping, SNP calling and dataset merging can be obtained from the DEST dataset mapping pipeline (see <https://dest.bio/sourcecode>).

I filtered the GDS object to restrict my focus on Single Nucleotide Polymorphism variants (SNPs) at the

for gene. I also performed a screening of the entire chromosome 2L to compare the levels of variation at the *for* gene relative to the variation of the genomic background. As annotated at <https://flybase.org/> (genome assembly version 6.32), the *for* gene is found on the left arm of chromosome 2 from base pairs 3,622,074-3,656,953. I further filtered the reads to get a subset of pools collected at least once during spring and winter across multiple years, ranging from 2014 to 2019 (see Table S1 for the exact number of season per locality). Although the data enabled me to assess the effect of seasonality on allelic variation at the *for* gene, I failed to test for an interaction between year and season, because both seasons could not always be sampled in the same year. Because the filtering process drastically reduced the number of pools in a few populations, I grouped together pools from the nearest neighboring localities such that the smallest populations had 5 pools. The resulting number of pools are distributed among 15 populations collected in Austria EU ($n = 6$), Charlottesville USA ($n = 6$), Denmark EU ($n = 5$), Esparto USA ($n = 5$), Finland EU ($n = 6$), France EU ($n = 10$), Germany EU ($n = 12$), Michigan-Wisconsin USA ($n = 9$), New York-Massachusetts USA ($n = 6$), Pennsylvania USA ($n = 18$), Russia EU ($n = 6$), Spain EU ($n = 7$), Tuolumne USA ($n = 5$), Turkey EU ($n = 13$), and Ukraine EU ($n = 38$). The DEST dataset provides the coordinates of each collection site, enabling one to obtain data about the seasonality of temperature and precipitation. These climatic variables were derived from the monthly values of temperature and rainfall for global land areas at a resolution of 30 s of a longitude/latitude degree spatial resolution (this is about 900 m at the equator) available at WorldClim v.2 (Fick and Hijmans 2017). In addition, I extracted log-transformed values of net primary production (NPP)—the net amount of solar energy converted to plant organic matter through photosynthesis—measured in units of elemental carbon. This variable represents the primary source of trophic energy for the world's ecosystems and can be obtained from the NASA Socioeconomic Data and Applications Center (sedac, Imhoff et al. 2004). These environmental variables were used in a genotype-environment association analysis as described below.

Quality control

To avoid the influence of atypical observations driven by samples with low coverage, I applied a minimum effective coverage N_{eff} filter of 28 (e.g., Nunez et al. 2022). I also removed pools and SNP loci with more than 20% missing genotypes. To do so, I used the *missingno* function from the “poppr” package of R v2.9.3 Kamvar, Tabima, and Grunwald (2014). Subsequently, I tested for deviations from Hardy–Weinberg equilibrium (HWE) using the function *hw.test* from the “pegas” package of R v1.1 Paradis (2010). This function calculated p-values of an exact test with a Monte Carlo permutation of alleles (1,000 replicates). I considered loci to be out of HWE if they deviated significantly in more than 50% of populations. This decision was based on a “false discovery rate” correction of the p-values.

Genetic differentiation and population structure

To test for genetic differentiation among populations at the level of the *for* gene, I estimated pairwise values of F_{st} adopting the method suggested by Weir and Cockerham (1984). The F_{st} values serve as a tool for describing the partitioning of genetic diversity within and among populations (Wright 1931). For example, large populations among which there is much migration tend to show little differentiation with values of F_{st} close to 0, whereas small populations among which there is little migration tend to be highly differentiated with values of F_{st} close to 1. I used the function *gl.fst.pop* from the package “*dartR*” v2.7.2 (Gruber et al. 2018), running 1,000 bootstraps across all loci to generate 95% confidence intervals of the values of F_{st} . When the confidence intervals did not include zero, the F_{st} value in question was considered significantly different from zero.

I estimated population structure based on admixture proportions. I performed this procedure for both the *for* gene and the chromosome 2L. Specifically, the admixture analysis uses a sparse negative matrix factorization method (SNMF), available in the R package “*LEA*” v3.2.0 (Frichot and Francqois 2015). Similar to Bayesian clustering programs such as CLUSTER, the function *snmf* in LEA estimates individual admixture coefficients from a matrix of allele frequencies. Assuming k ancestral populations, the function provides least-squares estimates of ancestry proportions and estimates an entropy criterion to evaluate the quality of fit of the statistical model through cross-validation. The entropy criterion enables one to choose the number of ancestral populations that best explain the data. Specifically, I used the function *snmf* in LEA to compute individual ancestry coefficients for a number of ancestral populations (k), ranging between $k = 1$ and $k = 10$. For each value of k , I ran the algorithm 50 times and calculated the cross-entropy for each run.

Seasonality analysis

To assess the effect of seasonality on allele frequencies at the *for* gene among populations, I used a constrained ordination analysis, which enables one to determine the genetic variation that can be explained by certain environmental variables or constraints. Accordingly, I performed a Constrained Correspondence Analysis (cca) through the function *cca* in the R package “*vegan*” v2.6-4. This function requires an independent variable (season), and a dependent variable (allele frequencies matrix). The output of this function decomposes the total variation into constrained and unconstrained components. To determine whether seasonality had a significant effect on the allele frequencies across populations, I used a permutation test using the *anova* method function. Accordingly, the data are permuted randomly and the model is refitted. When the constrained component in permutations is nearly always lower than

the observed constrained component, one says that the constraint is significant.

Detecting adaptation

To detect signatures of local adaptation, I performed a genotype-environment association analysis based on Latent Factor Mixed Models (LFMM) with a ridge penalty (Jumentier et al. 2022). Specifically, I first performed the analysis at the *for* gene alone, and then across the whole chromosome 2L, including *for*. The LFMM method is a univariate test that requires a matrix of allele frequencies as a dependent variable and an independent variable. Thus, I fitted three different models, each of which included a different predictor variable (e.g., precipitation, temperature, and NPP). The LFMM method also requires an estimate of the number of ancestral populations in the data (k), which relied on the admixture analysis discussed earlier. To correct for potential family-wise error rates, I fitted the models using the default genomic inflation factor correction (GIF) and a conservative False Discovery Rate (FDR) of 0.001 by converting the p-values to q-values. The FDR is the expected proportion of false positives among the list of positive tests (Storey and Tibshirani 2003). For example, an FDR threshold of 0.001 applied to a dataset with an ideal p-value distribution would produce 0.1% false positives (false discoveries) among the set of positive tests. However, if the distribution of p-values deviates from the ideal distribution, this same FDR threshold of 0.001 would produce more or fewer false discoveries among the set of positive tests, depending on the skew of the distribution.

Finally, I explored patterns of isolation by environment (IBE) and isolation by distance (IBD). I performed these analyses only on European samples considering that isolation by distance results in differences between populations at opposite ends of a more or less continuous group of populations. Unlike the European samples, the American populations were narrowly distributed on the west and east coasts, with no populations spanning the center of the country. In such cases, the dispersal distances might be too large for isolation by distance to be accurately detected. The IBE and IBD analyses require the estimation of distance matrices such as a genetic distance matrix according to Nei (1987), and both a matrix with ecological distances (e.g., precipitation distance) and a matrix with geographic distances (coordinates) based on Euclidean distance. To do this, I used a Multiple Matrix Regression with Randomization analysis (MMRR), which incorporates multiple regressions and can be extended to any numbers of predictor variables (Wang 2013). A multiple regression equation for distance matrices can be estimated using standard multiple regression technique, with the exception that tests of significance must be performed using a randomized permutation because of the non-independence of elements (Wang 2013).

3.3 Results

In general, none of the *for* loci nor populations were consistently out of Hardy-Weinberg equilibrium. I filtered out of the analyses only one sample that had more than 20% of missing genotypes, and 62 loci that had more than 20% of missing data. The quality control procedure generated a new dataset of 1,550 loci (SNPs), and 3,100 alleles from 151 pools distributed among 15 populations. Similarly, the quality control of the variants associated with the entire chromosome 2L resulted in a dataset of 931,161 loci, and 1,862,322 alleles from 151 pools distributed among 15 populations.

An analysis of genetic differentiation based on SNP variants of the *for* gene led to an overall F_{st} value of 0.032, but the pairwise differences in F_{st} ranged from 0 to 0.069. Interestingly, the analysis of genetic differentiation based on the *for* f_{st} values revealed two major clusters separating the pools collected in America from those collected in Europe (Figure 3.1). However, significant differentiation was also observed within each continent.

The genetic admixture analysis suggested a pattern of genetic clusters that approximates that observed from the F_{st} values. The *snmf* run at the *for* gene with $k = 9$ best accounted for population structure among all samples (Figure 3.2a). By contrast, a *snmf* run across chromosome 2L with $k = 8$ best accounted for population structure. Overall, I observed a distinct east-west gradient in population structure according to the admixture proportions (Figure 3.2b). This gradient seems to be stronger among the populations collected in America than those collected in Europe, although the structure of pools from Ukraine, Turkey, and Russia stands out (Figure 3.3).

According to the constrained correspondence analysis, an interaction between season and locality seemed to drive the difference in allele frequency across populations ($X^2_{29} = 0.076, p - value = 0.001$). Despite the substantial divergence of populations in response to seasonality, some populations seem to be overlapping and cannot be completely separated by the effect of seasonality (Figure 3.4).

From the latent factor mixed models performed at the level of the *for* gene and the entire chromosome 2L, I extracted locus-specific z-scores and adjusted p-values (Figure 3.5). Using $k = 9$ as suggested by the admixture analysis at *for*, the default GIF correction, and an FDR threshold of 0.001, I detected 2 candidate SNPs under selection in response to precipitation (Figure 3.5a). Interestingly, I found no loci under selection based on the effects of temperature and NPP. By contrast, the screening of the whole chromosome 2L polymorphism using $k = 8$ revealed only 52 candidate SNPs under selection in response to precipitation. Importantly, one of the SNPs under selection previously discovered at the *for* gene was also observed through the analysis of the entire chromosome 2L (Figure 3.5b).

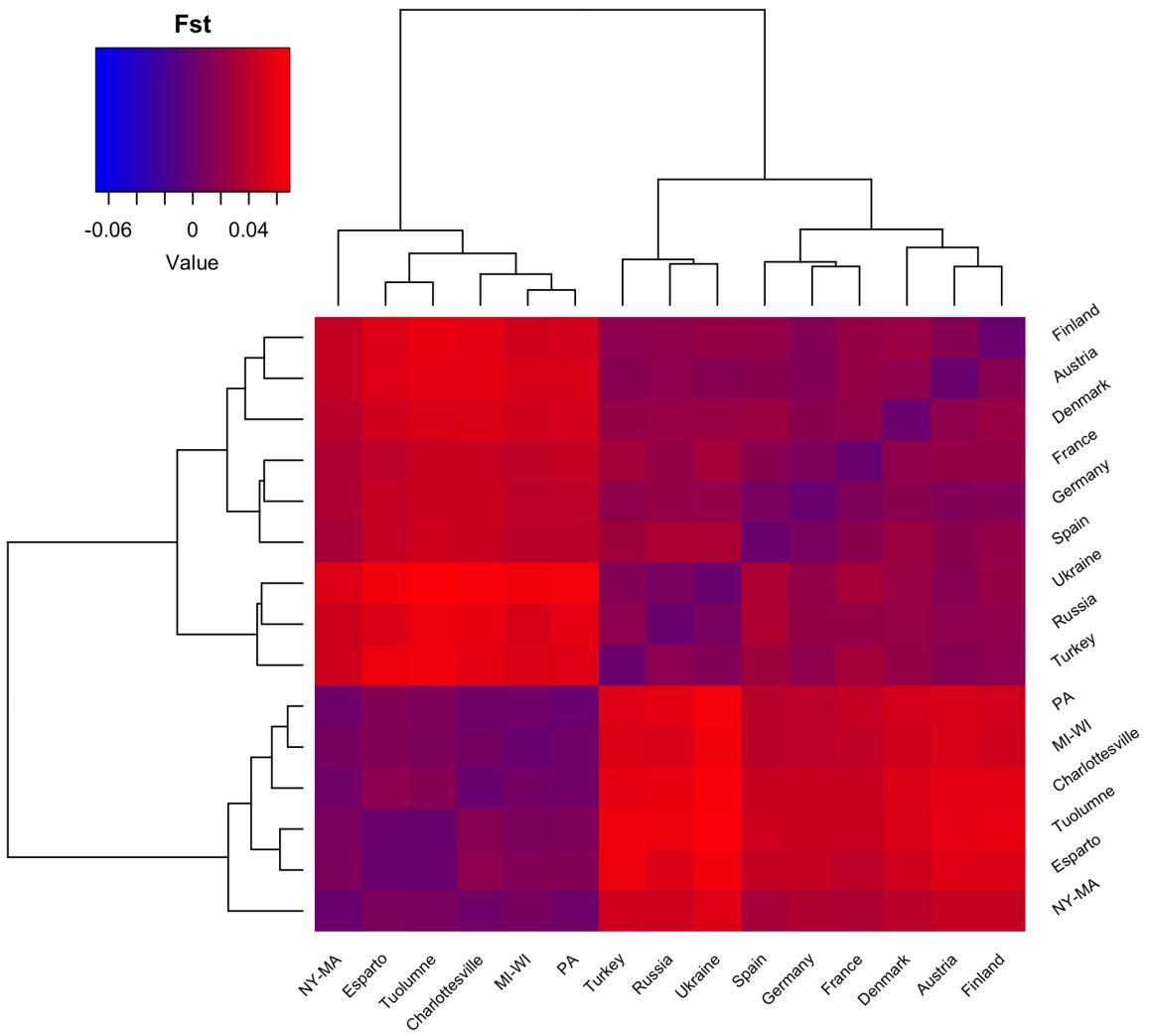


Figure 3.1: Heatmap depicting genetic differentiation among populations of *D. melanogaster* based on pairwise F_{st} values. In some cases, samples from adjacent localities were combined to avoid low sample sizes. Abbreviations are as follows: PA = Pennsylvania; MI-WI = Michigan and Wisconsin; NY-MA = New York and Massachusetts.

The IBE and IBD analyses based on the MMRR test showed an increase of genetic distance with geographic distance ($\beta = 4.728 \times 10^{-3}$, $t = 5.430$, $p - value = 0.001$). Similarly, the genetic distance increased with precipitation distance but the slope of the relationship was not significant ($\beta = 1.568 \times 10^{-3}$, $t = 1.433$, $p - value = 0.185$). These results suggested that populations are relatively similar at short distances, while this similarity decreases with geographic distance (Figure 3.6a-b).

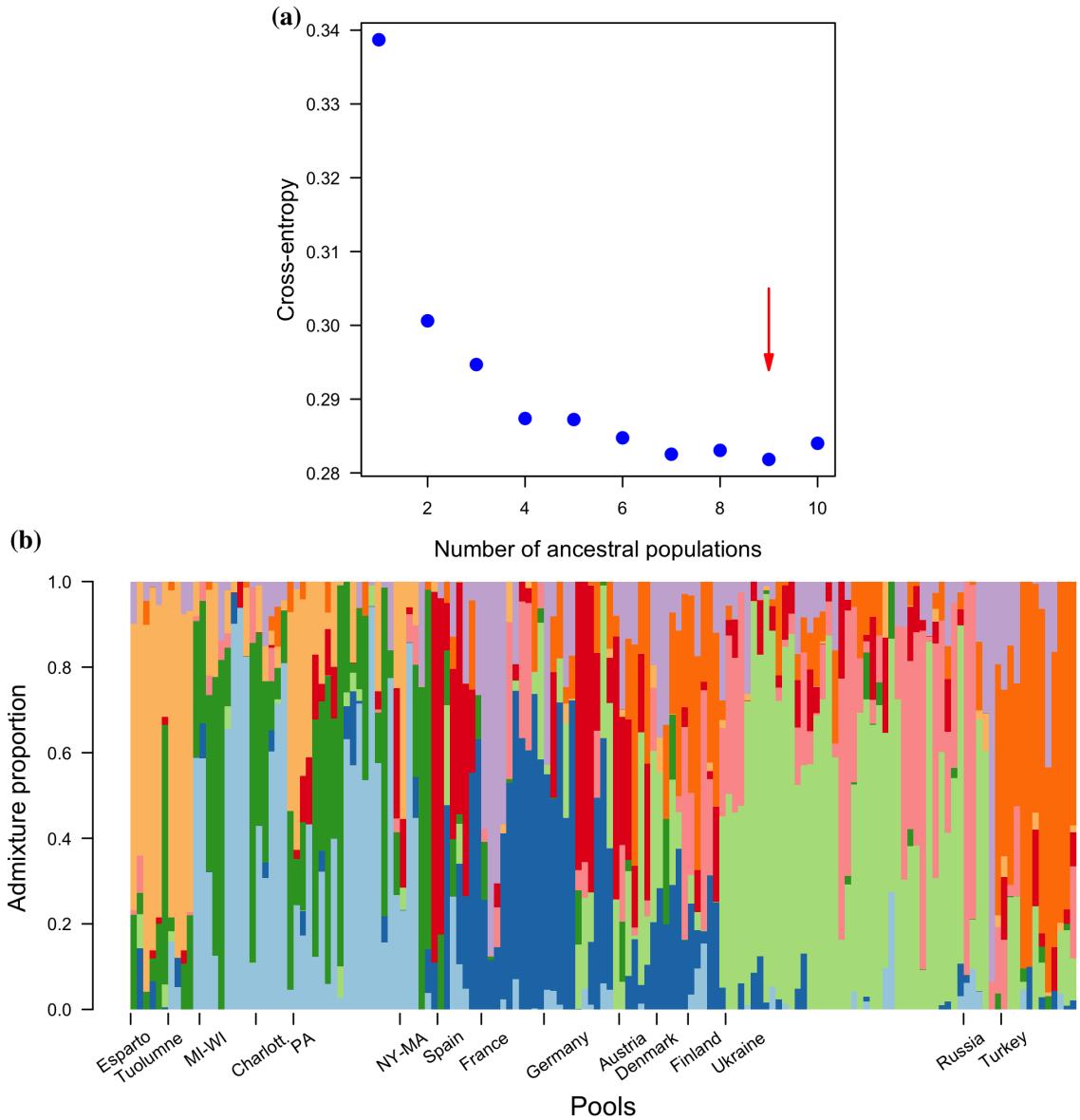


Figure 3.2: Population structure analysis based on individual ancestry coefficients for a number of ancestral populations. (a) Cross-entropy values for each *snmf* run with k ranging between $k = 1$ and $k = 10$. The red arrow indicates the most likely value of k . (b) Admixture proportion across populations of *D. melanogaster*. Colors indicate genetic clusters.

3.4 Discussion

This study shows that populations of *D. melanogaster* are geographically differentiated based on SNP variants of the foraging gene (*for*). Interestingly, the degree of genetic differentiation appears to be greater based on the allelic variation of the *for* gene relative to that of the genomic background. This finding was supported by estimates of the F_{st} index and individual admixture proportions among populations. Patterns of genetic differentiation among populations might have been shaped by both neutral processes such as demographic history, and adaptive forces such as environmental heterogeneity. Some evidence

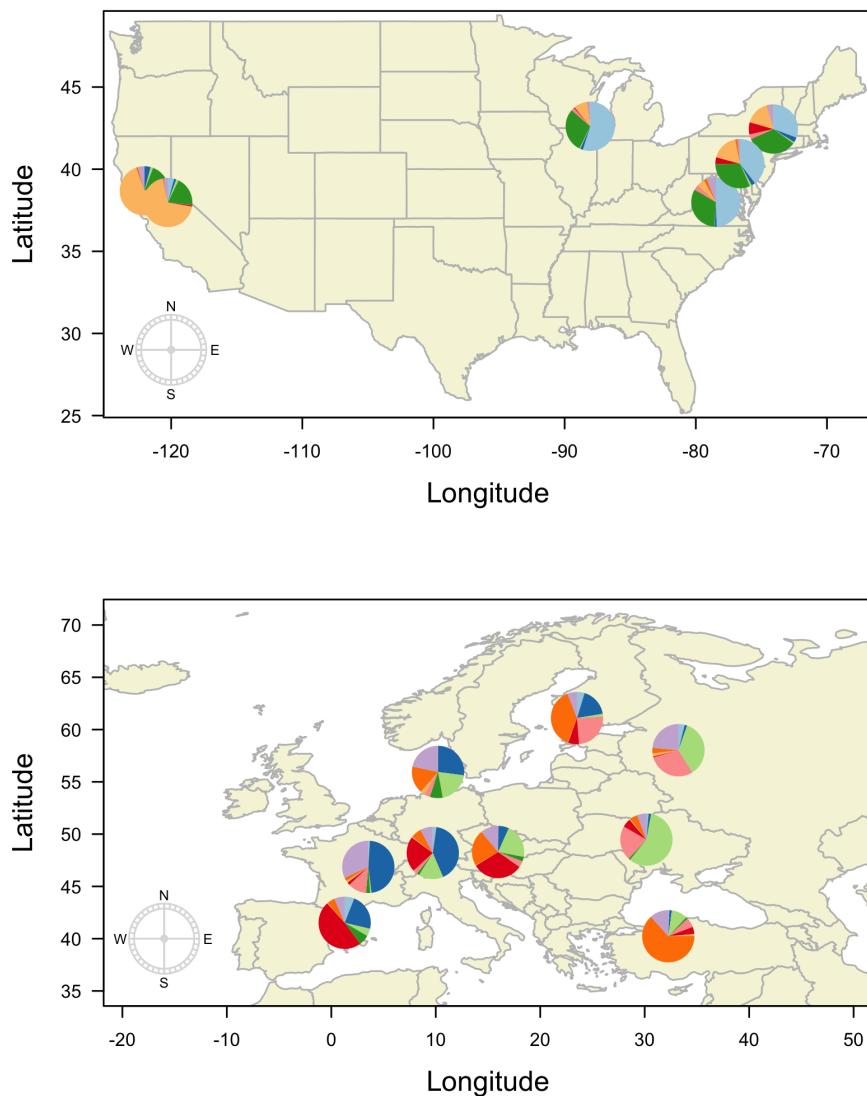


Figure 3.3: Mean admixture proportions across populations of *D. melanogaster* surveyed in America and Europe. Colors in the pie charts represent genetic clusters.

indicate that North American populations of *D. melanogaster* have been affected by African and European migrations associated with human cargo transportation and fruit trade around the world, contributing to genetic admixture worldwide in recent years (Bergland et al. 2016; David and Capy 1988; Kao et al. 2015; Lachaise et al. 1988). Numerous studies have also revealed clinal variation in phenotypes, chromosomal arrangements, and genotypes across environmental gradients for this species, implicating spatially varying selection (Flatt 2016; Hoffmann and Weeks 2007). This study suggests that genetic variation at the *for* gene seems to be influenced by seasonality, but this effect varies according to the site

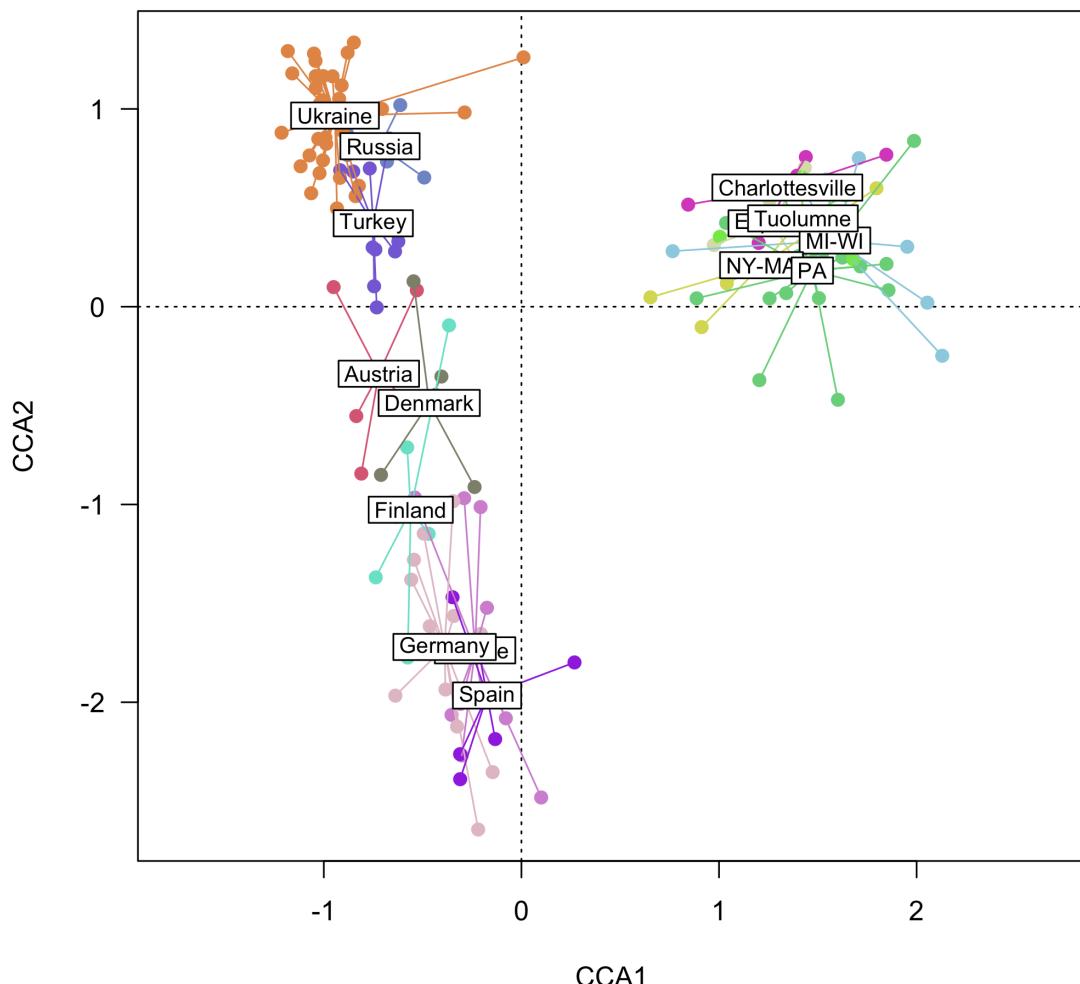


Figure 3.4: Constrained correspondence analysis depicting the difference in allele frequency across populations mediated by the interaction between season and locality. Filled dots represent pooled samples and "spider" diagrams connect the pools to the population centroid.

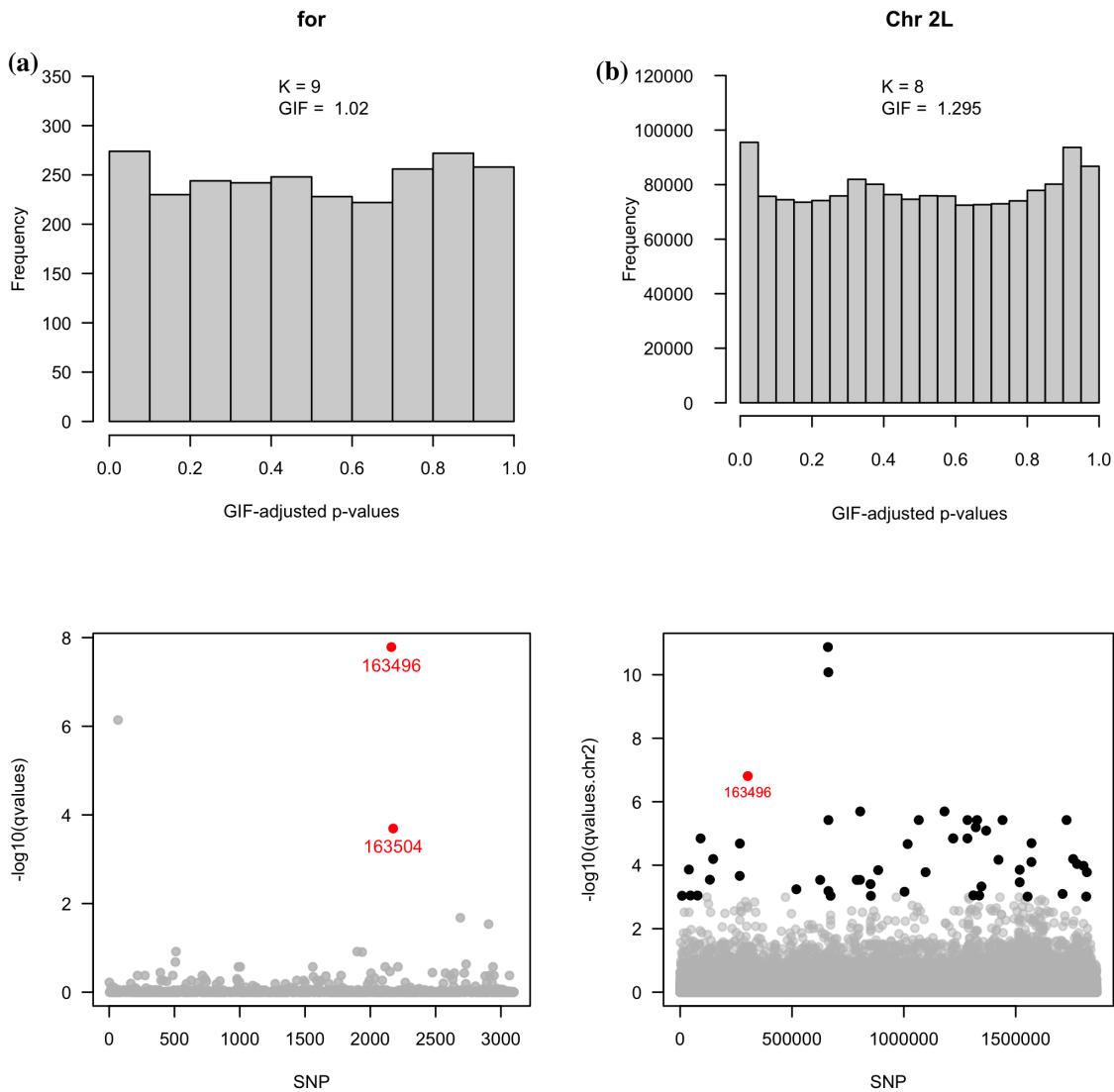


Figure 3.5: Genotype-environment association test based on a latent factor mixed model with ridge penalty. (a) Distribution of adjusted p-values using the default genomic inflation factor. (b) Manhattan plot of loci (SNPs) potentially affected by the PC1 predictor variable. Loci highlighted in red were considered to be under selection according to a False Discovery Rate of 0.05.

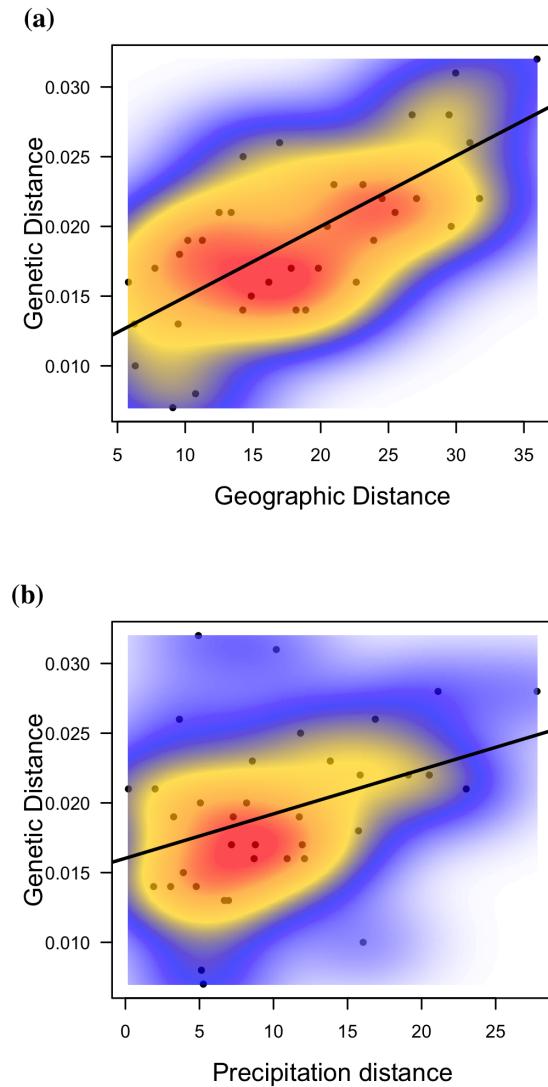


Figure 3.6: Patterns of isolation by distance and isolation by environment as suggested by the MMRR test. (a-b) Genetic distance as a function of geographic and precipitation distances. Colors represent estimated probability densities and the red line a smoothed local mean.

of collection. Although an effect of seasonality seems evident across populations, some populations cannot be separated by the effect of seasons. To understand such variation, the effect of natural selection should be disentangled from demographic events (Miller et al. 2020; Sexton, Hangartner, and Hoffmann 2014; A. Smith et al. 2020), and other unobserved factors. Those unobserved factors include uneven sampling designs, genome-sequencing biases, relatedness among individuals, gene interactions that affect phenotypic variation, and linkage disequilibrium within haplotypes (François et al. 2016). To deal with these factors, I applied a stringent correction to the p-values across the statistical test performed in genotype-association analysis (Balding 2006; Pearson and Manolio 2008; Korte and Farlow 2013). Displaying the empirical distribution of the adjusted p-values (see Figure 3.6) is a good way to show that confounding effects were removed from the analysis and that false discoveries could be controlled (Storey and Tibshirani 2003; McCarthy et al. 2008). Once confounding errors are removed, adjusting for multiple comparisons could then be achieved through the application of FDR control algorithms (Benjamini and Hochberg 1995; Storey and Tibshirani 2003).

According to the genotype-environment association analysis, I found two potential intronic variants under selection at *for*. One of the *for* variants remained under selection when the allelic variation of the genomic background was accounted for. The genotype-environment association analysis identifies loci that show strong correlations with one or more environmental variables, and corrects for confounding effects when the environment is correlated with population structure. When modeling the effect of the environment on genetic variation at the *for* gene, I found that seasonality strongly influences the allelic differentiation across populations of *D. melanogaster*. But the effect of seasonality is actually mediated by precipitation and temperature. Generally, abundant precipitation and radiation increase the rate of photosynthesis (Cramer et al. 1999), which translate into higher NPP conditions required for growth and reproduction of organisms at higher trophic levels. Consistent with this idea, the genotype-environment association analysis revealed that precipitation strongly influenced the variation in allele frequency across populations of *D. melanogaster*. If abundant precipitation causes NPP to vary spatially and temporally across the range of this species, genotypes better able to find food might prevail in environments with low NPP. Indeed, Williams, Rose, and Bradley (2004) found that food shortages in nature may often favor increased mobility in individuals of *D. melanogaster* to find food sources. Specifically, the authors showed evidence that flies become highly active when deprived of food. Yet, some organisms can adjust their foraging behavior to meet environmental changes in food availability (Schoener 1971). For example, northern pike and white-tailed deer may reduce food intake during winter, when food availability is low (Johnson 1966; Silver et al. 1969). In the context of the rover-sitter variants, a mechanism known as balancing selection could

explain the maintenance of these variants if they are exposed to environmental heterogeneity. Although this mechanism seems plausible in *Drosophila*, a recent study suggested that strong balancing selection at the *for* gene beginning recently (i.e., following migration out of Africa) is unlikely (Turner et al. 2015). The effect of precipitation as a spatially selective force could not be ruled out though, as the balancing selection test performed by the authors was based on a lineage-specific McDonald-Kreitman model, which does not consider the effect of the environment.

The positive correlations between genetic distance with both geographic and precipitation distances indicate that genetic differentiation between populations is consistent with signatures of IBD and potentially IBE. Accordingly, gene flow may be strong among populations that occur in close proximity and exploit similar environments. Different patterns of gene flow are expected under IBE and IDB models (Sexton, Hangartner, and Hoffmann 2014). One scenario predicts that with limited dispersal and no selection, genetic drift would cause populations to become more differentiated at greater distances, and that this process should be more pronounced as mean population size decreases (Wright 1943). Thus, under strict IBD, distance predicts differentiation as a result of dispersal limitation and drift, irrespective of environmental differences. A second scenario predicts gene flow to be strong among similar environments to a greater extent than predicted under IBD. However, adaptation to local environments can disrupt patterns of IBD, and subsequently IBE could arise if maladapted immigrants from different environments are selected against, causing strong barriers to gene flow. A good example of this scenario comes from plants growing on and near mine tailings, where adaptation to different soil types has occurred (Antonovics 1968). McNeilly and Antonovics (1968) showed that reproductive barriers arose through differences in flower bud development as a result of local adaptation to different soil types. Interestingly, a pattern of isolation by geographical and environmental factors has been reported recently in *D. melanogaster* (Yue et al. 2021). Using microsatellite genotypes, the authors showed an effect of precipitation on patterns of genetic variation across a latitudinal gradient. Similarly, a significant correlation between pairwise F_{st} and geographic distance was discussed in the context of IBD in a study that examined putatively neutral SNPs, mitochondrial haplotypes, as well as inversion and transposable element insertion polymorphisms (Kapun et al. 2020).

Currently, a number of studies have demonstrated that the foraging behavior of *D. melanogaster* can be strongly influenced by the *for* gene, but none of them have explored the environmental drivers of variation at *for* based on a genotype-environment association study. To my knowledge, this study provides the first evidence that naturally-occurring populations of *D. melanogaster* are genetically differentiated at the *for* gene as a result of environmental selective forces. Importantly, the patterns of genetic differentiation

at the *for* gene among populations remained evident when controlling for the genetic variation of the genomic background. Because the samples used in this study consisted of consensus sequences derived from pooled samples (33-40 flies), identifying foraging strains within populations could not be possible (sitters vs rovers). Regardless of this limitation, I showed evidence of an important genetic variation at the *for* gene in this study, but further evidence is required to support a hypothesis of a differential frequency of these strains in nature that can be attributed to balancing selection. Lastly, field studies could disentangle the contributions of selection and environmental structuring to patterns of gene flow. For example, one can conduct an experimental transplant study to understand how dispersal and habitat-specific selection interact to influence populations occupying heterogeneous environments. Although IBE and strong selection should promote local genetic adaptation, it is still unclear how often this is the case in *Drosophila*.

CHAPTER 4

FORAGING ACTIVELY CAN BE ADVANTAGEOUS IN HETEROGENEOUS ENVIRONMENTS

4.1 Introduction

A central goal of behavioral ecology is to determine how organisms exploit food in a given environment (MacArthur and Pianka 1966). In nature, food is distributed in patches that vary in size and density over time. In response to such environmental heterogeneity, organisms can adopt varying foraging behaviors that maximize their acquisition of energy (Schoener 1969). For instance, an active-foraging behavior is attributed to an organism that frequently abandons patches of food. By contrast, a sit-and-wait foraging behavior is attributed to an organism that rarely abandons patches of food. This behavioral dichotomy is currently known as “The Foraging-mode Paradigm”; a categorization that, albeit crude, remains useful to biologists for defining the extremes of a continuum. A seemingly tireless hummingbird that visits flowers in search of nectar, as opposed to a kingfisher that waits on a perch and swoop in the water when a fish passes by, are perfect examples to illustrate this dichotomy.

Since the early 1970s, researchers have developed mathematical models to specify which behavior is better suited for an organism to maximize energy intake in a particular environment, leading to the creation of the optimal foraging theory (Schoener 1971; Charnov 1976). However, the initial application of the optimal foraging theory was to explain the evolution of body sizes of organisms with little emphasis on their foraging mode. This theory was later extended in models that explicitly considered foraging modes as alternative strategies (e.g., Vitt and Congdon 1978; Janetsos 1982a). The models resulted in two main predictions: 1) Organisms should have a simple decision rule for leaving a foraging site. They should leave when the expected gain from moving surpasses the expected gain from remaining at the site. 2) If variation in foraging behavior affects the energetic benefits or costs, then growth rate, body size, and reproductive output should vary among individuals that forage differently. This expectation is supported by the idea that different behavioral strategies determine the life histories of organisms by limiting their acquisition and allocation of energy. An allocation tradeoff suggests that an increment in energy allocated to one function results in a decrement in energy allocated to other functions. Thus, an individual that acquire greater surplus energy may growth faster, have both a smaller body size and greater lifetime reproductive output (Stearns 1992; Roff 2002).

Such predictions were evaluated in a few elegant works fueled by the increasing interest in behavioral ecology at the time. The first empirical evidence derived from field studies of lizards (Vitt and Congdon 1978; Vitt and Price 1982). By comparing reproductive output among species, Vitt and Congdon (1978) and Vitt and Price (1982) showed that active foragers invested less in reproduction than did sit-and-wait foragers. The explanation proposed for this pattern was that carrying a voluminous clutch while pursuing a prey increases the probability of being killed by a predator or reduces the efficiency of foraging. Interestingly, a different study on orbweaver and sheetweb weaver spiders indicated that active foragers incur lower energetic costs than do sit-and-wait foragers (Janetos 1982b). Orbweavers decided whether to stay or leave the web based on the abundance of prey they capture in a day. By contrast, sheetweb weavers seemed to be sit-and-wait predators, staying on the web for a longer time and only leaving it at random. Surprisingly, the sheetweb weavers pay a much higher energetic cost for constructing a new web from body reserves than do orbweavers. Importantly, these analyses might have been confounded by the evolutionary relationships among species, because phylogenetic comparative methods were available only after Felsenstein's foundational paper (Felsenstein 1985).

Around the same time, Sokolowski (1980) discovered a gene in *Drosophila melanogaster* with major effects on foraging behavior. This gene, referred to as "for", influences the distance that fly larvae travel while foraging. Larvae were classified as either rover or sitter, with rovers traveling significantly longer distances than sitters when exposed to a substrate containing nutrients. Although the discovery of *for* stimulated active research into the evolution of foraging behavior and its connection to the life history, the current evidence among studies is rather conflicting. For example, while early observations in laboratory conditions suggest that, in nutrient-poor environments, rover larvae have higher survivorship and faster development than sitter larvae (Kaun et al. 2007), more recent observations indicate that the fecundity between rovers and sitters does not differ under adversity nor standard conditions (Burns et al. 2012). Therefore, the expectation of differences in the life history of organisms with varying foraging behavior requires further investigation.

The well-characterized behavioral polymorphism of *D. melanogaster* provides an excellent opportunity to resolve such discrepancy. Accordingly, I aimed to experimentally test two long-standing predictions of a relationship between foraging behavior and the life history. First, I hypothesized that the foraging behavior may depend on the distribution of food in the environment. In a patchy environment, my prediction is that the proportion of the area explored by rovers is expected to be larger than that of sitters while foraging. But this difference would become negligible if food is uniformly distributed in the environment. This prediction was based on recent evidence suggesting that adult rovers dispersed more than sitters

when the total amount of food increased with the number of patches (Edelsparre et al. 2021). A second hypothesis is that foraging behavior affects the energetic benefits and costs in ways that translate into life-history variation. In this case, I expected the sitter larvae to grow faster than the rover larvae in a uniform environment, while the opposite pattern was expected in a patchy environment. Furthermore, differences in growth were expected to be more pronounced when food was clumped rather than patchy. As described earlier, this expectation is supported by the idea that different behavioral strategies determine the life histories of organisms by limiting their acquisition and allocation of energy to growth (Reid, Prince, and Croxall 2000; Roff 2002; Bayne 2004; Angilletta, Steury, and Sears 2004).

4.2 Materials and Methods

Fly genotypes

The rover (*for^r*) and sitter (*for^s*) genotypes used in the experiments have isogenized *for^r* or *for^s* 2nd chromosomes, sharing isogenized X and 3rd chromosomes from the rover B15 genotype, as described in Bauer and Sokolowski (1985) and Sokolowski (1980). I maintained flies with these genotypes at 25°C in a 12:12 hr light/dark cycle at 60% relative humidity. I reared populations of flies in ~ 240 ml round-bottom plastic bottles, with a standard yeast-sugar-agar medium as suggested by Anreiter et al. (2016). Before the beginning of each experimental trial, I transferred the flies into empty bottles and capped them with grape plates containing a small amount of dry-active yeast to stimulate reproduction. After 22 hr, I removed the grape plates from the bottles and discarded all larvae from each plate with a dissecting probe. I then incubated the eggs that remained in the grape plates for 4 hr in standard conditions as described earlier. After 4 hr, I picked L1 larvae per genotype from the grape plates and placed them in food plates (i.e., yeast-sugar-agar medium). Lastly, I collected the testing larvae (L3) about 10 hr before wandering, which generally corresponds to 72-96 hr after hatching (Anreiter et al. 2016).

Locomotor performance assay

I estimated the locomotion of larvae in two types of environments by computing the proportion of area covered while foraging. One environment consisted of yeast paste distributed in patches in a matrix of Drosophila agar medium, whereas the other one consisted of a single patch in the same medium. I prepared these environments in 32 × 10 mm petri dishes. To make the patchy environment, I used a 12 ml insulin syringe to pour small drops of dry-active yeast mixed with water at a 1:2 ratio (weight to volume). Patches were separated from each other by a distance of 25 mm, creating a square grid pattern whose vertices consisted of 25 patches each. I used the same method to make the uniform environment,

but this time I poured the paste in such a way that a food clump formed at the center of each plate. I assured that the consistency of the paste, the volume used ($2 \mu\text{l}$), and the configuration of the food were the same among the test plates.

After setting up the test plates, I released an L3-stage larvae in each plate, randomizing the combination of genotype, environment, and position of release among plates. To randomize these factors, I used the *sample* function available in the free software R v.4.3.2 (2023-10-31, Team 2013), which enabled us to pick a sample of a specified size ($n = 1$ in this case) from a vector of predefined elements (e.g., a vector of two characters: rover and sitter). I then transferred plates to an incubator set up at 25°C and 60% relative humidity. After a period of 1 hr, I recorded the larvae for 30 min, using a camera held 30 cm above the plates. In each trial, I recorded four plates simultaneously as indicated in Figure (Figure 4.2). This experiment yielded data for $n = 92$ larvae; 46 larvae of each genotype were randomly divided into two groups ($n = 23$) to be tested in uniform and patchy environments. At the end of the experiments, all of the larvae were transferred back to food plates where they continued to develop.

To analyze the video recordings, I used the free software AnimalTA v.2.2.1 (Chiara and Kim 2023); a video-tracking software that enabled me to analyze videos recorded under the same conditions.

Growth assay

To quantify growth, I let L3-stage larvae develop in the same environments described earlier. I measured growth as the difference between the initial mass and the final mass of the larvae after a 24 hr. To record the initial mass, I gently washed the larvae with 1 or 2 ml of water and dabbed them dry with a paper towel to avoid any confounding factor when weighing the larvae. I then weighed the larvae using a micro-analytical balance (Mettler Toledo Model XPR6UD5 $6.1 \text{ gr} \times 5^{-7}$), and transferred them to test plates. The test plates were placed in an incubator set up at 25°C and 60% relative humidity. After 24 hr, I weighed and recorded the final mass of the larvae following the same procedure described above. This experiment yielded data for 27 rovers, and 31 sitters in the patchy environment, and 29 and 22 sitters in the uniform environment.

Data analysis

I fitted competing models to investigate the effects of genotype and environment on the proportion of area visited by the larvae in the test plates. Because the mass of the larvae might affect their locomotion, I corrected for the potential effect of mass in the models. Similarly, I modeled the effects of genotype and environment on the growth of the larvae. Ny analyses enabled us to test the main effects and the interactions between the independent variables. To do so, I fitted Generalized Least Squares models (GLS)

to account for heterogeneity in the data, and Ordinary Least Squares models (OLS). To evaluate the models' goodness of fit, I used information-theoretic criteria such as AICc. I ranked the candidate models accordingly and selected the most likely one for inferences (lowest value of AICc).

To produce a good visualization of my results and ensure that they are fully reproducible, I carried out all the analyses in the free software for statistical computing R v.4.3.2 (2023-10-31, Team 2013).

4.3 Results

The analyses supported a model describing the effects of genotype and environment on the proportion of area covered by the larvae while foraging (Table 4.1). Two lines of evidence aligned almost entirely with this result. First, rovers covered larger areas than did sitters within each environment, yet the area exploited by either genotype was generally larger in the patchy environment compared to that exploited in the uniform environment (Figure 4.1A). Second, the video-tracking experiment revealed that rovers generally traveled longer distances than did sitters, but I found no differences across environment (Figure 4.2).

Table 4.1: Parameters estimated by the most likely model describing the effect of environment on larval growth.

	Value	Std.Error	t-value	p-value
(Intercept)	1.713e-04	1.479e-05	1.158e+01	1.334e-20
environment	1.574e-04	3.102e-05	5.075e+00	1.644e-06

Although rovers traveled further and covered more area than sitters, both genotypes grew at similar rates. According to the analysis, a model describing only the effect of patchiness of the environment on growth was strongly supported. Larvae of both genotypes grew faster in uniform environments than they did in patchy environments (Figure 4.1B). In either type of environment, sitters grew slightly faster than rovers did. In addition, they spent a longer proportion of time in food patches than rovers did (Figure 4.2).

4.4 Discussion

The results suggest that foraging behavior is a plastic trait remarkably shaped by the distribution of food in the environment, which is consistent with the findings of recent investigations (e.g., Anreiter and Sokolowski 2019; Edelsparre et al. 2021). Based on theoretical models, I predicted that both genotypes would cover more area in a patchy environment compared to a uniform environment. This expectation relies on the fact that food was available over a larger area in a patchy environment; therefore, the

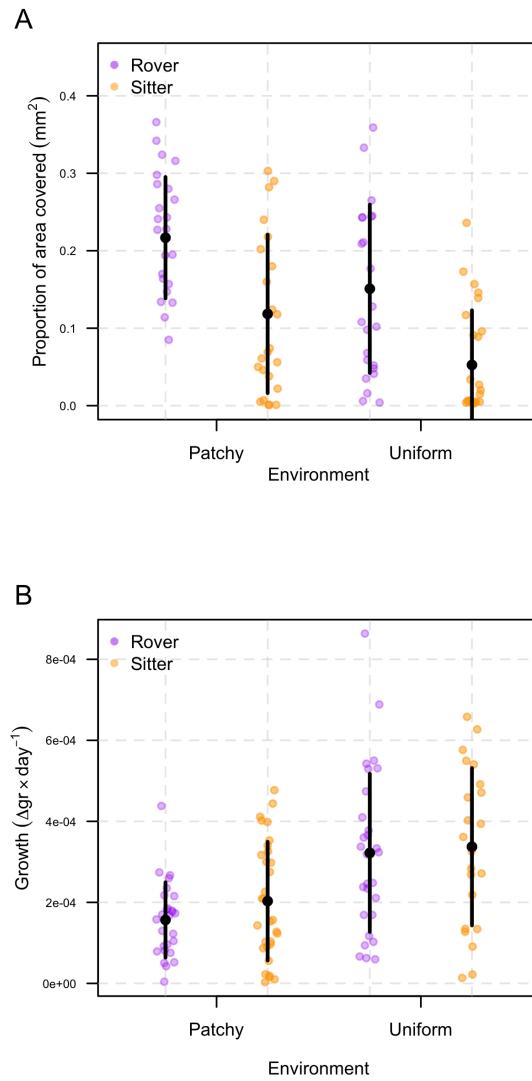


Figure 4.1: Statistical comparisons among the predictors involved in the experimental design of the study. A) Effects of genotype and environment on the locomotor activity of the larvae. B) Effects of genotype and environment on larval growth. Black dots represent the estimated means and the bars the standard deviation.

Figure 4.2: Animated visualization of the distance traveled by the larvae together with the proportion of time spent at specific sites of the test plates. A darker coloration indicates a longer proportion of time spent at a site (s). A) Patchy environment. B) Uniform environment. *For the best visualization, readers should view this illustration in Adobe Reader 9 or a later version: <http://www.adobe.com/products/reader/>.*

energetic return from moving would likely surpass that of remaining in a patch. By contrast, the uniform environment only contained a single clump of food in one location. In the latter case, leaving this sole patch of food would reduce the acquisition of energy. Following the same notion, I expected a patchy environment to cause a more pronounced difference in locomotion between genotypes. This predicted interaction between genotype and environment was not supported by my results. These findings suggest that the relationship between patch distribution, patch density, and the locomotion of organisms is likely more complex than expected.

A key assumption underlying my predictions was that organisms behave to maximize the net energy intake during foraging. Because the rate of energetic gain or loss greatly affects fitness (Reid, Prince, and Croxall 2000; Roff 2002; Bayne 2004; Angilletta, Steury, and Sears 2004), assessing which foraging strategy yields the highest surplus energy in a given environment becomes crucial to understanding the evolution of behavior (Schoener 1971; Charnov 1976). Often, investigating the connection between what is being optimized (i.e., foraging behavior) and a “fitness component”—such as growth—offers the opportunity to accomplish such a task (Flatt 2020). When testing the effects of foraging behavior on growth, I discovered that the uniform environment generally stimulated a faster growth than did the patchy environment. Interestingly, however, both genotypes grew to similar sizes in either environment even though rovers generally covered larger areas than did sitters while foraging. These observations were not entirely consistent with my predictions as I expected the rover genotype to decrease the locomotor activity and grow slower in the uniform environment. Because food was relatively abundant in each environment, and genotypes were tested individually, the density of food was not depleted at the same rate as it would be in the presence of competitors. Thus, there may have been little incentive to minimize daily foraging time and energy by rovers. The minimization of daily energy expenditure might not be necessary when food is relatively abundant, but a highly efficient energy-expensive foraging strategy, such as pursuing the food, would become advantageous as it saves time for other critical activities (Norberg 2021).

Based on the results of this study, the question remains which foraging strategy is better suited to maximize energy intake in a particular environment. The evidence indicates that foraging actively could be advantageous when food is either patchy or clumped. Two kinds of mathematical models provide support to this claim. As described above, an energetically demanding but efficient strategy of searching for food enables animals to save time for other activities, such as reproduction (Norberg 2021). In addition, eco-evolutionary models suggest that the ability of rovers to travel longer distances while foraging influences their dispersal (“high-dispersing” strategy). In its simplest form, dispersal can be defined as any movement with potential for genetic mixing (Ronce 2007), facilitating local adaptation via founder events, gene flow, and life history trade-offs (Hanski and Mononen 2011). If foraging actively promotes higher dispersal, such behavior may evolve fast and lead to the colonization of new environments (David Reznick and Ghilambor 2001). Although the dispersal ability of a larva is lower than that of an adult, previous investigations suggest that behaviors that are expressed early in life are closely integrated with a suite of life-history traits that enhance colonization ability, and often retain flexibility in expression throughout an organism’s life (Roff 1977). Such strategies are commonly described in insects that express winged

dispersive and wingless nondisperser morphs (Harrison 1980; Roff 1986; Zera and Denno 1997). In other animals, such as birds, highly dispersive behaviors are often associated with aggressive behaviors that can enhance survival and competitive ability in a novel environment (Dingemanse et al. 2003; Duckworth and Badyaev 2007).

Taken together, this study implemented a simple yet elegant way to test some of the long-standing predictions of the so-called “foraging-mode paradigm”. I suggest that foraging behavior is a plastic trait molded by the distribution of food in the environment. My findings support the notion that animals maximize their energy intake by adjusting their locomotion according to environmental heterogeneity. In wild populations of *D. melanogaster*, this could mean that rovers would influence the rate of population spread to a higher extent than would sitters. As such, foraging actively could have important implications for colonization or range expansion to novel habitats through the subsequent evolution of life-history traits. Although the interpretation of my results are limited to the larvae of *D. melanogaster*, evidence of correlated evolution of foraging behavior in larvae and adults enables us to make predictions at both developmental stages (Pereira and Sokolowski 1993; Hughson et al. 2018; Sokolowski 1980). It is important to point out that fitness should be measured over entire life cycles. Thus, the possibility exists that the appropriate time intervals for maximizing the benefits of foraging behavior is over a longer period than I consider in this study.

CHAPTER 5

CONCLUSIONS

I have argued throughout this work that the study of foraging behavior has become a paradigm in evolutionary biology because of its connection to the life history of animals. While some species forage widely over space to acquire food, others sit in place and wait for prey. A prevailing view is that different behavioral strategies determine the life histories of organisms by limiting their acquisition and allocation of energy to growth and reproduction. Also, it is often thought that widely foraging species have lower reproductive output than sit-and-wait species, because moving long distances imposes a risk of predation and a heavy reproductive load decreases the chance of escaping a predator. Despite these views, only a handful of studies have examined the effects of foraging behavior on life-history traits, and such studies have yielded conflicting results. Thus, the long-standing prediction of a relationship between foraging mode and life-history traits demands further investigation.

To address this issue, I first examined how the availability of resources shapes the evolution of life-history traits among 486 species of lizards, belonging to 34 families. Chapter 1 shows that abundant resources enables females to invest more in reproduction. This chapter indicates that precipitation is the most likely driver of the evolution of reproductive traits in lizards. Generally, abundant precipitation and radiation increase the rate of photosynthesis, which provides the energy required for growth and reproduction of organisms at higher trophic levels. We now know that lizards with larger amounts of energetic resources produce both larger hatchlings and larger clutches. Although I do not know the selective pressures responsible for this pattern, theoretical models tell us that parental care or intraspecific competition could play a role. In lizards, experimental studies of life-history evolution have lagged behind comparative studies. Though, phylogenetic path analysis enabled me to disentangle some evolutionary relationships among life histories. This analysis sharpens our focus on mechanisms underlying major evolutionary patterns, stimulating the development of a more general life-history theory.

Chapter 2 consists of an analysis of data for 485 species of lizards, representing 32 families, to test the traditional view about the relationship between foraging modes and reproductive output, as well as an opposing view based on life-history theory. In this chapter, I presented the first evidence that widely foraging species actually have greater reproductive output than sit-and wait species, which I attributed to

a greater rates of energy acquisition and mortality among mobile foragers. Such observation challenges traditional generalizations, calling into question basic assumptions of the foraging-mode paradigm.

In chapter 3, I further pursue this area of research with a genomic analysis involving *Drosophila melanogaster*. The molecular characterization of the foraging gene (*for*) in *Drosophila* suggests that flies can be divided in two genotypes: widely-foragers (or rovers) and sit-and-wait foragers (or sitters). This chapter presents results of the first genotype-environment association analysis that quantifies variation in the *for* gene among samples of *D. melanogaster* structured across space and time. My observations indicate that different patterns of gene flow as expected under models of isolation by environment and isolation by distance are likely driving the genetic differentiation among populations.

Given its behavioral polymorphism, *Drosophila melanogaster* provides a good opportunity to assess which foraging strategy yields the highest surplus energy in a given environment. Chapter 4 indicates that foraging behavior is a plastic trait remarkably shaped by the distribution of food in the environment. Active foragers and sit-and-wait foragers increase their locomotion when food is patchy rather than clumped, but the locomotion of the former is generally greater. Although both genotypes grow at a similar rate, my findings suggest that foraging actively can be advantageous in heterogeneous environments. The high-dispersing ability of widely foragers may facilitate local adaptation via founder effect, gene flow, and life history evolution.

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