UNIVERSIDADE DE LISBOA
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Intraspecific variation in functional traits in the wolf spider *Lycosa fasciiventris*: Implications for trophic cascades

"Documento Definitivo"

Doutoramento em Biodiversidade, Genética e Evolução

Jorge Filipe Pereira Henriques

Tese orientada por:
Prof. Doutora Sara Magalhães
Doutor Jordi Moya-Laraño

Documento especialmente elaborado para a obtenção do grau de doutor

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FCT – Fundação para a ciência e tecnologia

Documento especialmente elaborado para a obtenção do grau de doutor

2019

Acknowledgments

It has been a long way until I finally started to write this section, the only part in the whole thesis, which is deprived of any opinions and corrections and, as such, I feel the most responsibility in doing it properly. If someone is reading this and found its name missing…. I am sorry, I probably will realize you are missing here soon after delivering this thesis.

By finally writing these words I am getting the feeling that this thesis is finally coming to a term like the end of a long journey home. It has been a very rich experience, not without its lows but that, ultimately, contributed greatly to my individual growth, not just academically but also personal. It is also a reminder of the past experiences, the motivation that have drove me this way but also what compels me to go beyond. This last part is certainly a concluding chapter of a journey and, like any journey, this demand would not have been possible without the companionship of my fellow travelers. Therefore, in this section I will acknowledge all the people that made the completion of this thesis possible and get the change to acknowledge all those who contributed not only for the scientific part but all the other in-betweens.

First, I would like to express my highest gratitude to my two supersivors, Sara Magalhães and Jordi Moya-Laraño. Its thanks to them that I can be delivering this thesis, as without their guidance, this thesis would not have taken place and certainly would not have took the path it took to its final form. I want to thank you both for the guidance and all the "pruning" concerning this research project. I want to thank you for all the knowledge you transmitted me and all the help in guiding this ship to a safe port. Both your contributions were essential during this journey and I hope the end result of this project is something you can be proud of.

Although, before this thesis jumpstarted, I have to start by acknowledging the people who supported and were willing to listen and discuss my ideas and hopes within this project. As such I have to certainly express my thanks to both my co-tutors: Manuela Coelho and Cristina Branquinho for their patience and willingness to listen to my expectations and ideas and who did their best to council me properly and drive me into the direction to which I eventually ended up.

After I did not find what I thought to be a suitable project according to my expectations and desires I started looking for other directions and it was not long before Sara took me under her wings and together with Cristina proposed me a solution for my indecisions. That was when Jordi entered in the equation and we started to plan an exciting and challenging project which I took with eager ambition. And so, this PhD project was born and soon I found myself fully engaged in this new chapter.

It was in a blink of an eye that I was dispatched to Almeria for a “short” stay to learn more about this new world of arachnology and quantitative genetics. A short stay that ended up lasting 2 and a half years… During this time, I was well received in the EEZA and had the opportunity to meet new and interesting people. I was readily welcomed by the “spider team” with whom I worked through the next years. Thanks Lolli, Celeste, Pillu, Mariángeles, Eva, Iñaki. A special thank you note to Eva De Mas for all the warmth and attention that she gave me during my whole stay in Spain. Not only was Eva essential in this project but also introduced me to the Andalusian wilderness and my stay in Almeria would have been the same without those photo trips in Cabo de Gata.

I wish to thank all the other colleagues from “Chumbo” that made every break a enjoyable moment and made my stay in Almeria an enjoyable experience. Thanks Bea, Cristina, Manu, Monica EG, Monica M, Raul, Jesus, Paco and many others that I may be forgetting.

I also want to thank the EEZA technicians which helped in every need that I had during the long days in the lab. I am talking specially about German who I bugged from time to time to ask for some material or just for some random reallocation of shelfs, the perfect workout in the EEZA. Thanks also to Pillar for listening to some of my ideas and Angel for the effort to work with the spectofotometer. I would also like to acknowledge Ramon and Alberto for the tech support when something went wrong.

Also, a big thank you to all the Portu’s of Spain, who certainly contributed to a joyful stay in Almeria, which always found a way to convince me to leave work and go for some fiesta. I will forever remember all the relaxing moments that I had the privilege to share with you, either in the sunny beaches of Cabo de Gata, just diving or chilling or in Vila Tritón. For sure, without all of you my stay would have been more lonesome and a lot more boring. I will also miss all the discussions during padel, the “noisy” guitar lessons (thank you “Yoyes”), and the beautiful trips in the *Posidonia* forests. By the way, this group of people included Gustavo, Morillo, Paula, Paco, Giussi, Ary, Antonio, Juan An, Juan, Pollo and Nieves y claro Milka y Pongo. Muchas Gracias Chic@s!!!

I would like to leave a special thank you to Gustavo for all the coffees and beers shared and for all the adventures lived in Almeria, some of them which I will not address here... “Unita, hein…” :P. Also, to Morillo, who always had a kind and wise word to share in any situation along with the whole philosophical discussions that took place occasionally.

Back in Lisbon, I had the most pleasure in being hosted in the Mite Squad, the powerhouse that is the group of Sara. I wish to thank each and any one of you for receiving me and letting me feel welcomed. This group includes Flore, Leonor, Diogo, Miguel, André, Lucie, Inês S., Inês F., Inês C.P., Cátia, Jéssica, Maud, Alpedrinha, Alexandra, Catarina, Agnieszka and Raul! It is indeed a remarkable group of individuals, not without a high degree of intraspecific variation. I would like to acknowledge all the workout you provide me once a month and the rollercoster of joy and amusement that is travelling with you either to congresses or just hiking!!

Also, I want to thank all the colleagues from Lisbon, past and present, which transmit me their excitement and inspiration and the occasional (or not) beer just around the corner. I would like to thank Paula Gonçalves for all conversations and counselling as well as the companionship. Also, to Adriana Silva for all the support, the long talks and all the other great moments during this PhD. Thank you Gonçalo for the all beers and the workout sessions. Many thanks also to Adrià, João Carvalho, Flávio, Sara, Vera and many others which I had the pleasure to share an office space, play sports from time to time and share many discussions.

I am very grateful for the long-lasting relationships that have lasted over these years which endured despite the long distance and my recurrent absence. I am truly lucky to count as friends people from the places where I previously lived/worked despite my absence and, one may say, negligence. To all of you, that I am constantly delaying a reunion: Many thanks for not losing your faith in me!!! I would like to specially acknowledge Joana and Fox, for always having a bed available when I needed to crash. I would also like to thank João Batista and Rui Pimpão for all the meetings throughout the years, no matter in what places of the world we are, even if in front of a screen as it has been a long time since the three of us have met in person.

I want also to express my gratitude to all my friend from my hometown with whom I grew up with, who constantly remind me of my roots and who I am, and of course, for all the meetings that make my visits to this hometown feel a warm homecoming.

For last, but certainly not the least, I would like to express my highest gratitude to my family, specially to my parents and sister for all the support, warmth and motivation not only for this PhD but for all the other stages that my career and life in general have been through. I also welcome this opportunity to apologize for not being there in the meaningful moments and for your comprehension for my everchanging life plans and absence.

Resumo

Os ecossistemas e as comunidades são constituídos por indivíduos que interagem entre si. A estrutura dessas comunidades é definida pela magnitude e pela natureza dessas interações. Uma das interações biológicas mais importantes num ecossistema é a interação entre predadores e presas. As populações naturais são constituídas por um conjunto diverso de indivíduos, não existindo dois indivíduos iguais do ponto de vista funcional. Esta diversidade individual gera diversidade nas interações entre organismos, variando assim o impacto que estes têm na dinâmica populacional e consequentemente na estrutura das comunidades.

Com a ressurgente e crescente atenção dada às diferenças entre indivíduos dentro duma mesma população, o impacto da variação intraespecífica tem recebido cada vez mais destaque em estudos de ecologia. Dados recentes indicam que a variação intraespecífica é surpreendentemente alta, podendo mesmo ultrapassar a variação observada entre espécies diferentes, levantando a questão sobre de que modo esta variação afeta os processos ecológicos. Nesta tese, foquei-me na variabilidade em características relacionadas com a procura de alimento ou o evitamento de tornar-se alimento doutros. Estudos recentes têm demonstrado que esta variação é bastante alta e pode afetar fortemente as interações entre predadores e presas e consequentemente a estrutura das comunidades.

Para um melhor entendimento dos potenciais efeitos da variação intraespecífica nas comunidades, é fundamental avaliar as fontes de tal variação. Assim, é importante determinar se esta variação é devida a fatores genéticos ou ambientais, pois isso irá determinar se os impactos desta variação têm um significado evolutivo ou se estes efeitos afetam apenas processos puramente ecológicos.

Para além disso, uma análise completa do efeito da variabilidade individual nos ecossistemas deverá incluir o estudo da associação/correlação entre características, pois estas podem determinar o leque de valores de cada característica que é expresso. A expressão destas características está também dependente das condições ambientais às quais os organismos estão expostos e assim sendo, possíveis interações com o ambiente devem também ser considerados completando o “quadro” da arquitetura genética.

O principal objetivo desta tese é medir estas fontes de diversidade em características relacionadas com a interação entre presas e predadores e testar o impacto potencial desta diversidade na estrutura das cadeias tróficas. Como modelo de estudo usámos a aranha-lobo *Lycosa fasciiventris*, um predador canibal em cadeias tróficas de solos, que habita em zonas áridas. De modo a medir a diversidade de várias

características, assim como avaliar a origem de tal variação, procedeu-se á realização de vários cruzamentos levando à obtenção de famílias de meios-irmãos. Assim, cruzou-se cada macho com duas fêmeas distintas. Dentro de cada família, separou-se a progenia em dois grupos, tendo uma parte sido alimentada com o triplo da quantidade de alimento dos restantes. Este último passo permite avaliar o impacto do ambiente nestas características e avaliar possíveis interações com o ambiente. Após o nascimento, as crias foram retiradas das suas mães e isoladas em recipientes individuais de modo a evitar canibalismo entre estas. Durante o seu ciclo de vida foram medidas características morfológicas, fisiológicas e comportamentais que incluíram tamanho e condição corporal ao nascerem, taxa de crescimento, eficiência de assimilação, rácio de tamanho presa-predador e características comportamentais associadas ao canibalismo como atividade, ousadia, cautela e exploração.

Com este desenho experimental pôde-se medir efeitos genéticos aditivos, efeitos maternais e interações ambientais (interações entre o genótipo e o ambiente e interações entre efeitos maternais e o ambiente).

Nesta tese analisámos uma série de padrões comportamentais em resposta a pistas depositadas por conspecíficos. As características comportamentais associadas a resposta a perigo de canibalismo foram ousadia, cautela, exploração e atividade. Estas respostas foram avaliadas ao colocarmos cada aranha no interior de uma arena experimental onde metade desta continha pistas de outros conspecifícos, excluindo graus de parentesco, tendo sido o seu comportamento gravado e analisado através de imagens vídeo. A ousadia avaliou-se como a percentagem de tempo passado na zona com pistas em comparação com a zona sem pistas, cautela como a redução de velocidade observada quando os indivíduos se deslocavam na zona com pistas e exploração como a diferença nos índices de atividade na zona com pista em comparação com a zona limpa.

Apesar de ter sido observada uma elevada variação fenotípica, esta não se deve a efeitos genéticos ou maternais. No entanto, estas características apresentam fortes correlações entre si. Assim, indivíduos com maiores índices de ousadia são mais cautelosos e apresentam um menor índice de exploração quando se deslocam em locais onde houve deposição de pistas provenientes de conspecíficos. A forte correlação entre estas três características comportamentais sugere a existência de um síndrome comportamental, apesar deste não ser explicado por fatores genéticos. Este síndrome indica que a expressão destas características não é independente, sendo os valores duma delas constrangido ao das outras. Este constrangimento leva à manutenção de diversidade intraespecífica permitindo a existência de um continuo de estratégias no que toca á resposta a pistas de conspecíficos no seio da população, promovendo a diversidade em diversos contextos ambientais. Estes padrões comportamentais aparentam ser explicados pela presença de pistas (i.e., ambiente social). A perda de peso verificada em indivíduos durante a recolha de pistas (que assumimos estar associado á produção de pistas) parece apresentar uma base genética e consequentemente a variação no ambiente social parece apresentar o potencial evolutivo para a manutenção de diferentes estratégias para lidar com canibais, através de efeitos genéticos indiretos.

Quanto às restantes características avaliadas, os dados revelaram que grande parte da variância é devida a efeitos maternais. As características para as quais encontrámos uma forte predominância de efeitos maternais foram o tamanho e a condição corporal dos indivíduos ao nascer, a eficiência de assimilação, a taxa de crescimento e o rácio de tamanho entre predador e presa. Adicionalmente, deslindamos interações entre os efeitos maternais e o ambiente, não havendo indícios da existência de interações entre genótipo e ambiente. Foram detetadas interações entre os efeitos maternais e o ambiente para a eficiência de assimilação e a taxa de crescimento. Para além disso, estas duas características apresentam uma correlação maternal positiva entre elas, indicando que indivíduos com taxas de crescimento superiores apresentam também uma maior eficiência de assimilação. Também encontrámos uma correlação maternal negativa, entre o tamanho ao nascer e o rácio que determina o tamanho máximo de presa que um indivíduo pode capturar, indicando que indivíduos que nascem com um tamanho corporal superior mostram uma preferência por presas de menores dimensões mais tarde no seu ciclo de vida. A significância destes parâmetros mostra uma clara predominância de efeitos maternais como fonte de variação intraespecífica e covariação entre as diferentes características.

Por último, avaliou-se o impacto da diversidade intraespecífica na intensidade de cascatas tróficas. Para tal, foram constituídos mesocosmos onde a diversidade intraespecífica foi manipulada e os seus efeitos na cascata trófica avaliados. Calcularam-se diferenças entre famílias devido a efeitos maternos e usou-se o grau de diferenciação entre famílias para designar indivíduos a usar em cada unidade experimental (mesocosmo). Também se mediu a variação nos caracteres devida ao ambiente e os seus impactos na cascata trófica. Para isso designaram-se aranhas consoante o ambiente onde estas foram criadas (muito ou pouco alimento) e constituíram-se mesocosmos com variabilidade ambiental, onde os indivíduos provinham de ambos os ambientes, ou sem variabilidade ambiental onde os indivíduos provinham do mesmo ambiente (muito ou pouco alimento). Adicionalmente, foi constituído um tratamento de controlo onde não foram colocados predadores. Assim os mesocosmos foram constituídos segundo as seguintes condições experimentais: alta diversidade maternal + ambiente homogéneo

(pouco alimento); diversidade maternal + ambiente homogéneo (muito alimento); baixa diversidade maternal + ambiente homogéneo (pouco alimento); baixa diversidade maternal + ambiente heterogéneo (pouco + muito alimento); controlo (sem predadores). Com este desenho experimental, pôde constatar-se que a variância maternal para características de predadores afeta a magnitude das cascatas tróficas, dado que mesocosmos com maior diversidade intraespecífica apresentaram cascatas tróficas com maior intensidade que mesocosmos com baixa diversidade intraespecífica. Este efeito é provavelmente produzido por efeitos indiretos produzidos sobre o comportamento da presa. O grau de canibalismo também varia entre tratamentos, podendo explicar, ainda que parcialmente, as dinâmicas observadas. Como esta variação é essencialmente devida a efeitos maternais, os resultados destacam a importância desta fonte de variação, não apenas como fonte de diversidade, mas também pelos seus impactos em processos ecológicos. Apesar da notória diferença na taxa de canibalismo observada para os mesocosmos onde indivíduos provinham de ambientes diferentes, isto não se traduziu num efeito significativo na cascata trófica. Adicionalmente, também não se observaram diferenças entre os contextos ambientais, não importando se indivíduos provinham de ambientes com muito ou pouco alimento.

Assim, os dados apresentados nesta tese demonstram que os efeitos genéticos indiretos, maternais e ambientais são importantes fontes de diversidade em características associadas a interações tróficas e que, através destas, podem modular os processos ecológicos, nomeadamente as cascatas tróficas.

Resumindo, o trabalho desenvolvido ao longo desta tese permite-nos entender mais profundamente quais os fatores que regulam a diversidade intraespecífica de características associadas ao consumo (de conspecíficos e de presas) e como estes podem impactar importantes processos ecológicos como as cascatas tróficas.

Palavras-chave (5): Genética quantitativa, variação intraespecífica, canibalismo, predação, Lycosidae

Abstract

Intraspecific variation has recently been acknowledged as an important factor affecting ecosystems. Indeed, levels of intraspecific variation in natural populations can be very high, even surpassing interspecific variation. In particular, variation in foraging traits can significantly impact ecological processes. The aim of this thesis was to measure the relative strength of drivers of intraspecific variation in foraging traits in the cannibalistic wolf-spider *Lycosa fasciiventris* and its potential impacts on trophic interactions and consequently trophic cascades.

We evaluated trait variation using a half-sib split brood design, by crossing each male with two females and separating offspring from each family into 2 different rearing environments. The offspring was scored for several morphological, physiological and behavioral traits and heritability, maternal effects and environmental interactions (genotype-by-environment and maternal-by-environment interactions) were determined as well as genetic and maternal correlations. Maternal effects were predominant over additive genetic effects in all traits and their correlations, except in traits related to cannibalism, in which both genetic and maternal effects were absent. In this case, trait variation was explained by the social environment faced by spiders (conspecific cues).

We then evaluated the impacts of intraspecific variation in ecological processes by performing mesocosms experiments. To this aim, we experimentally manipulated intraspecific variation and evaluated its effects upon trophic cascades. Results showed that variation in foraging traits due to maternal and environmental effects can modulate ecological processes, namely trophic cascades. Mesocosms with higher trait diversity displayed stronger trophic cascades, attributable not to a higher prey mortality but to a change in their behavior, while environmental diversity was reflected in a higher rate of cannibalism but not enough to dampen trophic cascades. The results of this thesis highlight the importance of maternal effects and other indirect genetic effects as drivers of intraspecific variation, modulating ecological processes and shaping community structure.

Keywords: Quantitative genetics, intraspecific variation, maternal effects, cannibalism, foraging traits, Lycosidae

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C**areful sneaker: bolder spiders move more cautiously in presence of conspecific cues**

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CHAPTER I

General introduction

Predator-prey interactions are key to understanding the mechanisms underlying food webs. However, predator foraging traits have been classically considered homogeneous within a specific population, despite the fact that traits vary within populations, and this variation may shape predator-prey interactions, potentially affecting food web structure and dynamics.

The importance of intraspecific variation in ecological systems has been highlighted in recent studies (Schreiber et al. 2011; Violle et al. 2012). Indeed, it has been shown that this variation can be as large as that across species (Albert et al. 2010). Therefore, intraspecific variation may influence community structure and ecosystem function to a similar degree as variation among species (Palkovacs and Post 2009). This is particularly true if such variation is expressed in traits responsible for the outcome of predator-prey interactions such as foraging and antipredator traits.

In this thesis, I will evaluate the origin and degree of variation in predator foraging traits and test how it affects top down control in a simple food web. In this introduction, I will describe the foraging traits under study, then refer to potential sources of intraspecific variation for those traits. Subsequently, I will explore the importance of such variation in modulating ecological processes. Finally, I will provide a brief description of the biology of the model species used in this study, the wolf-spider *Lycosa fasciiventris*, and then present the thesis outline.

1. Foraging traits and predator-prey interactions

Predation is one of the most important ecological interactions shaping community structure and ultimately ecosystem services. Classic approaches describe predator-prey interactions based on functional responses, i.e. the predator per capita consumption rate as a function of prey density, and numerical responses, i.e. changes in predator density in response to changes in prey density (Angerbjörn et al. 1999). These approaches suffer from an over-simplification of predator-prey interactions, by a) assuming that individuals are functionally equivalent and b) ignoring that prey consumption results from the integration of various behavioral and physiological traits in both predators and prey. Therefore, characterizing predator-prey interactions solely based on the functional and numerical response is insufficient for a proper prediction of the impact of predators upon prey populations and consequently food web dynamics.

In predators, foraging or trophic traits are any morphological, behavioral or physiological traits determining the ability to successfully capture prey (Gravel et al. 2016). One of the most important foraging traits is body size, which is expected to determine "who eats who" in a given food web (Woodward et al. 2005; Brose et al. 2006) Specifically, predator-prey size ratio is a central feature in trophic interactions defining the threshold size ratio at which a predator is able to subdue and consume prey. However, this ratio is not fixed, as it can vary for example with the degree of prey limitation (Costa-Pereira et al. 2018). Additionally, this ratio may vary across individuals, with some being more prone to attack, kill and consume larger prey than others (Brose et al. 2008; Okuyama 2008). From this point of view, this ratio can be considered an individual trait.

However, body size can change throughout individual ontogeny, and this strongly impacts who eats whom (Magalhães et al. 2005a; de Roos and Persson 2013). Therefore, studies using this trait in the foraging context should complement the analysis by including the individuals' growth rate. This trait measures changes in body size throughout ontogeny. Variation among individuals in growth rates can expand the diet breath of predators and potentially lead to a greater incidence of intraspecific predation, i.e., cannibalism (Moya-Laraño 2011).

Body condition (i.e., the energy reserves stored within individuals) can also severely affect predation rates, because variation in this trait determines different motivational status and physiological needs (Vrede et al. 2004; Woodward et al. 2005; Moya-Laraño et al. 2008). This, in turn, can be reflected in prey selection levels and in foraging activity.

Besides varying in their ability to pursue and subdue prey, predators may also differ in their ability to efficiently convert food into biomass. Such assimilation efficiency is defined as the amount of mass gained per amount of prey consumed. Assimilation efficiency is expected to affect both the growth rate of predators and the amount of prey eaten. Therefore, this trait can deeply impact food web dynamics (Metcalfe et al. 1995; Jones et al. 2002).

Despite the importance of morphological traits in defining predator-prey interactions, behavioral traits can also affect the outcome of predator-prey interactions. Behavioral traits may also display high diversity within populations, with many recent studies emphasizing the consistency of these differences over time and across contexts (Sih et al. 2004; Sih and Bell 2008; Dall et al. 2012). Consistent behavioral variants are defined as behavioral types or personalities while correlation in behavioral traits are defined as behavioral syndromes (Pruitt et al. 2012).

Besides consumption, predators can affect prey in non-consumptive ways, interfering with their activity. In response to predators, prey may develop specific sets of behaviors, often disrupting their normal foraging activity when predation risk is perceived. Anti-predator behaviors may include avoidance of locations with predators, reduced locomotion, increased cover-seeking or vigilance (Lima and Dill 1990; Lima 1998). These responses may also have a physiological component, a stress response which is characterized by increased energy input into functions to support prey escape and/or maintain a state of alertness (Hawlena and Schmitz 2010; Van Dievel et al. 2016).

The display of these responses relies on the perception of predation risk, which is often assessed through chemical cues emitted by predators (kairomones). Prey can also extract information from environmental cues related to a given predator. For example, they may detect predators through its excreta, which may provide information on the kind of predator or its diet (Persons et al. 2001; Magalhães et al. 2005b), or via the alarm pheromone emitted by conspecifics (Janssen et al. 1997). As such, upon recognition of predation risk, behavioral traits in prey can determine the outcome of predator-prey interactions (Réale et al. 2007).

1.1. Living in a cannibal world: Trophic interaction between conspecifics

Predator-prey interactions can also occur within a species, which is referred to as cannibalism. Cannibalism is a widespread phenomenon, occurring in a wide diversity of generalist predators (e.g. amphibians (Anderson et al. 2013), fish (Pereira et al. 2017) and insects (Bayoumy and Michaud 2015)). Cannibalism may be beneficial, given that it allows the acquisition of high-quality food sources and the elimination of potential exploitative predators (Polis 1981). Its possible costs include injury or death, transmission of pathogens and parasites, lower inclusive fitness if prey and predator are kin, and a reduction in the number of potential mates (Elgar and Crespi 1992). It has been hypothesized that cannibalism evolved primarily as a means of foraging for calories and nutrients when supplies are limited (Wise 2006). In line with this, a few studies show that starvation levels strongly affect rates of cannibalism (Samu et al. 1999; Roberts and Gavery 2012; Gavín-Centol et al. 2017).

Cannibalism has been studied mostly from the perspective of the factors promoting its occurrence (Roberts et al. 2003) or the traits that underly such biotic interaction (Gavín-Centol et al. 2017). However, beyond the context of sexual cannibalism in which males are forced to meet potential cannibals (e.g. Moya-Laraño et al. 2004) few studies have focused on the anti-predator traits responsible for avoidance of cannibalism (but see Sih 1992)). Studying behavior towards cannibalism can be very challenging as it is not straightforward to assess if individuals are behaviorally reacting towards conspecifics as potential predators or as potential prey. Individuals involved in these interactions can fulfill the roles of predators and prey at the same time and therefore, properly determining if these are displaying anti-predator or foraging strategies is not a trivial task.

2. Intraspecific variation in foraging traits

Several studies have demonstrated that phenotypic trait variation within species (intraspecific variation) can be as high as trait variation across species (interspecific variation) (Albert et al. 2010; Violle et al. 2012; Barabás and D'Andrea 2016). While trait variation within populations is known to be the raw material for natural selection, ecological studies tend to disregard potential variation among individuals. However, it has recently been acknowledged that intraspecific trait variation can substantially alter ecological dynamics (Bolnick et al. 2011). Indeed, individual variation in resilience to abiotic factors (Meyer et al. 2009), resource use (Bolnick et al. 2003) or competitive ability (Duffy 2010) can lead to variation in demographic parameters such as recruitment (Ortego et al. 2007).

Given that foraging determines predator-prey interactions, it is expected that variation in traits related to this ecological function strongly impact the outcome of species interactions and community structure as predicted by theory (Schreiber et al. 2011; Barabás and D'Andrea 2016; Jonathan et al. 2016). This prediction is confirmed in a few empirical studies. For example (Post et al. 2008), showed that intraspecific variation in feeding morphology (gape width and gill raker spacing) had an impact on the strength of the trophic cascade caused by predators.

To properly understand the impacts of intraspecific variation in ecological processes, we need to identify the underlying sources of intraspecific variation. Knowing if such variation possesses an underlying genetic basis is of major importance as genetic variation allows trait evolution, which can alter the mean strength of interspecific interactions or allow coevolutionary dynamics that may promote coexistence (Saloniemi 1993). This eco-evolutionary feedback is a topic that has recently been reborn under the label of eco-evolutionary dynamics (Schoener 2011).

2.1. Drivers of phenotypic variation

Quantitative genetics provides the framework to quantify the sources of phenotypic variation underlying a particular trait. Depending on the breeding design used, the most basic approach allows partitioning variance components into additive genetic (σ_a^2), maternal (σ_m^2) and remaining residual variance (σ_{res}^2), being the total phenotypic variance

(σ^2_{phen}) the sum of these components (Falconer and Mackay 1996; Lynch and Walsh 1998).

$$\sigma^2_{phen} = \sigma^2_a + \sigma^2_m + \sigma^2_{res}$$

2.1.1. Additive genetic effects

As additive genetic variance is the only variance component that responds to selection, the focus in quantitative genetic studies is generally to estimate this variance component.

Narrow-sense heritability (h^2) is defined as the proportion of the total phenotypic variance (σ^2_{phen}) that is due to additive genetic effects (σ^2_a).

$$h^2 = \frac{\sigma^2_a}{\sigma^2_{phen}}$$

Heritability can be estimated from the degree of resemblance between relatives (Falconer and Mackay 1996). One of the most commonly used methods to estimate heritability is the regression of offspring phenotypes on those of their parents (parent-offspring regression). However, this method requires information from both the parent and offspring generations. Another popular method is sib analysis. Within sib-analysis there are three types: half-sibs, full-sibs and combinations of both. These family structures allow to partition phenotypic variance into within and among-family variances. Specifically, the half-sib design, which is applied in this study, allows to disentangle additive genetic effects from maternal effects. A typical paternal half-sib design involves the random mating of *N* males to *n* different females. All the progeny of a given male is thus unrelated to progeny of other males. The main advantage of this design, and one of the reasons of why I applied it here, is that one can have quantitative genetic estimates on juveniles without needing to rear all animals to adulthood, which is particularly cumbersome for long-leaving organisms. This is particularly important when the questions involved do not necessarily have to be tested on the adult stages, which in large arthropods are the least frequent phenotypes.

Surprisingly, however, only a handful of experimental studies have measured additive genetic variation in predator foraging traits (Hedrick and Riechert, 1989; Henryon et al., 2002; Jia et al., 2002; Metcalfe et al., 1995; Nachappa et al., 2010). These have reported substantial genetic variation in foraging traits including behavioral (Hedrick and Riechert 1989), physiological (Henryon et al. 2002) and morphological (Páez and Dodson 2017).

2.1.2. *Maternal effects*

Intraspecific variation in foraging traits may also be due to maternal effects. Beyond direct gene transfer, maternal effects are defined as the causal influence of the maternal phenotype on the offspring phenotype (Wolf and Wade 2009). This includes the indirect genetic contribution of a mother to its offspring, but also the influence of the maternal environment on offspring phenotypes (Mousseau 1998). Apart from a few exceptions (e.g. Mcadam et al., 2002; Taylor et al., 2012), the role of maternal effects in defining trait variation has not been deeply explored and consideration of this driver of phenotypic variation is urged in future studies.

2.1.3. *Environmental effects*

As most metabolic and developmental pathways are influenced to some degree by the environment, the expression of most quantitative traits is not under purely genetic control. Therefore, considering the environmental conditions in which individuals developed is of most importance as it can affect the expression of genetic variance and other components of variance, such as maternal effects.

Genotype-by-environment interactions (G x E; Robinson and Qvarnström, 2014; Wade, 2014) occur whenever the genetic variance changes according to environmental conditions while maternal-by-environment interactions whenever the maternal variance components changes according to the environment in which offspring grow (M x E; Vega-Trejo et al., 2018). The existence of G x E interactions in a population indicates that different genotypes respond to environmental changes in different ways. In extreme cases, the ranking of genotypes may be altered simply by a change in the environment. G x E in foraging traits has been studied for a wide range of taxa in environmental setups such as food availability and temperature (e.g. Vieira et al., 2000). In contrast, only very recently has M x E been specifically measured (Vega-Trejo et al. 2018).

Since maternal effects can also have a genetic basis, both G x E and M x E interactions can contribute to maintain genetic variation, as these allow for different phenotypes to be favored across different contexts, leading to their maintenance within the population.

2.1.4. *Correlation among traits*

A complete understanding of how variation in foraging traits may affect predator-prey interactions requires addressing correlations among such traits.

Traits may be correlated among each other forming either behavioral syndromes, when these are exclusive to behavioral traits, or functional syndromes, when these correlations include functional traits in general. The existence of such syndromes imply that individuals cannot fully express the range of behavioral/functional responses within a population. If traits are positively correlated, it means that individuals cannot express, at the same time, the highest value for one trait and the lowest for another. As an example, there is often a positive correlation between boldness and exploration (Mazué et al. 2015). While exploration may be adaptive because individuals are more likely to find resources, boldness may more likely expose individuals to predation. Thus, correlations may limit the trait variation that is actually expressed, resulting in suboptimal phenotypes. However, since two correlated traits may each show an advantage in different contexts, these correlations may also enhance the maintenance of intraspecific variation within populations.

Similarly to variation, phenotypic covariation can be decomposed into additive genetic, maternal and residual (co)variance components (Dochtermann and Roff 2010). Additive genetic variances and covariances are jointly included in what is defined as the **G** matrix with the trait’s additive genetic variances in the diagonal and the additive genetic covariances between traits in the off-diagonal. Variance-covariance matrices for the other variance components (maternal and residual) can also be estimated.

If trait covariation is explained by additive genetic effects, those traits are genetically correlated. Genetic correlations have the potential to alter and constrain the rate and direction of the response to selection (Cheverud 1996; Roff 1997). Despite its importance, only few studies deal with correlations among foraging traits. Among the exceptions, for example a genetic correlation between growth rates and risk taking behavior has been demonstrated in salmonids (Biro et al. 2004).

3. Ecological consequences of trait variation

As described above, understanding trait variation is key for a deeper understanding of how individuals interact, the outcome of such interactions and ultimately, its impact on community structure and ecosystem processes. Differences in traits of predator species are known to alter prey abundance and composition, impacting ecosystem functioning (Schmitz and Suttle 2001). Classical ecological studies have explored how predator identity and abundance structure prey communities and alter trophic cascades (Schmitz et al. 2000), where a trophic cascade is the propagation of predator impacts down in the food web (Pace et al. 1999). However, these studies generally overlook intraspecific trait variation, which can have important impacts in community structure (Bolnick et al. 2011) and even for food web persistence (Moya-Laraño et al. 2014) and the associated ecosystem processes.

Intraspecific variation in foraging traits implies that individuals within a predator population experience different interactions, potentially attacking different prey types or species (Bolnick et al. 2003), or being vulnerable to different predators (Reimchen 1992), or parasites (Wilson et al. 1996). Therefore, it is of great importance to determine how this intraspecific variation affects the structure and dynamics of populations, communities and ecosystems.

A small but growing set of theoretical studies suggest that intraspecific variation in foraging traits can have profound effects on populations (Doebeli 1997), predator-prey interactions (Rudolf 2008) and coexistence among competitors (Hart et al. 2016). Some of these theoretical predictions have been tested by experimentally manipulating intraspecific variation. Indeed, empirical studies have demonstrated that diversity can enhance population productivity (Crutsinger 2006) or stability (Agashe 2009; Hughes and Stachowicz 2009), increase the abundance or diversity of higher trophic levels (Crutsinger 2006), alter rates of nutrient cycling (Madritch et al. 2006; Crutsinger et al. 2009) and allow eco-evolutionary feedbacks concerning predator-prey interactions (Post and Palkovacs 2009). Some of these studies focus on bottom-up effects of trait variation in resource species (Crutsinger 2006). Moreover, manipulation of intraspecific variation in predators suggests that variation in top trophic levels can strongly impact the strength of top-down control (Harmon et al. 2009; Raffard et al. 2019). For example, Palkovacs and Post (2009) showed that variation in foraging traits among different predator populations of a fish impacted community structure in plankton communities and consequently on the biomass of basal resources.

In cannibalistic systems, higher intraspecific variation in foraging traits (e.g. body size) results in a higher diet breadth, increasing the range of edible prey in lower trophic levels but also conspecifics leading to an increase in rates of cannibalism (Rudolf 2007; Moya-Laraño 2011). High rates of cannibalism can reduce the abundance of predators, consequently increasing the abundance of prey, weakening trophic cascades (Rudolf 2007). However, if rates of cannibalism are low, higher intraspecific variation in predator foraging traits may result in a higher strength in top-down control due to a complementary effect resulting from a wider dietary breadth. For example, higher intraspecific variation in predator size may lead to a higher diet breadth concerning prey resulting in higher predation pressure in prey size classes which would be released from predation otherwise. This way intraspecific variation may strengthen trophic cascades.

Trait variance per se can also affect population size, stability or interspecific interactions (Bolnick et al. 2011), but if a genetic basis is absent, it is deprived of evolutionary potential. Therefore, to fully understand the two sides of the coin, how intraspecific variation affects the environment, and *viceversa* how the environment affects the maintenance and the evolutionary changes in intraspecifc variation, one must understand whether the functional traits involved have a genetic basis.

4. Study system

4.1. Biology of *Lycosa fasciiventris*

Wolf spiders (Lycosidae) are one of the most diverse spider families in the world, with more than 2438 species described as on October 17th 2019. This high species diversity is only surpassed by Salticidae, Linyphidae and Araneidae (World Spider Catalog 2019). Their world-wide distribution, relatively large body size, abundance and conspicuous synapomorphies, such as eye arrangement and brood care, make them well-known spiders even among the general public. Additionally, this family has been chosen as a model organism for many ecological and behavioral studies (Royauté and Pruitt 2015). In particular, Lycosa species have been widely used as a model organism for studies of cannibalism, territoriality (Moya-Laraño et al. 2002), sexual size dimorphism (Fernández-Montraveta and Moya-Laraño 2007), anti-predator behavior (Persons et al. 2001; Williams et al. 2006), homing (Ortega-Escobar 2011) and venom composition (Zhang et al. 2010), among others.

Most female spiders in temperate zones live for only one season as adults. Wolf-spider mothers carry the egg sac attached to their spineretes. After hatching, the spiderlings cling to the dorsal surface of their mother's abdomen and start slowly dispersing from the female that same fall (Parellada 1998). Spiders of this genus are generalist predators that feed on an array of mid to large size arthropods including conspecifics (Moya-Laraño 2002; Moya-Laraño et al. 2002; Gavín-Centol et al. 2017). Females may cannibalize males during courtship or during or after mating. When females cannibalize males during courtship, a phenomenon termed pre-copulatory sexual cannibalism, they may obtain nutritional benefits from consumed males enhancing their survival and fecundity. Due to its aggressive and cannibalistic behavior this genus has been used as model (Rabaneda-Bueno et al. 2014) for studies concerning behavioral syndromes related to pre-mating sexual cannibalism by spillover aggression of females (i.e. the "aggressive spillover hypothesis" (Arnqvist and Henriksson 1997; Kralj-Fišer et al. 2013)). Also, spiders of this family have been widely used for studies concerning anti-predator responses and recognition of chemical cues (e.g. Persons et al., 2001). In particular, our model species, the wolf spider *Lycosa fasciiventris* (Dufour 1835) is a moderately sexually size dimorphic, non-burrowing wolf spider with an annual life cycle, inhabiting semiarid lands in the Iberian Peninsula (Parellada 1998; Gavín-Centol et al., 2017; Planas et al., 2013). The fact that it completes its life cycle in one year and that it does not build borrows makes this species easy to maintain and handle in the laboratory.

Wolf spiders are not widely studied as model organisms for quantitative genetic studies, although we could still find some reporting quantitative genetic estimates in Lycosids. In particular, we found a study by Hendrickx et al. (2008) reporting heritability and maternal effects for growth rates and egg size and another study assessing the heritability of spider ballooning by Bonte and Lens (2007).

Figure 1. 1 - Cannibalistic interaction between two female wolf spiders as captured in the wild (Photo: Eva De Mas).

5. Thesis outline

With this thesis, we aim to address the evolutionary and ecological implications of intraspecific variation on foraging traits.

We tackle this by assessing the sources of phenotypic variation of foraging traits, specifically, on the analysis of phenotypic variation in behavioral patterns related to cannibalistic interactions and on the drivers of intraspecific variation in foraging traits. Finally, we assessed how intraspecific variation in foraging traits modulates trophic cascades.

In Chapter II, we assessed the sources of phenotypic variation in behavioral patterns related to anti-cannibalism behavior. Specifically, we exposed spiderlings to conspecific cues and monitored behavioral patterns through video-tracking towards those cues. The traits measured were boldness, cautiousness, exploration and activity. We assessed genetic variation through using a half-sib design. Despite high phenotypic variance, no genetic or maternal effects were found for any behavioral trait assessed. Significant phenotypic correlations were found between boldness, exploration and cautiousness. Indeed, bolder individuals were more cautious and less exploratory, suggesting a continuum of strategies to cope with cannibals. These patterns were explained by conspecific cues (i.e. the social environment) to which spiders were exposed. Cue production, indirectly estimated from the mass loss by the spider which released the cues, had an additive genetic basis and therefore, variation in the social environment may allow maintaining different strategies to cope with cannibals via indirect genetic effects.

In chapter III, we measured the sources of intraspecific variation in foraging traits. For that, we performed a half-sib design to estimate genetic, maternal and environmental effects. Additionally, a split brood design was performed, by providing two different quantities of prey, to evaluate environmental effects and their interactions with genetic and maternal effects. The traits under study were body size and body condition at birth, assimilation efficiency, growth rate and predator-prey size ratio. Results showed a predominance of maternal effects and negligible narrow sense heritability for all traits. Additionally, only maternal correlations were found, with no significant genetic correlations and some maternal-by-environment interactions without genotype-by-environment interactions detected. Overall, the findings in this chapter show that variance in foraging traits is mostly due to maternal and environmental effects.

In chapter IV, we evaluated the ecological effects of intraspecific variation in foraging traits. To this aim, we performed a mesocosms experiment in which we manipulated intraspecific variation either through maternal or environmental effects and evaluated its effects upon trophic cascades. Our results show that mesocosms with higher intraspecific variation had stronger trophic cascades than mesocosms with lower variation. Since this intraspecific variation was mainly due to maternal effects, the results also highlight the importance of maternal effects not only as sources of intraspecific variation but also as drivers of ecological processes. These findings run counter to the hypothesis that cannibalistic predators dampen the strength of trophic cascades.

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CHAPTER II

Careful sneakers: bolder spiders move more cautiously in presence of conspecific cues

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Chapter II – Careful sneakers: bolder spiders move more cautiously in presence of conspecific cues

Abstract

Cannibalism is an important source of nutrient income, as well as of predation risk for organisms. Although several studies unraveled the sources of variation in the response of organisms to predation risk, those addressing the response to cannibalism are relatively scarce. Here, we monitored the response of the cannibalistic wolf spider *Lycosa fasciiventris* to conspecific cues. We evaluated the genetic variance of traits underlying this response using a half-sib design. We used video-tracking to measure activity (total time spent moving), boldness (proportion of time spent in patches with / without conspecific cues), exploratory behavior (difference in activity in patches with / without conspecific cues) and cautiousness (difference in speed in patches with / without conspecific cues).

Despite high phenotypic variance, no genetic or maternal effects were found for any behavioral trait. Additionally, we found significant correlations between boldness, exploratory behavior and cautiousness. A principal component analysis suggests a behavioral syndrome including those traits, but contrary to what is most commonly reported, we did not find a positive relationship between activity and boldness. Indeed, bolder individuals were more cautious and less exploratory, suggesting a continuum of strategies to cope with cannibals: avoidance, i.e., moving away from arenas with conspecific cues, or stealthiness, i.e., reducing conspicuousness by moving more slowly and less often but spending more time in patches with conspecific cues. Which of these strategies is adopted depends on the rate of weight loss of the individual releasing the cues, for which we found a genetic basis. Therefore, variation in the social environment may allow maintaining different strategies to cope with cannibals via indirect genetic effects.

Chapter II – Careful sneakers: bolder spiders move more cautiously in presence of conspecific cues

Introduction

Behavioral traits can be compiled into five main categories (Réale et al. 2007): i) activity, i.e., the overall movement per unit of time of an individual; ii) exploration, the change in activity patterns when confronted to a new situation/environment; iii) boldness, the level of risk in the individual's change in behavior when exposed to a threat; iv) aggressiveness, the agonistic reaction towards conspecifics; and v) sociability, the individual's reaction to the presence or absence of conspecifics, excluding aggressiveness. Such variation in personality traits may be due to genetic (Van Oers et al. 2005) or environmental factors (Réale et al. 2007). Moreover, these behaviors are often correlated with each other, forming behavioral syndromes (Sih et al. 2004a). An often reported syndrome in the literature involves a positive correlation between exploratory behavior and boldness, as individuals displaying high activity levels in the absence of predators (higher foraging activity) tend to incur into more risk taking behaviors in the presence of predators (e.g. Sih et al. 2003; Mazué et al. 2015). Conversely, individuals, taking less risks and hiding more in the presence of predators tend to display lower activity also when predators are absent. The occurrence of syndromes limits the trait variation that is actually expressed and may result in suboptimal behavior across contexts (Sih et al. 2004b) such as not displaying appropriate anti-predator behavior when in the presence of predators.

Anti-predator behavior is one of the most common behaviors in ecosystems and has been the subject of many studies (e.g. Wilson et al. 2010a; Mazué et al. 2015). It includes the avoidance of locations with predators, reduced locomotion, increased cover-seeking and increased vigilance (Lima and Dill 1990; Lima 1998). Displaying such responses can be costly, for example due to the loss of foraging opportunities (Verdolin 2006). To avoid unnecessary costs, individuals are expected to adjust their antipredator response to predation risk.

One particular type of predator-prey interaction is cannibalism. Cannibalism differs from common predation in that a given organism can either be a predator or a prey (but see Magalhães et al. (2005)). Cannibalism is widespread among a range of taxa, including amphibians (Anderson et al. 2013), fish (Pereira et al. 2017) and arthropods (Montserrat et al. 2006; Bayoumy and Michaud 2015). Studies on cannibalism generally analyze the factors underlying the propensity to cannibalize. Indeed, food availability is probably the most important ecological factor influencing the occurrence of cannibalism (Dong and Polis 1992), but other factors such as prey quality (Montserrat et al. 2006), population density, relatedness, size differences and habitat complexity (Wise 2006) can also determine the occurrence of this trophic interaction. A few studies also showed that maternal and/or genetic effects can account for the latency to incur in cannibalism (Hvam et al. 2005; Johnson et al. 2010). In contrast, no study so far has analyzed the behavioral responses towards cannibalistic conspecifics and its underlying genetic basis.

To fill this gap in the literature, we here measure the response to cannibalism in a wolf spider, by measuring several traits of spiders exposed to chemical cues from conspecific cannibals. We tested whether such traits have an underlying genetic basis and if they are affected by the intrinsic state (e.g. body condition) and the social environment (e.g. the nature of chemical cues). Although spiders are a model system to study cannibalism (Wise 2006), the response to the risk posed by cannibals and its genetic basis has been overlooked.

Wolf spiders (Lycosidae) are generalist predators, hunting by movement and exhibiting little prey preference (Nentwig 1986) with the ability to explore chemical cues from both predators (Persons and Rypstra 2001) and prey (Persons and Rypstra 2000). Lycosids may exhibit a variety of defensive tactics, including prolonged periods of immobility, reduced walking speed and avoidance of patches containing predator cues (Persons et al. 2001). As cannibalism is a common intraspecific interaction in this group of spiders (Elgar and Crespi 1992), it is expected that they will respond to any type of cues from conspecifics.

To test this, we monitored the behavioral response to conspecific cues in spiderlings of *Lycosa fasciiventris*. Specifically, we performed a half-sib design to assess the underlying genetic basis of boldness (increased residence time in conspecific patch; Sloan Wilson et al. 1994), cautiousness (reduction in speed when travelling through the conspecific patch; Hedrick 2000) and exploratory behavior (as relative activity in conspecific patch; Mazué et al. 2015). We also tested whether these traits correlate with each other.

Material and Methods

Spider collection

Individuals of *Lycosa fasciiventris* were collected from June 23rd to July 27th 2015 in four different localities within the Almeria province (South-East Spain), in dry temporal washes ("ramblas"). One locality near Boca de los Frailes village (36.8036°N, 2.1386°O), other near Carboneras village (36.9667°N, 2.1019°O), other near Almanzora river (37.3414°N, 2.0078°O) and the last locality around Paraje las Palmerillas, Estación Experimental Cajamar (37.7917°N, 2.6891°O). They were kept in the laboratory in individual tanks (22 x 18 x 18 cm) with the bottom filled with 2-3 cm of soil collected from one of the sites. Two wooden blocks (10 x 8 x 1 and 3 x 5 x 1 cm) were added to each tank to provide shelter. Only sub-adult females were used to ensure that individuals were all virgin. All individuals (adult males and sub-adult females) were fed once a week with size-matched crickets (*Gryllus assimilis)* purchased from a pet supply store (Exofauna, Spain). Spiders had access to water ad libitum through a 40-ml vial filled with water and covered with cotton. Vials were checked and refilled, if necessary, every 2-3 days. Holding tanks were placed in a climate chamber with simulated outdoor climatic conditions (day and night temperature cycles, photoperiod with light bulbs of 54W, mimicking natural sunshine, and a relative humidity of 50-65%). Climatic conditions were adjusted to the preceding weekly average conditions in the Almeria province, with day-night temperature oscillations (ranges: temperature, 18.7-34.3 ºC; photoperiod, 17:7-16:8 hours light-dark photoperiod). These settings were used for reasons beyond this current study (i.e. offspring produced were used later for an experiment in the wild, so similar conditions were necessary).

Breeding design

To estimate additive genetic and maternal effects, we performed a half-sib breeding design (Falconer and Mackay 1996; Lynch and Walsh 1998). To this aim, 52 males were each mated with two virgin females to generate families of paternal half-siblings. Traits were measured in 12 full-siblings from each dam removed from the female back 42±8 days after they hatched and placed in a container. They were then sucked using a potter and placed in separate cylindrical containers (15 cm height and 6 cm of diameter) inside a growth chamber with controlled temperature and humidity (25±1ºC, 70±5%, and 16-8 hours light-dark photoperiod). The bottom of each container was covered with filter paper providing a substrate for both locomotion and absorption of excreta. Filter papers were checked weekly for signs of degradation and replaced if necessary. Containers had an opening at the center bottom where a plastic tip was inserted and filled with cotton. Water was provided ad libitum by capillarity through this cotton string submerged in a reservoir below each container (Moskalik and Uetz 2011). To prevent common environmental effects within the growth chamber, the 1248 spiderling containers were randomly spatially arranged within the chamber. Each week, spiderlings were fed with fruit flies (*Drosophila melanogaster*), originated from cultures produced in the lab. These constitute an optimal food source for spiderlings due to their size and easiness to handle. Flies were reared in a nitrogen rich medium supplemented with high quality dogfood to ensure increased survival and growth of the spiderlings (Jensen et al., 2011).

As the data collected here was part of a wider study, a portion of the offspring within each dam family (3 out of 12) were reared in a richer environment by providing them three times the amount of food than that provided in the standard treatment. These experimental treatments allowed to evaluate environmental effects which are further explored elsewhere.

Behavioral analysis

We tested how spiderlings behave in the presence of conspecific cues. Behavioral trials were carried out in small petri dishes (5.5 cm Ø) with the bottom covered with filter paper divided in two even patches: one half containing intact filter paper (control) and the other half impregnated with conspecific cues (Fig. 2.1). Chemical cues (along with other types of cues such as silk, which they release frequently even at the juvenile stages, and prey remains) were collected in filter paper by previously enclosing spiderlings in a small petri dish with the bottom covered in filter paper and feeding it with 10 fruit flies (*D. melanogaster*). Spatial position of the paired filter pater was randomized to eliminate any potential side bias. Spiders were assigned randomly to each petri dish except that care was taken to avoid any direct relatedness (sibling) between the focal individuals and the one that produced the cues. We also imposed the constraint that the spiders releasing the cues and those responding needed to come from the same feeding regime.

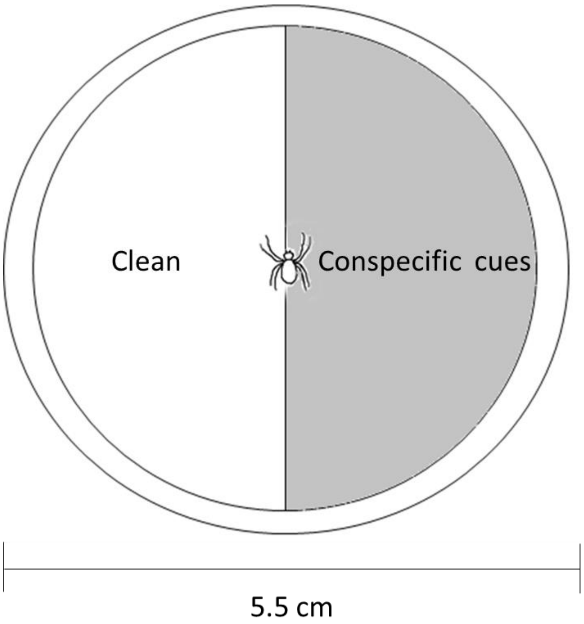

Figure 2. 1– Experimental setup for testing behavioral responses towards predation risk. Spiderlings were placed inside the petri dish and allowed to move freely between a clean patch and a patch containing conspecific cues, for a period of 3 hours.

All individuals were measured and weighted. Body size (*BS*) was assessed by measuring the carapace width (Hagstrum 1971). The width of the abdomen was used to assess body condition (*BC*) by regressing the abdomen width to the carapace width (Jakob et al. 1996). Abdomen width represents body condition in spiders as it is in this structure that nutrients and body fats are stored (Jakob et al. 1996; but see Moya-Laraño et al. 2008). Measurements were performed with a stereoscopic magnifying glass (Leica MZ125) with a precision of 0.1mm. Weight was measured to the nearest 0.1mg using a high precision scale (Mettler Toledo XP26). Weight loss of the eliciting individual was calculated as the relative weight loss during cue collection and may be used as a proxy for the quantity of cue released, such as through excretion: $WL = \frac{M_{t_0}-M_t}{M_{t_0}}$, where WL is weight loss, M is spider body mass at either time t or at the onset of the trial (t_0). To this end, animals were fed a known amount of food, and then weighed at two time points. Further details can be found elsewhere (Chapter III), as this procedure was part of an experiment testing for the genetic basis of assimilation efficiency.

Locomotor behavior (time spent moving, distance travelled, speed and time spent on a given patch) was measured by monitoring spiders through video recordings retrieved from a video camera (Sony® HDR CX-150) placed overhead. Spiders were recorded in blocks of 15 Individuals included in each of three chambers, and a simple videotracking software was implemented by one of us (ARM) which allowed estimating movement at 25 frames/s. For testing for possible variation in behavior due to oscillations in room temperature we recorded the temperature of the chamber for all trials.

Activity was estimated as the relative amount of time a spiderling spent moving; *activity = time spent moving / total recording time.* Contrasts in behavioral patterns between sides were estimated recurring to a difference/sum ratioborrowed from the relative interaction intensity (RII) index described by Armas et al. (2004), because it has been shown to be highly statistically efficient. We thus applied the following formula $R_{trait} = (trait_{cues} - trait_{control}) / (trait_{cues} + trait_{control})$. Using this general formula, we calculated exploratory behavior as differences in activity between patches; *Exploration index* = $(activity_{cues} – activity_{control}) / (activity_{cues} + activity_{control})$, cautiousness as the difference in mean velocity when walking within the conspecific patch; *cautiousness index* = $(velocity_{control} – velocity_{cues}) / (velocity_{cues} + velocity_{control})$, and boldness, estimated as the proportion of time spent on a dangerous area: *boldness index* = $(\%time_{cues} – \%time_{control}) / (\%time_{cues} + \%time_{control})$.

As we registered the identity of the individuals that produced the cues, we also assessed if there was any effect of the phenotype (body size, body condition and weight loss) and identity of such individuals on eliciting behavioral responses. The latter was used to test for genetic and maternal effects of eliciting behaviors via cue releasing, assuming that individuals losing more weight had dropped more excreta and other elements on the filter paper. We present these data later in supplementary materials.

Statistical analyses

All analyses were performed using the statistical software package R v.3.5.2 (R Core Team 2019). Additive genetic and maternal effects were estimated through variance component partitioning. Generalized Linear Mixed Models were implemented through recurring to the MCMCglmm package (v.2.12; Hadfield 2010) in a Bayesian framework using Markov chain Monte Carlo (MCMC) sampling. Sire, dam and block were included as random factors. Age and treatment were introduced as covariates. Priors were estimated using the phenotypic variance for each trait divided by the number of random terms of the model and *nu* (degree of belief) of 0.2, so that not excessive weight is put on the specific variance values. As a sensitive analysis, different priors were tested to check their influence on the estimates by using different *nu* values and attributing different proportions (from 0.025 to 0.95) to each random variance components (Wilson et al. 2010b). Genetic and maternal variance components were assessed by comparison of models containing one or both variance components (sire and dam) or none of those variance components (null model). Models were compared through the DIC criterion and the best models were those presenting the lowest DIC. Models that differed with DIC values < 2 were considered not to differ significantly from each other (Burnham et al. 2011).

To test whether other traits affected the behavioral responses, we built models by fitting each behavioral trait with morphological and physiological traits from focal (BS_{focal} and BC_{focal}) and elicitors ($BS_{elicitor}$ and $WL_{elicitor}$) and temperature as fixed effects.

Behavioral syndromes were identified through principal components analysis (PCA) (library psych). Parallel analyses and scree criterion where used to select the number of components to retain. PCA axes were interpreted as meaningful when superior to 0.5 (Budaev 2010).

Phenotypic correlations between traits were assessed by calculating the phenotypic variance-covariance (VCV) matrix using multivariate mixed models. Behavioral traits were assumed to approach Gaussian distributions and included in a multivariate model that included sire, dam and block as random effects whenever these explained part of the variance. The phenotypic covariance matrix **P** was partitioned into the **G** (additive genetic effects) matrix, the **M** (maternal effects) matrix, **B** (covariance between blocks) and residual covariances **R**, where **P = G + M + B + R**. Therefore, the phenotypic VCV matrix was calculated as the sum of the other resulting matrices. The resulting matrix is symmetrical, the diagonal indicates the variance in a given trait and upper and lower triangles correspond to pairwise covariances among traits. Correlations were calculated from the VCV matrices, following the standard definition of a correlation (i.e. $r_{XY} = \frac{C_{XY}}{\sqrt{V_x V_y}}$). The multivariate mixed model was implemented in a Bayesian framework using Markov chain Monte Carlo (MCMC) sampling in the package “MCMCglmm” (Hadfield 2010).

Results

We did not find any evidence for genetic or maternal variance for any of the behavioral traits measured (Table 2.1). Indeed, the best fitted model was the null model (no genetic or maternal effects). However, we did find genetic and maternal effects for weight loss of the eliciting individuals (Table 2.1).

Table 2. 1 – Model comparison of additive genetic and maternal effects on traits measured on focal individuals. Δ DIC is the difference between DIC values against the null model. **V_{sire}** – variance among sire families, **V_{dam}** – variance among dam families. **EXP** – exploratory behavior, **BOLD** – boldness; **CAUT** – cautiousness, **ACT** – activity, **WL** – weight loss.

TRAIT	model	DIC	Δ DIC
EXP	null	-379.1901	0
	V_{sire}	-376.9469	2.2432
	V_{dam}	-373.6407	5.5494
	V_{sire} + V_{dam}	-372.4227	6.7674
BOLD	null	731.1975	0
	V_{sire}	734.7541	3.5566
	V_{dam}	732.9282	1.7307
	V_{sire} + V_{dam}	735.7251	4.5276
CAUT	null	-809.9145	0
	V_{sire}	-810.9039	-0.9894
	V_{dam}	-808.1648	1.7497
	V_{sire} + V_{dam}	-808.1882	1.7263
ACT	null	-579.646	0
	V_{sire}	-577.684	1.962
	V_{dam}	-579.2916	0.3544
	V_{sire} + V_{dam}	-577.1419	2.5041
WL	null	-1514.646	0
	V_{sire}	-1519.452	-4.806
	V_{dam}	-1518.578	-3.932
	V_{sire} + V_{dam}	-1519.483	-4.837

As observed in figure 2.2, Individuals tended to avoid the side of the experimental arena that contained conspecific cues (average boldness index was -0.137±0.022). However, individual variation in this response was very high (-0.999 to 0.999). We also found high variation in the remaining behavioral traits, with means close to 0 and very wide ranges (Figure 2.2). Indeed, average cautiousness was around -0.015±0.004 with a range between -0.723 and 0.769 and average exploratory activity was 0.029±0.006 with range from -0.414 to 0.653.

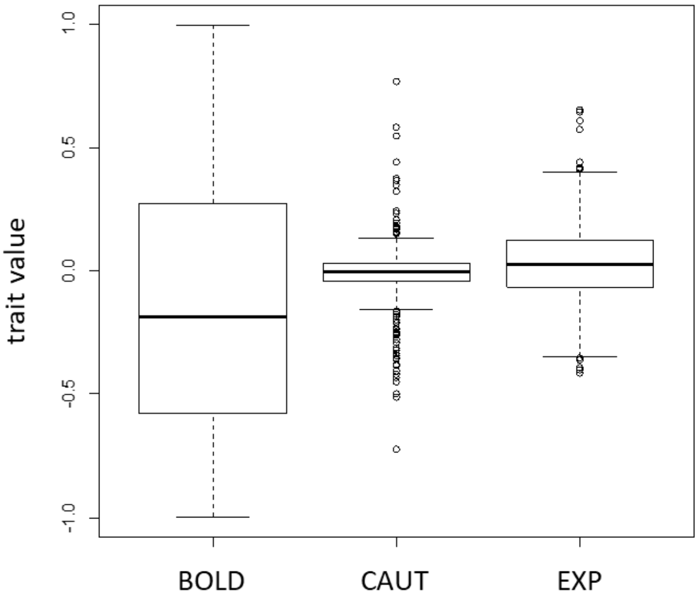

Figure 2. 2 – Boxplot showing the variation observed in the behavioral traits measured in response to conspecific cues. **BOLD** – boldness; **EXP** – exploratory behavior; **CAUT** – cautiousness.

The PCA analysis revealed that boldness, cautiousness and exploratory activity were grouped in one of the principal components while activity was placed in the other principal component (Table 2.2).

Table 2. 2 – Principal components (Varimax rotated) analysis of behavioral traits related to response towards predator cues.

behavioral trait	loadings	
	RC1	RC2
activity	0.01	**0.95**
exploratory behavior	**-0.64**	-0.35
cautiousness	**0.80**	-0.12
boldness	**0.87**	0
% variation explained	63	37

This suggests a behavioral syndrome formed by these three traits. Indeed, when we check the association among traits without partitioning our variance components, we found significant phenotypic correlations among the behavioral traits assessed, namely a strong positive correlation between boldness and cautiousness, a strong negative correlation between boldness and exploratory behavior and a weaker correlation between cautiousness and exploratory activity (Figure2.3). Additionally, albeit small, we found a negative and significant phenotypic correlation between exploratory behavior and activity, which was not immediately obvious in the PCA (Figure 2.3).

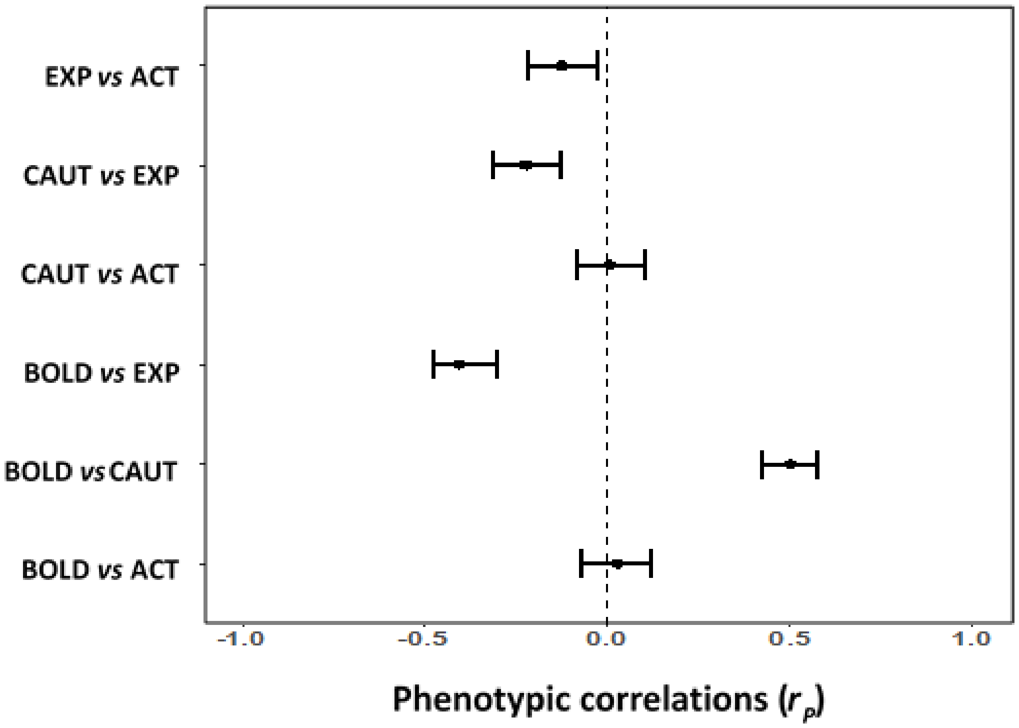

Figure 2. 3 – Phenotypic correlations (r_p) among the behavioral traits measured in this study. Points represent the posterior mode for the estimates measured and the intervals represent Bayesian credible intervals (95%). Significant estimates are those that do not overlap zero (dashed line **BOLD** – boldness; **EXP** – exploratory behavior; **CAUT** – cautiousness; **ACT** – activity.

We observed a significant positive effect of weight loss by the elicitor on boldness and a negative effect on exploratory behavior (Table 2.3). Activity was negatively affected by the body condition of the focal individual and cautiousness was not significantly explained by any of the traits fitted (Table 2.3).

Table 2. 3 – Regression coefficients for the focal and elicitor traits fitted to explain variation of behavioral traits in response to predation risk. Significance of parameter estimates is given as *p< 0.05; ** p < 0.01; *** p < 0.001. **BSR** - Body size ratio, **BS** – Body size; **BC** – body condition; ***$BS_{elicitor}$*** – body size of the elicitor; ***$WL_{elicitior}$*** – weight loss of the elicitor.

TRAIT	BS	BC	BS_elicitor	WL_elicitor
BOLD	0.063 (-0.067 to 0.185)	-0.053 (-0.175 to 0.056)	0.047 (-0.074 to 0.160)	0.111 (0.011 to 0.207) *
CAUT	0.043 (-0.086 to 0.176)	-0.066 (-0.174 to 0.054)	0.015 (-0.099 to 0.125)	0.069 (-0.030 to 0.164)
EXPL	0.194 (-0.107 to 0.134)	0.058 (-0.047 to 0.176)	-0.019 (-0.137 to 0.085)	-0.119 (-0.219 to -0.024) **
ACT	-0.047 (-0.169 to 0.079)	-0.115 (-0.232 to -0.004) *	0.013 (-0.106 to 0.114)	-0.001 (-0.100 to 0.094)

Discussion

In this study, we aimed to measure the sources of variation in traits associated to avoidance of cannibalism in a solitary wolf spider, namely boldness, cautiousness and exploratory behavior. We did not find any evidence of genetic or maternal effects for the set of traits assessed. Overall, juvenile spiders tended to avoid areas with conspecific cues as the calculated boldness index was predominantly negative. The other behavioral traits exhibited low average values but a high variance across individuals. Furthermore, we found strong correlations among traits. Indeed, bolder individuals tended to move more cautiously in arenas with cues from conspecific individuals. Additionally, weight loss by the elicitor, which had substantial genetic variation, had a significant effect on boldness and on exploratory behavior. None of the behavioral patterns were influenced by body size of either focal or elicitor individuals. As differences in body size should promote cannibalism, the fact that this trait did not affect the responses measured, may indicate that i) individuals were unable to assess the body size of elicitor by released cues alone, or ii) body size differences were not large enough for an individual to perceive conspecifics as small enough to be a potential prey or big enough to be a potential threat. Since spiders were exposed to cues of spiders reared under the same food regime, this is consistent with what we found in Chapter IV, where the tendency towards cannibalism in mesocoms with spiders coming from the same rearing environment (and relatively more close to each other in body size) was much lower. This result differs substantially from that of Persons and Rypstra (2001) for interspecific interactions among wolf spiders, where the smaller species (the IGP prey) showed a stronger avoidance response towards relatively larger individuals of the IGP predator. It would be interesting to test if within a cannibalistic context, larger differences in body size among individuals deal with stronger avoidance responses.

The negative regression coefficient observed between activity and body condition of the focal individual shows that individuals possessing higher amounts of energy reserves tended to display lower activity, as documented for subadults and adults of another congeneric wolf spider (Moya-Laraño 2002; Moya-Laraño et al. 2003). This is also in agreement with previous work showing that food deprived spiders moved more frequently and therefore travelled further than better provisioned individuals (Walker et al. 1999). Individuals possessing higher amounts of energy reserves probably need to move less in search for food. This is also likely to place them in risky situations less often than starving individuals. This is in line with the asset protection principle, which states that the more assets individuals possess, the less willing they are to incur into risky situations (Clark 1994).

Behavioral traits measured in response to conspecific cues were correlated. Indeed, individuals that spent more time in the side containing conspecific cues (i.e., bolder individuals) tended to decrease their activity and mean velocity. The wolf spider visual system is strongly biased towards movement (Rovner 1996). Thus, decreasing movement is likely to reduce conspicuousness towards conspecifics. Lower conspicuousness in the presence of conspecific cues can also be a hunting strategy to avoid being detected by potential conspecific prey. Indeed, it is difficult to interpret such behavioral response strictly as anti-predator behavior, given that cannibal conspecifics can be both predator and prey. Additionally, the negative phenotypic correlation found between activity and exploratory behavior shows that individuals possessing a propensity to explore more actively the conspecific patch tend to display lower overall activities, perhaps due to being intrinsically cautious individuals that feel stressed upon entering a patch with conspecific cues. The hypothesized behavioral syndrome here identified is discrepant from the commonly described boldness-exploration behavior (e.g. Mazué et al. 2015). As described above, instead of the commonly found positive correlation (bolder individuals tend to explore more) we here report a negative correlation between these two behavioral traits. Moreover, bolder individuals are also more cautious in conspecific patches. This may indicate that the previously described syndrome may be more complex than initially thought and that its selection and expression may be context dependent.

In this study, we also found out that among individual variation in behavioral traits was very high but had neither a genetic nor a maternal basis, suggesting high behavioral plasticity within the population in response to conspecific cues. As the mean trait values for both cautiousness and exploratory behavior were around zero, we would assume that no behavioral response would occur towards conspecific cues, had we ignored the underlying trait variation. Overall, our data shows a complex phenotype in response to conspecific cues. Assessing this behavior variability was only possible by considering the correlation among traits and would have been missed if only the isolated traits and their means were considered.

We found a high diversity of behavioral traits and a behavioral syndrome where bolder individuals were more cautious and less exploratory, suggesting a continuum of flexible strategies to cope with cannibals. At the extremes, we can observe that some individuals displayed avoidance of conspecific cues, as these moved away from the patches with conspecific cues whereas others showed to be stealthy, reducing its conspicuousness by moving more slowly and less often, but spending more time in patches with conspecific cues. Along this continuum of strategies, the behavior expressed is context dependent as the rate of weight loss of the individual releasing the cues seems to significantly explain these patterns.

Indeed, among the morphological and physiological traits, only weight loss from the elicitors significantly contributed to explain behavioral traits in response to conspecific cues, namely boldness and exploratory behavior. Since a great proportion of the cues likely correspond to excreta, animals losing more weight were likely those that also released more cues. Alternatively, higher weight loss may be related to animals that have higher voracities, as recently found in another wolf spider (Rádai et al. 2017), which could be associated to their willingness to attack conspecifics (Arnqvist and Henriksson 1997).

As weight loss, and consequent cue release, is genetically determined, it has the potential to evolve and modulate evolutionary routes, beyond its impact at the ecological level. Variation in this trait fuels intraspecific variation in behavioral traits, which, despite not possessing genetic variation, varied according to the social environment (i.e. conspecific cues), thus still allowing some room for the evolution of indirect genetic effects (Wolf et al. 1998). One testable prediction for this "evolution of indirect effects hypothesis" is that for indirect genetic effects to evolve, animals should be flexible in their behaviors across contexts but consistent within contexts. In that respect, it would be interesting to study the context-dependent repeatability of the behaviors that respond to the weight loss of the elicitor. That is, do spiders show a consistent response in the degree of boldness, exploration and cautiousness under cues of a potentially dangerous conspecific? Does this pattern change when the same spider is exposed to a less dangerous conspecific (i.e., one that losses less weight)? And, is this new response consistent?

In conclusion, we found that the behavioral traits associated to cannibalistic interactions were correlated but not heritable and therefore we cannot state the existence of a behavioral syndrome. However, correlations among behavioral traits are likely to be adaptive as increased exposure to risk was balanced by reduced conspicuousness. Despite not possessing a genetic basis, behavioral patterns were driven by the social environment (i.e. conspecific cues) which, in turn, possessed a genetic basis and therefore potential to evolve. Our data shows that taking the phenotypic gambit (Hadfield et al. 2007) can sometimes lead to misleading conclusions towards the evaluation of behavioral syndromes as phenotypic correlations may not necessarily be traduced into behavioral syndromes, as seen in this study. Regardless of whether we call this a syndrome or not, in future studies, it will be interesting to study the behavioral flexibility of this continuum of strategies and their adaptive value in front of cannibalistic conspecifics.

Acknowledgments

This study was supported by a PhD grant (PD/BD/106059/2015) attributed to Jorge Henriques by the Portuguese Science and Technology foundation (FCT), by the grant P12-RMN-1521 from the Andalusian government and by the grant CGL2015-66192-R from the Spanish Ministry of Economy and Competitiveness, both partially funded by the European Regional Development Found, both attributed to Jordi Moya-Laraño and to the FPU scholarship (FPU13/04933) from the Spanish Ministerio de Educacion, Cultura y Deporte to Dolores Ruiz-Lupión.

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CHAPTER III

Maternal effects as a relevant source of intraspecific variation in foraging-related traits of a predator

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Chapter III – Maternal effects as a relevant source of intraspecific variation in foraging-related traits of a predator

Abstract

Intraspecific variation in foraging traits may affect the ecology and evolution of communities. Still, knowledge on the relative contribution of environmental *vs* genetic effects on these traits is as yet largely incipient, particularly in predator foraging traits. We performed a half-sib design to estimate genetic, maternal, and environmental effects on foraging-related traits in the predatory wolf spider *Lycosa fasciiventris* (Dufour, 1835). We measured body size and body condition at birth, assimilation efficiency, growth rate and predator-prey size ratio. Environmental effects were assessed by providing spiderlings with two different quantities of prey. Results show high maternal variance and negligible values of narrow sense heritability for all traits. Assimilation efficiency and growth rate were maternally correlated and showed maternal-by-environment interactions. This variation in converting food into growth may be adaptive in heterogeneous environments. Another maternal correlation indicated that individuals with larger body sizes were less willing to take risks to attack larger prey later in life. Overall, these findings show that variance in foraging-related traits is mostly due to maternal and environmental effects. This may potentially drive evolutionary change through indirect genetic effects, while holding the potential to shape food web structure and dynamics by modulating predator-prey interactions.

Keywords: Quantitative genetics, maternal effects, foraging traits, Lycosidae

Chapter III – Maternal effects as a relevant source of intraspecific variation in foraging-related traits of a predator

Introduction

Foraging is a key process in ecosystems. Like most ecological traits, foraging traits have typically been considered as invariant within populations. However, several recent studies have shown that intraspecific variation in such traits can actually be quite high (Estes et al. 2003; Palkovacs and Post 2009; Agashe and Bolnick 2010; Howeth et al. 2013; Costa-Pereira et al. 2018a; Des Roches et al. 2018; Raffard et al. 2018). This variation strongly impacts the outcome of species interactions (e.g., Bolin et al., 2018) and community structure (Post et al. 2008), as predicted by theory (Schreiber et al. 2011; Barabás and D'Andrea 2016; Jonathan et al. 2016).

To understand the potential impact of phenotypic variation on ecological interactions, it is crucial to identify the origin of such variation (Bolnick et al. 2011). Indeed, theory predicts that the effect of intraspecific variation upon the outcome of predator-prey interactions depends on the relative strength of environmental *vs* genetic variation (Schreiber et al. 2011; Cortez 2018). Additionally, the occurrence of genetic variation for foraging traits raises the possibility that such traits evolve at a rapid pace. This evolution, in turn, has the potential to modify predator-prey interactions. For example, genetically diverse prey led to the stabilization of predator-prey dynamics via the evolution of resistance to predation (Yoshida et al. 2007). Surprisingly, however, only few experimental studies have measured genetic variation in predator foraging traits (Hedrick and Riechert, 1989; Henryon et al., 2002; Jia et al., 2002; Metcalfe et al., 1995; Nachappa et al., 2010).

Intraspecific variation in foraging traits may also be due to maternal effects. Remarkably, the role of maternal effects in intraspecific variation of traits relevant to predator-prey interactions and food webs has been largely overlooked. Beyond direct gene transfer, maternal effects are defined as the causal influence of the maternal genotype or phenotype on the offspring phenotype (Wolf and Wade 2009). In earlier studies in several taxa, maternal effects for foraging traits were either absent (Hedrick and Riechert 1989) or not tested (Metcalfe et al. 1995; Nachappa et al. 2010) in all but one case (Henryon et al. 2002). Moreover, maternal effects were described for traits linked to foraging such as body size (Heath et al. 1999; Lindholm et al. 2006) and growth (Räsänen et al. 2005). Additionally, the expression of both genetic and maternal effects is contingent upon the environment in which organisms occur. Although the importance of such maternal-by-environment and genotype-by-environment interactions (M x E and

G x E, respectively; Charmantier and Garant, 2005; Wood and Brodie, 2015) is undisputable, their effect upon foraging traits remains largely unexplored.

Several traits in predators and prey, beyond predation rates, define predator-prey interactions (Lima 1998). Indeed, although most studies addressing the genetic basis of foraging traits focus on the functional response of predators (e.g., Jia et al., 2002), foraging actually results from the expression and integration of various behavioral and physiological components, including those that affect movement and consumption (Nachappa et al. 2010). In particular, both predator body size (BS) and body condition (i.e., the nutrient stored independently of the animal fixed structural body size, BC) severely affect predation rate, because larger and/or hungrier predators have higher and different physiological needs (Vrede et al. 2004; Woodward et al. 2005; Moya-Laraño et al. 2008). Additionally, larger predators are expected to be better able to subdue larger prey. Indeed, the predator-prey size ratio is a central food web feature determining the outcome of predator-prey interactions (Brose et al. 2006, 2008). Despite being generally considered a species or population parameter (Barnes et al. 2010), this ratio can also be viewed as an individual trait (Okuyama 2008). Indeed, predator-prey size ratio (PPSR, i.e., the threshold size ratio at which a predator is able to subdue and kill a prey) may vary across individuals, with some being more prone to attack, kill and consume larger prey than others (Brose et al. 2008; Okuyama 2008). How efficiently individuals convert food into their own biomass is also an important feature of predator-prey interactions. Indeed, predator assimilation efficiency can strongly impact individual and population growth and therefore food web dynamics (Metcalfe et al. 1995; Jones et al. 2002; Wilder et al. 2013).

Predator traits involved in predator-prey interactions may not be independent from each other. For instance, a significant negative correlation between consumption and development time was found in predatory mites (Nachappa et al. 2010). If these correlations are genetic, they have the potential to alter and constrain the rate and direction of the response to selection (Cheverud 1996; Roff 1997). Thus, providing a complete view of the genetic architecture of traits involved in predatory interactions is essential to predict the response of predator populations to selection. Unfortunately, studies addressing correlations among foraging traits are scarce (but see Biro et al. (2004) and Nachappa et al. (2010). Also, even beyond foraging traits, maternal correlations have been broadly disregarded with only a few examples available in the current literature (Hoque et al. 2008; García et al. 2009; Taylor et al. 2012). However, maternal correlations may be more important than previously thought because they may reflect either a) a combination of traits that jointly provide a fitness advantage to the offspring, and that are consequently co-transmitted, or b) relevant physiological constraints in the female provisioning of her offspring (Hsu et al. 2016).

Here, we investigate the sources of intraspecific variation for foraging traits in the soil predator Lycosa fasciiventris (Dufour, 1835), a non-burrowing wolf spider inhabiting the Iberian Peninsula. Spiders of this genus are generalist predators which feed on an array of mid to large size arthropods including conspecifics (Moya-Laraño 2002; Gavín-Centol et al. 2017). Specifically, we assess the role of additive genetic and maternal effects as components of phenotypic variation and how these effects interact with the environment to which offspring are exposed through experimental manipulation of food availability. The traits assessed are ecologically relevant in the context of trophic interactions of this species, as predator-prey interactions involving wolf-spiders are strongly biased, for instance, towards size (Rypstra and Samu 2006). In particular, we measured body size and body condition at birth, assimilation efficiency, growth rates and predator-prey size ratio of the offspring. Identifying the relative contribution of environmental, maternal and genetic effects to variation in foraging traits will shed light into their potential evolutionary trajectory and their importance in shaping the ecological community.

Material and Methods

Spider collection

Individuals of Lycosa fa*sciiventris* were collected from June 23rd to July 27th 2015 in four different localities within the Almeria province (South-East Spain, in dry temporal washes (“ramblas”). One locality near Boca de los Frailes village (36.8036°N, 2.1386°O), other near Carboneras village (36.9667°N, 2.1019°O), other near Almanzora river (37.3414°N, 2.0078°O) and the last locality around Paraje las Palmerillas, Estación Experimental Cajamar (37.7917°N, 2.6891°O). Each of these individuals were then kept in the laboratory in a tank (22 x 18 x 18 cm) with the bottom filled with 2-3 cm of soil collected from one of the sites. Two wooden blocks (10 x 8 x 1 cm and 3 x 5 x 1 cm) were added to each tank to provide shelter. Only sub-adult females were used to form the laboratory population, to ensure that they were all virgin. All individuals (adult males and sub-adult females) were fed once a week with size-matched crickets (*Gryllus assimilis* Fabricius, 1775) purchased from a pet supply store (Exofauna, Spain). Spiders had access to water *ad libitum* through a 40 ml vial filled with water and covered with cotton. Vials were checked and refilled, if necessary, every 2-3 days. Tanks were placed in a climate chamber with simulated outdoor climatic conditions (day and night temperature cycles, photoperiod with light fluorescent tubes of 54 W -mimicking natural sunshine- and a relative humidity of 50-65%. Climatic conditions were adjusted to the preceding weekly average conditions in the Almeria province, with day-night temperature oscillations (ranges: temperature, 18.7-34.3 °C; light-dark photoperiod, 17:7-16:8 hours).

Breeding design (cf. chap. II)

To estimate the sources of variation of traits associated to predation, as well as the interactions among such traits, we performed a half-sib breeding design (Lynch and Walsh, 1998; Roff 1997) in two different environments. To this aim, 52 males were each mated with two virgin females to generate families of paternal half-siblings. Female body size and body condition (check below for details) were measured prior to mating and the number of offspring (clutch size) was counted after emergence. Further details on the staged matings can be found elsewhere (Gavín-Centol et al. 2017).

Traits were measured in 12 full sibs from each dam. For logistic reasons we did not rear the animals until maturation, and hence we could not determine the sex of the scored offspring. After hatching, spiderlings of wolf spiders climb to the female back and can stay there for prolonged periods. In burrowing wolf spider this period can be several months (e.g. Humphreys, 1983). We removed the spiderlings from the female back approximately 42 days after they hatched. Since females lay up to 600 offspring in a single egg sac, we aimed at approaching a random sample of spiderlings collected from each female. To this end, we gently removed all the offspring from the back of the mother with the help of a paintbrush and placed them in a 5 cm Ø container that was gently shaken each time a group of 2-3 spiderlings were sucked with a pooter, until we obtained 12 individuals. Then the spiderlings were individually placed in separate cylindrical containers (5 cm height; 6 cm Ø) inside the growth chamber. The bottom of the containers was covered with filter paper, providing a substrate for both locomotion and absorption of excreta. Filter papers were checked weekly for signs of degradation and replaced if necessary. The containers had an opening at the center of the bottom where a plastic tip was inserted and filled with cotton. Water was provided *ad libitum* by capillarity through this cotton string submerged in a reservoir below each container (Moskalik and Uetz 2011). The 1248 spiderling containers were randomly arranged within the growth chamber to ensure that individuals belonging to the same family were spatially interspersed. This procedure was followed to prevent resemblance between individuals within families due to common environmental effects, thus ensuring that similarity among sibs is only due to shared maternal and/or genetic effects.

Each week, spiderlings were provided with fruit flies (*Drosophila melanogaster* Meigen, 1830) originated from cultures produced in the laboratory. These constitute an optimal food source for spiderlings due to their small size and easiness to handle. Nutrient enriched flies were obtained by rearing them in a nitrogen rich medium supplemented with high quality dogfood to ensure increased survival and growth of the spiderlings (Jensen et al., 2011). To measure environmental effects in trait variation during offspring development, food availability was experimentally manipulated. Within each dam family, 3 out of the 12 spiderlings were reared in a richer environment by providing them three times the amount of food than that provided in the standard treatment. Initially, a single fly was offered to the spiderlings in the standard treatment and 3 flies in the richer treatment. This quantity was adjusted to 3 and 9 when individuals were approximately 6 months old due to higher food demand for a proper offspring development. A lower sample size was assigned to the food rich environment because we anticipated relatively higher offspring mortality in the poor environment.

Trait measurement

Given that several traits were measured on many individuals, it was not possible to standardize age across all measurements. Instead, the order in which individuals were scored was randomly assigned independently for each trait, and we registered the age at scoring for later use as a covariate in statistical analyses (see below).

Offspring body size (BS) was assessed by measuring carapace width (Hagstrum 1971). Offspring abdomen width was used to assess body condition (BC) by regressing the abdomen width on carapace width for all the offspring individuals in the sample and taking the residuals (Jakob et al. 1996; Moya-Laraño et al., 2008). This was done because abdomen width is considered a good proxy for body condition in spiders, as it is in this body part that nutrients and body fats are stored (Jakob et al. 1996; Moya-Laraño et al.

2008). These measurements were taken to the nearest 0.1mm with a dissection microscope (Leica MZ125). Both traits were measured when individuals were isolated and always by the same measurer (Eva De Mas), which displayed an intra-observer repeatability for both traits > 0.91.

Individual growth rate (GR) was calculated as the mass gained per day (mg/day) approximately 6 months after being retrieved from the mothers. Assimilation efficiency (AE) was calculated as the ratio of mass gain, by individual, to the amount of prey consumed. To this aim, prior to the experiment spiderlings were starved for one week to standardize hunger level, then isolated in small petri dishes (5.5 cm Ø). The petri dishes contained a piece of cotton soaked in water at its center to avoid water deprivation throughout the experimental procedure. The bottom of each petri dish was covered with filter paper providing a substrate for optimal locomotion and absorption of excreta and other secretions. Individuals were then provided with a total of 10 nutrient enriched fruit flies (*Drosophila melanogaster*) and left for 36h. Subsequently, the spiderling, each alive prey and prey remains were collected and weighted. AE was calculated as the amount of mass gained (mg) by each spiderling per amount of prey consumed (mg) according to the following equation:

$$AE_t = \frac{M_t - M_{t_0}}{P_{t_0} - P_t - R_t} \tag{1}$$

where AE_t is assimilation efficiency at time *t* (36h), M_t and M_{t_0} is spider body mass at time *t* and at the onset of the trial (t_0), respectively, P_t and P_{t_0} are alive prey at time *t* and t_0 respectively, and R_t corresponds to prey remains at time *t*. Mass was measured to the nearest 0.1 mg using a high precision scale (Mettler Toledo XP26). We also attempted to measure predation rate, assessed as the number of flies captured during the first 36 hours. However, most spiderlings killed the 10 flies placed inside the petri dish in that time period and hence unfortunately we had no resolution to asses this trait.

Prior studies addressing predator-prey size ratios (PPSR) either offered prey of different size to predators and measured their choice (Evans 1976; Hirvonen and Ranta 1996; Matlock Jr 2005) or they measured the size distribution of prey consumed by predators and compared it with that present in the environment (Costa-pereira et al., 2018b and refs therein). Both these set-ups include a possibility for predators to choose among prey of different size. Here, we were interested in singling out the acceptance of prey of a given size by predators. Prey size is likely to be correlated with risk of injury to the predator, particularly when the prey is a cricket which has the ability to kick spiders with their back legs (Gnatzy and Otto 1996), often causing damage to wolf spiders (Rovner 1980). Additionally, pursuing, catching and subduing larger prey may require more energy from the predator (Griffiths 1980). Moreover, PPSR has been validated as an important factor affecting predation risk of crickets exposed to spiders (Binz et al. 2014) as well as successful predatory events between wolf spiders and crickets (Rypstra and Samu 2006). Therefore, we sequentially offered 5 field crickets (*Gryllus assimilis*) of decreasing size to each spider and recorded the size at which each spider attacked, subdued and killed the prey. Specifically, the length of the first cricket offered was 5±0.2 times (5x) the carapace width of the spider, and if this cricket was not caught, then a second cricket with length 4±0.2 times (4x) the carapace width of the spider was offered and so on until a last 1x cricket was offered if the spider had not caught any of the formerly offered crickets. PPSR was evaluated as the 1x-5x cricket length/spider carapace width ratio at which the spider attacked and killed the cricket. Therefore, a higher value of our test result (1x-5x) means that the spider is successful at hunting relatively larger prey (high PPSR). Crickets were weighted, and their length determined from a calibration curve, previously generated with the weight and length of 40 crickets: $L = 3.22 + 0.32\log(M)$; $R^2 = 0.99$; $p < 0.0001$; where L is cricket body length (in mm) and M is cricket body mass (in mg). All morphological measurements were taken to the nearest 0.1mm under a dissection microscope (Leica MZ125).

None of the crickets were used in more than one trial. To standardize hunger levels across individuals, randomly-assigned spiders were left to starve for seven days before entering the experimental setup. Both individuals (prey and predator) were placed inside the arena (7.5 cm Ø) isolated from each other within enclosed inverted plastic vials (3 cm Ø) and in opposing sides of the arena. At the beginning of the interaction trial, both prey and spider tubes were gently lifted simultaneously and left to interact for 6 minutes. If after 6 minutes the spider did not attack and kill the cricket, the spider was enclosed in the vial and the cricket removed. Spiders were then left to recover in the vial for 30 minutes until a new cricket from the next immediately lower ratio was presented. This experiment ended as soon as the spider attacked and killed a given cricket or if the spider did not catch the smallest (1x) cricket.

Statistical analyses

Variance components (additive, maternal and residual) and interactions with food treatment (GxE and MxE interactions) were estimated using univariate mixed effects models through Bayesian inference using the MCMCglmm package (R 3.4.3 (Hadfield 2010; R Core Team 2019). In all models, we fitted food treatment and age as fixed factors. For PPSR, body condition was also fitted to account for the effects of spider condition on behavior, as in wolf spiders body condition reflects hunger levels and has been linked to decreased foraging effort (e.g., Moya-Laraño (2002); Moya-Larano et al. (1998)).

For each trait, we tested a set of 9 plausible models (Table B.1), which included all possible interactions among genetic, maternal and environmental random factors. Additionally, for AE and PPSR, block was also included as a random effect. For traits displaying environmental effects, we also tested, genotype x environment (GxE) and maternal x environment (MxE) interactions. The best fitted models were those which presented the lowest DIC (DIC is the Bayesian analogous to the Akaike information criterion - AIC). We considered that a difference between DIC values (ΔDIC) > 2 indicated that the two models differed from each other (Burnham et al. 2011).

When environmental interactions were detected, we measured V_{sire} and V_{dam} (as well as genetic and maternal correlations, see below) only in the standard environment, as the number of replicates in the rich environment was too low. Narrow-sense heritability (h^2) was estimated as the proportion of additive genetic variance to the total phenotypic variance ($h^2 = V_A / V_P$) while maternal effects (m^2) were estimated as the proportion of maternal variance to the total phenotypic variance ($m^2 = V_M / V_P$). Priors used in this analysis were slightly informative and generated by partitioning the phenotypic variance evenly among each random term (Wilson et al. 2010) and given a low degree of belief ($nu = 0.2$). Univariate models were run for 200 000 interactions, a burn-in of 5000 and a thinning interval of 100, ensuring more than 1000 effective samples for each term in the model.

Additional models were run including mother body size (carapace width) and body condition (abdomen width), as well as clutch size. These traits were used as potential proxies of the maternal environment as well as of the genetic ability of the female to accrue resources and provision her offspring. We also included sampling site as a random effect to test whether it could contribute to increasing the explained phenotypic variance either because it could be an additional proxy for the maternal environment or because it could be a sign of genetic divergence, as populations were collected as far as 83 Km apart.

Multivariate generalized linear mixed models also in MCMCglmm were used to estimate genetic and maternal correlations for each pair of traits. Again, we included food treatment and age as a fixed effect and genetic terms (sire and dam) as random effects. Multivariate analysis allows the estimation of variance-covariance matrixes for each variance component, therefore the **G** (additive genetic), **M** (maternal) and **R** (residual) matrices. Genetic correlations (r_A) were calculated using the **G** matrix obtained through this multivariate approach as described in (Wilson et al. 2010), and following the equation:

$$r_A = \frac{COV_{A(xy)}}{\sqrt{\left(var_{A(x)}\right)\left(var_{A(Y)}\right)}} \tag{2}$$

where $COV_{A(xy)}$ is the additive genetic covariance between two characters X and Y, and $var_{A(x)}$ and $var_{A(Y)}$ are the additive genetic variance of X and Y, respectively. Maternal correlations (r_M) were calculated similarly but instead of variance and covariances for additive genetic effects, the expression was modified by using maternal variances ($var_{M(x)}$ and $var_{M(y)}$) and covariances ($COV_{M(xy)}$). Priors were 2x2 diagonal matrices where the diagonal corresponded to the variance for each trait and off-diagonals were the covariance between traits. Priors were generated by partitioning the phenotypic variance for each trait evenly among each random term and setting the prior covariance to 0. Model chains were run for 300 000 interactions, a burn-in of 5 000 and a thinning interval of 100 ensuring effective sample sizes above 1000.

A sensitivity analysis was run for all univariate and multivariate models by testing several *nu* parameters (0.2 – 2.2) and revealed negligible difference in the estimates obtained among the models tested. Moreover, we also tested for priors with varying proportion of the raw phenotypic variance attributed to the residual variances (0.025 and 0.95) (Wilson et al. 2010), leaving the remaining to be shared equally between the genetic and maternal components. Only the most robust results were considered, i.e., the ones which did not change substantially depending on the *nu* parameter or the prior variances. We evaluated convergence of the models by visual inspection of the time series plots of the model parameters and ensured that autocorrelation values were less than 0.05 for all parameters included to ensure independence of samples in the posterior distribution (Wilson et al. 2010).

Ninety-five percent credible intervals (CI) for the heritability estimates, maternal effects and correlations were calculated from the posterior distributions using the highest-posterior-density function (HPD interval, package MCMCglmm, Hadfield 2010). Covariances were supported when 95% credible intervals excluded zero and when the model with sire and/or dam random effects had lower DIC values than null models. Because variances are bounded above zero, support of variances estimates was assessed by comparing the DIC values between fitted models.

Results

All traits showed significant and substantial maternal effects while heritability estimates were low or negligible (Table 3.1, Table 3.2). No GxE interactions were found in any of the traits under study. Instead, we found evidence of MxE interactions for some traits (Table 3.1, Table B.1). The food treatments and individual age showed a significant effect on both GR and AE (Table B.2). Best candidate models (lowest DIC) are displayed on Table 3.1. For GR, this model contained only the maternal component and MxE interactions (Table 3.1, Table B.1), displaying substantial maternal effects (m^2 = 0.442; 0.211 to 0.777). Similarly, the best candidate models fitted for AE were the ones containing the maternal and MxE interaction variance components, although the occurrence of additive genetic effects could not be rejected (Table 3.1, Table B.1). In the standard environment, we found substantial maternal effects (m^2 = 0.312; 0.099 to 0.667) and, despite low, significant heritability estimates (h^2 = 0.099; 0.029 to 0.329). In contrast to the last two traits, PPSR was not affected by food treatment or age but was significantly affected by individual BC (Table B.2). The best fitted model (lowest DIC) for this trait contained only the maternal effect component and differed substantially from the null model (Table 3.1, Table B.1), yielding maternal effects (m^2 = 0.181; 0.065 to 0.439) but no MxE interactions. For BS, we found evidence of both genetic and maternal effects (Table 3.1, Table B.1), despite low heritability (h^2 = 0.106; 0.028 to 0.458). This trait presented exceptionally high maternal effects (m^2 = 1.628; 1.271 to 1.988). Finally, for BC we also found evidence for genetic and maternal effects through DIC comparison

(Table 3.1, Table B.1) presenting a low heritability (h^2 = 0.179; 0.024 to 0.446) but also exceptionally high maternal effects (m^2 = 0.836; 0.568 to 1.182).

Female body size contributed significantly to explain variation in offspring body condition, whereas female body condition and clutch size were significant predictors of offspring body size (Table 3.2). Inclusion of these maternal traits decreased the maternal effects estimated in offspring body size by 7%, (m^2 = 1.52; 1.183 to 1.942), and in offspring body condition, by 18%, (m^2 = 0.686; 0.456 to 1.055). A reduction in the maternal variance due to inclusion of maternal traits shows that these partially explained the maternal effects observed, albeit weakly.

Finally, inclusion of site did not produce any significant changes in the maternal effects observed (Figure B.1) and no significant genetic correlations among traits were found (Table B.3). A substantial negative maternal correlation was observed between BS and PPSR (r_M = -0.449; -0.711 to -0.063), and a positive maternal correlation between AE and GR, (r_M = 0.596; 0.195 to 0.787) as shown in Figure 3.1 and Table B.3.

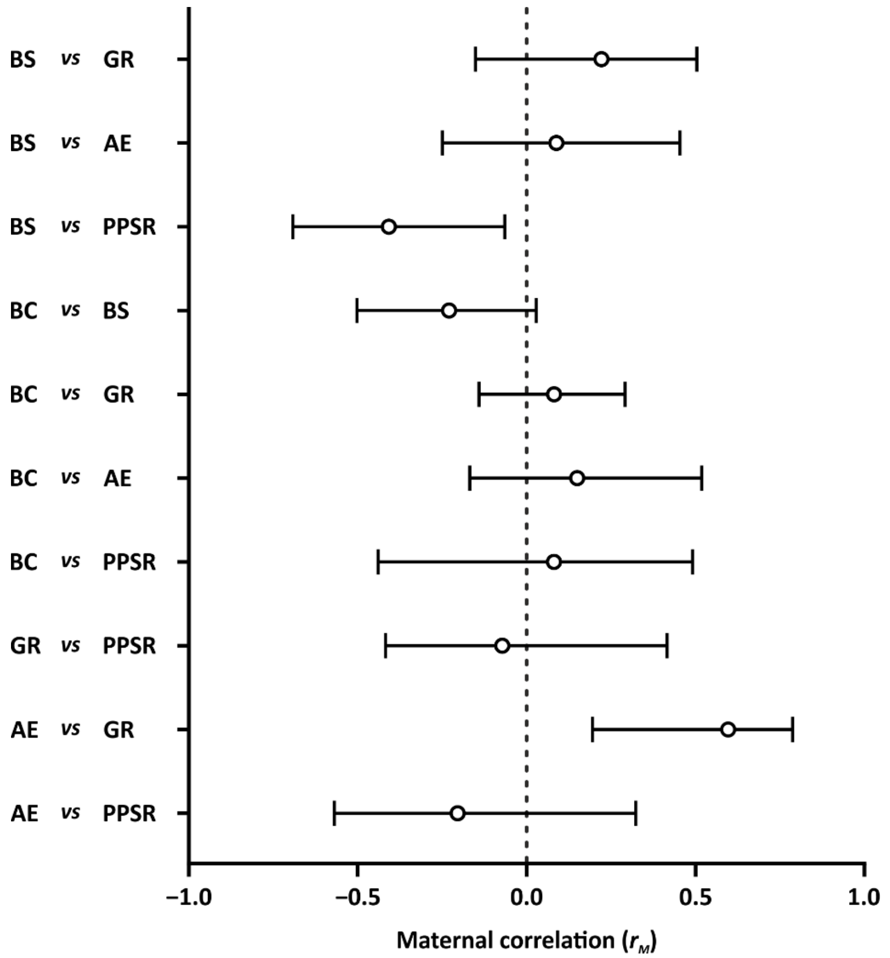

Figure 3. 1- Maternal correlations (r_M) among the traits measured in this study. White points represent the posterior mode for the estimates measured and the intervals represent Bayesian credible intervals (95%). Significant estimates are those that do not overlap zero (dashed line). **BS** – body size, **BC** – body condition, **GR** - growth rate, **AE** - assimilation efficiency, **PPSR**- predator-prey ratio.

Table 3. 1– Candidate models for each trait selected through the DIC criterion. As none of the models selected displayed genotype-by-environment interactions, this estimate is absent from this table. Traits: **BS** – body size; **BC** – body condition, **AE** – assimilation efficiency, **GR** – growth rate; **PPSR** – predator-prey size ratio. VCs - variance components: $\mathbf{V_A}$ - additive genetic variance, $\mathbf{V_M}$ – maternal variance, $\mathbf{V_{MxE}}$ - maternal-by-environment interaction. ΔDIC is the difference between DIC values against the best null model (lowest DIC). V_{sire} – variance among sires, V_{dam} – variance among dams, $V_{dam\ x\ treatment}$ – variance for the interaction between the maternal component and the environment, V_B – block variance, V_R – residual variance.

Trait	VCs	DIC	ΔDIC	$\mathbf{V_{sire}}$	$\mathbf{V_{dam}}$	$\mathbf{V_{dam\ x\ treatment}}$	$\mathbf{V_B}$	$\mathbf{V_R}$
BS	$\mathbf{V_M}$	-5219.10	-609.86		5.943x10^{-4} (4.332x10^{-4} to 7.985x10^{-4})			7.573x10^{-4} (7.004x10^{-4} to 8.192x10^{-4})
BS	$\mathbf{V_A + V_M}$	-5219.75	-609.61	3.573x10^{-5} (7.883x10^{-6} to 1.672x10^{-4})	4.862x10^{-4} (3.825x10^{-4} to 7.591x10^{-4})			7.650x10^{-4} (6.969x10^{-4} to 8.188x10^{-4})
BC	$\mathbf{V_M}$	-3927.49	-280.04		7.796x10^{-4} (5.3797x10^{-4}to 1.049x10^{-4})			2.194x10^{-3} (2.030x10^{-3} to 2.395x10^{-3})
BC	$\mathbf{V_A + V_M}$	-3927.32	-279.88	1.140x10^{-4} (2.671x10^{-5} to 3.394x10^{-4})	6.361x10^{-4} (3.939x10^{-4} to 9.425x10^{-4})			2.242x10^{-3} (2.025x10^{-3} to 2.379x10^{-3})
AE	$\mathbf{V_M + V_{MxE}}$	-778.153	-44.471		1.682x10^{-3} (7.659x10^{-4} to 3.030x10^{-3})	1.039x10^{-3} (3.015x10^{-4} to 2.103x10^{-3})	1.431x10^{-3} (7.901x10^{-4} to 3.549x10^{-3})	1.993x10^{-2} (1.788x10^{-2} to 2.221x10^{-2})
AE	$\mathbf{V_A + V_M + V_{MxE}}$	-776.296	-42.614	1.392x10^{-3} (6.802x10^{-4} to 2.726x10^{-3})	1.253x10^{-3} (4.315x10^{-4} to 2.401x10^{-3})	9.747x10^{-4} (3.412x10^{-4} to 2.046x10^{-3})	1.424x10^{-3} (5.699x10^{-4} to 3.135x10^{-3})	1.990x10^{-2} (1.762x10^{-2} to 2.184x10^{-2})
GR	$\mathbf{V_M + V_{MxE}}$	-4566.94	-172.96		1.389x10^{-5} (7.334x10^{-6} to 2.561x10^{-5})	5.727x10^{-5} (3.607x10^{-5} to 8.460x10^{-5})		1.909x10^{-4} (1.680x10^{-4} to 2.079x10^{-4})
PPSR	$\mathbf{V_M}$	1570.48	-8.16		3.846x10^{-2} (1.187x10^{-2} to 9.836x10^{-2})		6.850x10^{-2} (2.249x10^{-2} to 1.409x10^{-1})	7.286x10^{-1} (6.637x10^{-1} to 8.507x10^{-1})

Table 3. 2 – Heritability (h^2) and maternal effects (m^2) for morphological, physiological and behavioral traits and posterior estimates of the maternal traits fitted. Values were estimated from the mode of the posterior distributions obtained in the statistical model; the 95% Bayesian credible intervals are presented between brackets. **BS** – body size, **BC** – body condition, **GR** - growth rate, **AE** - assimilation efficiency, **PPSR**- predator-prey ratio, **Mother BS** – mother body size, **Mother BC** – mother body condition, **CS** –clutch size.

Model	BS	BC	AE	GR	PPSR
Model 1					
Estimates					
h^2	0.106 (0.028 to 0.458)	0.179 (0.024 to 0.446)	0.099 (0.029 to 0.329)	NS	NS
m^2	1.628 (1.271 to 1.988)	0.836 (0.568 to 1.182)	0.312 (0.099 to 0.667)	0.442 (0.211 to 0.777)	0.181 (0.065 to 0.439)
Model 2					
Estimates					
h^2	0.110 (0.027 to 0.505)	0.139 (0.034 to 0.496)	0.107 (0.029 to 0.365)	NS	NS
m^2	1.520 (1.183 to 1.942)	0.686 (0.456 to 1.055)	0.333 (0.090 to 0.649)	0.514 (0.235 to 0.798)	0.174 (0.061 to 0.435)
Mother BS	0.163 (-0.054 to 0.378)	-0.303 (-0.468 to -0.145) ***	-0.088 (-0.241 to 0.063)	-0.046 (-0.209 to 0.116)	0.068 (-0.067 to 0.209)
Mother BC	0.183 (-0.010 to 0.358) *	0.084 (-0.073 to 0.237)	-0.012 (-0.141 to 0.138)	0.019 (-0.128 to 0.1457)	-0.009 (-0.086 to 0.075)
CS	-0.318 (-0.573 to -0.057) **	0.129 (-0.077 to 0.358)	0.088 (-0.082 to 0.256)	0.030 (-0.166 to 0.225)	-0.001 (-0.001 to 0.002)

For BS and BC, treatment was not fitted as a fixed effect because measurement of this traits was prior to applying the respective food treatment. Models and estimates for assimilation efficiency and growth rate were based only on individuals reared in the standard environment due to the existence of environmental interactions. Regression coefficients for the maternal traits presented are standardized (i.e., variables with mean=0, var=1). NS – Not significant. Significance of parameter estimates is given as *p< 0.05; ** p < 0.01; *** p < 0.001.

Discussion

In this study, we assessed the relative contribution of genetic, maternal, and environmental effects for variation in foraging traits in the soil top predator *Lycosa fasciiventris*, a wolf spider with generalist feeding habitats. Overall, we found that phenotypic variation in these traits was mainly determined by maternal effects, whereas genetic variation was either inexistent or low. The environment, i.e., the food treatments, significantly affected growth rate and assimilation efficiency and interacted with the maternal variance, leading to maternal-by-environment (MxE) interactions. In contrast genotype-by-environment (GxE) interactions were not detected. Moreover, we found that correlations among traits were largely explained by maternal effects, namely between body size (BS) and predator-prey size ratios (PPSR) and between assimilation efficiency (AE) and growth rate (GR), with no evidence supporting the occurrence of genetic correlations among traits. Thus, overall, this study highlights the importance of maternal effects for variation in foraging traits.

We found strong maternal effects and low heritability for offspring body size (BS), offspring body condition (BC), growth rate (GR) and assimilation efficiency (AE), as found in other organisms (Heath et al. 1999; Henryon et al. 2002; Mcadam et al. 2002; Van Der Westhuizen et al. 2004; Lindholm et al. 2006; Noble et al. 2014). The traits we measured have been shown to be highly correlated to fitness in several organisms (Ritchie 1990; Carroll et al. 1997; Hunt and Simmons 2000; Rauter and Moore 2002). Therefore, the low levels of additive genetic variance found here may be due to natural selection depleting genetic variance (Houle 1992, Hoffmann et al., 2016). Similarly, recent studies have pointed out that personality traits may have important consequences for fitness (Smith and Blumstein 2008) and consequently low levels of additive genetic variance are also expected in these traits, as it is the case for behavioral traits in general (Roff 1997). This may explain the low genetic variance in threshold predator-prey size ratios, which can be a proxy for aversion to potentially dangerous prey.

Our set-up does not allow disentangling dominance from maternal effects. Also, we cannot pinpoint the mechanistic basis of the effects we found. Indeed, the contribution of mothers to their offspring may occur via resource provisioning (Johnson et al. 2014), hormones (Groothuis and Schwabl 2008; McGlothlin and Ketterson 2008) or other maternal factors (Mousseau and Fox 1998). Here, we found that maternal effects persisted in traits expressed long after maternal care has ceased, such as PPSR, measured approximately 9 months after birth. This suggests that maternal effects are mediated by long-lasting mechanisms, such as hormones, although others cannot be discarded.

Still, we attempted to disentangle the effect of specific maternal traits on offspring traits (Sinervo and Doughty 1996; Noble et al. 2014). In particular, we reasoned that larger females (fixed structural body size) or females in better body condition could better provision their offspring. However, accounting for maternal traits did not explain the maternal variance observed in most traits, except for offspring size and condition, in which the maternal variance was reduced by 7% and 18%, respectively. Therefore, we do not have strong evidence that the high variance found in maternal effects can be attributed to these particular maternal traits.

The regression coefficients between mother and offspring traits may shed light on the factors shaping the traits observed. Indeed, we found a negative effect of clutch size on offspring size, which may indicate a trade-off between offspring size and number, expected from life-history theory (Fox and Czesak 2000), and often reported in the literature (Einum and Fleming 2000, 2004; Uller and Olsson 2005). Also, we found that clutch size increases with mother body size, as commonly found in ectotherms, this being the basis for the fecundity selection hypothesis for bigger body size in females (Fairbairn 1997) and at the proximate level probably indicates morphological constraints related to the abdominal space available (Honěk 1993). Further, we found a positive effect of mother body condition on offspring body size, suggesting that females in better condition are able to better provision their eggs, leading to offspring of larger size at hatching. Together, these results suggest that the mother physiological state and size can regulate the offspring number-size trade-off, as also shown in lizards (Uller and Olsson 2005) and fish (Gagliano and McCormick 2007). In contrast, we observed a negative coefficient for mother size on offspring body condition. This suggests that smaller mothers produced offspring better provisioned at birth. Possibly, females with smaller body sizes, having experienced more food deprivation throughout their ontogeny, invest in producing offspring of better quality, with higher chances of surviving in poor environments. This has been found in previous studies with lizards and crickets (Stahlschmidt and Adamo 2015; Wang et al. 2017).

Apart from such covariance between maternal and offspring traits, we also found significant correlations among maternal effects. The negative maternal correlation found between body size and PPSR indicates that if females provisioned offspring to be born to a larger size, the latter displayed lower PPSR later in life. This may be explained by the fact that larger spiders have access to more prey and they are less likely to being preyed upon (Verdeny-Vilalta et al. 2015). This could then allow taking lower risks when confronted with potentially dangerous (bigger) prey. Spiders provisioned to be smaller at birth, in contrast, may choose to take more risks later in life, as larger prey are more profitable, and this will allow growing at a higher rate, allowing to compensate their growth to resume their post-embrionic development without a disadvantage relatively to spiderlings that are provisioned to born larger. However, we found a significant positive effect of body condition (measured during the trial) on PPSR, indicating that better fed individuals tend to attack and subdue bigger prey sizes. This seems contradictory with the earlier finding that hungrier spiders (with lower body condition) tend to be more voracious (e.g., Moya-Laraño et al., 2003) which could imply a higher rate of prey acceptance. Possibly, relatively heavier spiders have higher chances of subduing larger crickets, as spiders jump on top of crickets to do so. Thus, PPSR is governed both by a maternally-transmitted factor that has repercussions in the offspring later in life and by intrinsic state characters (e.g., body condition).

A positive and significant maternal correlation was also found between assimilation efficiency and growth rates. Such correlation has been found in several studies, although its source was either purely environmental or genetically determined (e.g., Thodesen et al. 1999). To our knowledge, this is the first time that maternal effects have been found to affect this correlation. Most likely, some females provision resources to their offspring in such a way that these have high assimilation efficiency and growth rates, whereas others have offspring with low assimilation efficiency and growth rates. Individuals with high assimilation efficiency and growth rates may be able to become bigger without the additional cost of handling and searching, minimizing the exposure to predation and cannibalism. In contrast, individuals with low assimilation efficiency and growth rates may pursue other life history strategies in order to meet their energy requirements to achieve maturation, such as finding food more efficiently, achieving maturation later or maturing at smaller body sizes. Therefore, this differential provisioning of the offspring could serve to ensure growth in a large array of environments. In fact, females of this species accrue food and lay egg sacs in the peak of the Thermo-Mediterranean summer (August), in the dry season and before the first rains, when rainfall and thus productivity of the next fall are difficult to predict (Lázaro et al. 2001). Therefore, different maternal strategies could be adaptive in different years depending on the conditions, allowing the maintenance of many of them from fluctuating selection. Moreover, the fact that both traits were also involved in maternal x environment interactions (MxE) suggests that mother-induced variation in life-history strategies vary across environments (Figure B.2).

By promoting variation in foraging traits, maternal effects can differentially impact food web structure, even without the occurrence of standing genetic variation. Additionally, this effect may vary with the environment where mothers or their offspring occur. In heterogeneous environments, such as semi-arid ecosystems, maternal effects may thus be an important source of intraspecific variation, allowing offspring to cope with environmental uncertainty. Cannibalistic systems are particularly sensitive to such variation. For example, variation in body sizes throughout the season increases the possibilities of cannibalistic events (Moya-Laraño 2011). The adaptive value of sets of maternally correlated traits should be the focus of further research. Even beyond morphology and physiology, maternal effects can modulate PPSRs through personality, further contributing to a diversification of trophic interactions and consequent impacts upon food webs, community structure and ultimately ecosystem functioning. Future studies should thus take in consideration how maternal effects drive intraspecific variation not only in top predators, but across all trophic levels, including how this will modulate the architecture of food webs and dynamics of trophic cascades.

Acknowledgments

This study was supported by a PhD grant (PD/BD/106059/2015) attributed to Jorge Henriques by the Portuguese Science and Technology foundation (FCT), by the grant P12-RMN-1521 from the Andalusian government and by the grant CGL2015-66192-R from the Spanish Ministry of Economy and Competitiveness, both partially funded by the European Regional Development Found, both attributed to Jordi Moya-Laraño and to the FPU scholarship (FPU13/04933) from the Spanish Ministerio de Educacion, Cultura y Deporte to Dolores Ruiz-Lupión.

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CHAPTER IV

Multidimensional intraspecific maternal functional diversity in a top predator strengthens a trophic cascade

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Chapter IV – Multidimensional intraspecific maternal functional diversity in a top predator strengthens a trophic cascade

Abstract

Ecologists have recently recognized the importance of intraspecific variation in communities and ecosystems (hereafter, ecological effects). However, to date almost no study has considered the role of variation in more than one trait nor in the multidimenstional nature of intraspecific variation (i.e., multidimensional intraspecific functional diversity, MIFD) on ecological effects. Here, we describe a novel methodology to experimentally manipulate multidimensional intraspecific functional diversity (MIFD) to study its ecological effects. We use it for the first time to manipulate the level of MIFD in the foraging traits of a top predator, and test the effects on a trophic cascade. In a mesocosm experiment, we assembled simple communities including a species of wolf spider, a cricket prey and lettuce as the basal resource, and manipulated the provenience of spiders (from different feeding environments; i.e., environmental diversity) or the level of MIFD calculated from 4 independent axes of trait variation, estimated as maternal effects in a half-sib design. We show that mesocosms with spiders having higher MIFD experiment stronger trophic cascades than mesocosms with lower MIFD. This was not due to higher mortality of crickets but most likely to a change in cricket behavior. Environmental diversity resulted in a higher rate of spider cannibalism driven by the large differences in body sizes from the spiders reared in different environments. However, this rate of cannibalism was not enough to release crickets from predation threat, and thus the strength of the trophic cascade was not dampened. Our results highlight the importance of maternal effects as a source of ecological effects. Moreover, these findings suggest that multidimensional individual variation in cannibalistic predators, instead of increasing predation rate, may induce ecological complementarity and undermine the dampening of trophic cascades.

Keywords: Intraspecific variation, multidimensional functional diversity, maternal effects, food webs, *Lycosa fasciiventris.*

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Introduction

How biodiversity affects ecosystem functioning is a central question in ecology (Loreau et al. 2001; Hooper et al. 2005; Balvanera et al. 2006), as the loss of biodiversity from global change may lead to an irreversible impairment of ecosystem processes, with potentially unprecedented consequences for our planet (Estes et al. 2011; Ceballos et al. 2017). Studies on the ecological effects of biodiversity have traditionally focused on interspecific diversity (Tilman et al. 2014). Recently, however, several reviews and a few experimental studies have acknowledged the role of intraspecific variation in population dynamics, ecological communities and ecosystem processes. Indeed, meta-analyses show that the ecological effects of intraspecific variation can be as strong as are those of interspecific variation (Des Roches et al. 2018; Raffard et al. 2019).

In particular, as it is the case with intraguild predation during interspecific interactions (Finke and Denno 2004, 2005), when top predators are cannibalistic, higher diversity of traits within predator populations can dampen trophic cascades because populations with high intraspecific diversity may be more prone to cannibalism (Moya-Laraño 2011). However, following with the analogy with interspecific diversity (Griffin et al. 2013), if cannibalistic rates are not higher when diversity in functional traits increases, complementarity may lead to strong top down control (Thébault and Loreau 2003).

Thus, intraspecific variation may be an important component of functional diversity (Carmona et al. 2016) when the traits involved are “effect” traits; i.e., those that have an effect on the ecosystem (Diaz and Cabido 2001). Furthermore, when these traits have a genetic basis and may also respond to changes in the environment (“response” traits), rapid evolution may follow, potentially giving rise to eco-evolutionary dynamics (Schoener 2011). Therefore, partitioning the genetic and environmental components of intraspecific variation is highly relevant for understanding how this variation affects ecosystem functioning (Bolnick et al. 2011). Additionally, the ecological effects of maternal variance have been rarely tested, especially their impact across trophic levels (e.g., top-down control). This is at odds with the fact that maternal effects may greatly affect population dynamics (Mousseau and Fox 1998; Wilson et al. 2005).

However, with a few exceptions, most studies addressing the effects of intraspecific variation on ecosystems did not identify the source of trait variation.

Additionally, the ecological effects of intraspecific functional diversity have generally been investigated only in one single dimension or trait (but see Pruitt *et al.* 2016, 2017).

In this study, we experimentally manipulated multidimensional intraspecific functional diversity (MIFD) in a cannibalistic predator, the wolf spider *Lycosa fasciiventris*, and tested its effect on a trophic cascade with herbivorous crickets as prey and lettuce as the basal resource. Our approach is novel in that we define MIFD based on the maximum number of detected ecological degrees of freedom; i.e., analogously to "genetic degrees of freedom" (Schluter 2000) we define it as the number of relevant orthogonal axes of a PCA on genetic variation of effect traits. These ecological degrees of freedom may include enough trait diversity as to induce orthogonal and complementary ecological effects. When this phenotypic matrix (P) has a strong genetic component (i.e., the G matrix explains a large proportion of the P matrix), obvious consequences for eco-evolutionary dynamics follow.

We focused on spider traits that can affect either directly or indirectly predator-prey interactions: predator growth rate, assimilation efficiency, threshold prey-predator size ratios (i.e., the relatively largest prey that the spider is able to attack and kill) and developmental time. The latter trait can affect predator-prey interactions independently of growth rate because in a food web context developing faster may entail reaching a size refuge earlier to minimize predation risk (especially from conspecifics), and also may improve the offspring chances to catch a larger array of prey (Verdeny-Vilalta et al. 2015). All these traits showed substantial and orthogonal maternal effects in a PCA (i.e., four ecological degrees of freedom) and we therefore used them to manipulate maternally determined MIFD distances. As early life-experiences, such as access to food resources, can influence offspring phenotype throughout their life, we also tested whether carry-over-effects could influence trophic cascades. These are defined as any events or conditions experienced by offspring in a given season or stage that influences individual performance in subsequent seasons or stages (Harrison et al. 2011). In this study, we assessed such effects by rearing offspring from each female in two food environments (rich and poor) and assessed if the rearing environment could affect the strength of the trophic cascade by replicating some of the above treatments with spiders coming from rich or poor environments. Additionally, we tested for the effect of environmental diversity by including another treatment in which we mixed spiders originated in these two contrasting environments, therefore increasing the environmental variability of the phenotypes.

We believe that this study entails an important step forward into our understanding of how biodiversity affects ecosystem processes and offers a new approach that allows manipulating MIFD.

Methods

Experimental design

The experimental treatments aimed at testing two components of intraspecific diversity, maternal MIFD (high vs low) and environmental diversity (animals from a single vs diverse rearing environments) on a trophic cascade. In addition, it aimed at testing the carryover effect of rearing environmental quality (rich or poor) on top down control. Each basic experimental arena was a 57x38x25cm mesocosm with 4 spiders, 8 crickets of variable size and 4 renewable lettuce disks, forming a trophic chain. Because we targeted a minimum of 20 replicates per treatment and we had only 364 spiders available for the experiment, we could not have all the combinations to conform to a full bi-factorial design. Thus, we ran the experiment with 4 treatments and devised a series of *a priori* comparisons to test each of the predictions. To compare high *vs*. low maternal MIFD, we established two MIFD treatments in which all spiders had been reared in poor feeding environments: High Maternal Distance and Poor Environment (HMD_PE) *vs* Low Maternal Distance and Poor Environment (LMD_PE). In order to test whether carryover effects from environmental quality (rich or poor) was important, we established a treatment with high MIFD and with spiders reared in a rich environment (HMD_RE) and compared the results with the HMD_PE treatment above. Finally, to test whether environmental diversity dampened a trophic cascade, we established a fourth treatment with low maternal distances and with 2 spiders that had been reared in a poor environment and two others having been reared in a rich environment (Low Maternal Distance and Diverse Environment, LMD_DE). We then compared the results of this treatment against the LMD_PE treatment above. We also established a 5thControl treatment, with crickets and lettuce but no spiders, to test for the occurrence of a trophic cascade by comparing cricket mortality and behavior, as well as lettuce consumption in the treatments with spiders against the Control. We ran the experiment in blocks, each in a separate laboratory room containing 5 mesocosms (one for each treatment) which were spatially arranged at random. Each block lasted for 72 hours and had one observer. We could run up to 3 of these blocks per week with three different observers (one in each room). Thus, the experiment lasted for almost 3 months.

Details of the experimental set-up

Mesocosms included 2 pairs of conspecific *L. fasciiventris* predatory spiders, 8 herbivorous insects *Gryllus assimilis* (Fabricius) and 4 *Lactuca sativa (var. capitata)* leaf discs as a basal resource, representing the three levels of a trophic chain. Each mesocosm was divided into 4 quadrants through mobile wooden barriers (one longitudinal and two transversal). The aim of these barriers was to direct the order in which interactions occurred. At the beginning of the trial (first 24h) the transversal barrier was removed, allowing two pairs of spiders to interact to each other but not with the spiders of the other pair. Similarly, 4 crickets were placed in half of the experimental arena, isolated from the other 4. After 24 hours the longitudinal barrier was removed, allowing interactions among all the individuals that survived during the first 24 hours. Since cannibalism can occur quite rapidly, this procedure aimed at better controlling the interactions in the mesocosm and better mimicking what occurs in nature, as 4 spiders will barely meet at the same time in the wild. We added a layer of approximately 1cm depth sieved soil at the bottom of the mesocosm. Before the experiment, all the spiders and crickets were weighted in a precision scale (Mettler Toledo XP26). The carapace and abdomen width of the spiders were measured in a stereoscopic magnifying glass (Leica MZ125). To allow identification of each individual spider interacting within the mesocosm, one of their right legs was marked with a small mark using nail polish (e.g., Moya-Laraño et al. 2003).

Two crickets were assigned to each spider: a small one (with a length two times the carapace width of the spider, 2X) and a larger one (with a length four times the carapace width of the spider, named 4X). The length of the crickets was calculated from weighing them and using a Length-Mass calibration curve (Chapter III). These two sizes of prey were chosen to include variation on prey availability to the spiders, as we know that not all spiders are equally prompt to attack large prey (Chapter III). The lettuce leaf discs were cut with a hole puncher of 28 mm diameter and situated on top of a wet cotton inside a Petri dish (5.5 cm Ø) to maintain its turgidity. The petri dishes were placed in the middle of each quadrant of the mesocosms, and the wet cotton work as a water source for both the spiders and the crickets. We determined the average initial area to be (Mean ± SE) 6.36 ± 0.422 cm^2 by scanning 36 discs and analyzing the images with the program Image J (Abràmoff et al. 2004).

At the beginning of the experiment, the spiders were located one in each quadrant and allowed to acclimate for 16h (overnight), having only access to the water source. At the starting of the trial, the spider was enclosed within a plastic cup next to the mesocosm wall and the respective two crickets were also enclosed in inverted cups but at the opposite end of the container than the spider. Between quadrats, spiders were placed at opposite sides at the beginning of the trial (before removing the barriers), so that when individuals were released and the first barriers removed, the spiders had minimum chances to directly interact antagonistically with each other, and therefore we provided the opportunity for them to decide upon pursue or avoid conspecifics and/or crickets.

After adding the lettuce discs and wet cotton to the petri dish the transversal barrier and the vials enclosing predators and prey were removed, starting the trial. Every 24h the lettuce discs were removed and scanned. Unconsumed lettuce area (cm^2) was determined using Image J (Abràmoff et al. 2004). The lettuce discs were then replaced by fresh ones and the longitudinal barrier was removed, allowing the remaining spiders and crickets to interact. We recorded the number of predation events (if a spider preyed upon a cricket), the number of cannibalistic events (if a spider preyed upon a conspecific spider) and the spider and cricket location at each quadrant, as well as how many crickets were on the lettuce spot. Observations were repeated 3 times per day, starting at 9am and every 4 hours.

Half-sib design, rearing environments and predator traits

To assess the genetic, maternal and environmental components of phenotypic variation of traits, we ran a half-sib design in which 50 males were mated to 100 females. The details of this experiment, and how traits were recorded are described in previous chapters (Chapter II, Chapter III). In short, 12 spiderlings of each female were reared in the laboratory during approximately 9 months, time during which several traits were measured in the surviving spiders. A third of the spiders from each dam was randomly assigned to a rich rearing environment (3-9 *Drosophila* flies per week) and the remaining, in which we anticipated higher mortality, to a poor rearing environment (1-3 *Drosophila* flies per week). After all traits were measured, the present experiment began with the surviving individuals (N=364). Sample size was slightly higher for trait measurement as some spiders died during the time from trait measurement until the experiment began (Table 4.1). Although a larger array of traits than the ones used here were measured, to assess MIFD we just used the traits whose results were readily available at the end of the experiment and did not need further complex analyses (e.g. analyze video recordings):

individual growth rate, assimilation efficiency, prey-predator size ratio and developmental time. We predicted that all these traits could affect predator-prey interactions either directly or indirectly, through their correlation with other unmeasured traits. Individual growth rate is the rate of increase in mass in a period of 6-7 months. Assimilation efficiency is the ratio between the amount of gained mass by the spider divided by the amount of ingested mass. Prey-predator size ratio is the threshold ratio at which the spider attacks and kills a prey, determined by an assay in which 5 prey were offered sequentially from largest to smaller. Developmental time was the time elapsed until the spider reached the 5th instar. Note that none of the spiders used in the present experiment had reached maturation.

Assessment of ecological degrees of freedom and manipulation of MIFD

Our approach to manipulate MIFD had three main steps: 1) Calculating the ecological degrees of freedom via PCA, which aims at ensuring orthogonality among the traits involved in MIFD, 2) calculating phenotypic or genotypic (maternal) multidimensional distances among individuals, genotypes or families, and 3) assigning individuals to treatments according to their genetic or maternal multidimensional distances. As in Chapter II and Chapter III we used MCMCglmm to calculate the variance components of the half-sib design. Since the procedures followed are described in detail there, here we just briefly summarize them. We ran a generalized linear mixed model with normally distributed errors for each of the four traits. Differently than previous chapters, here we included rearing treatment as a random factor, a degree of believe parameter ν of 1, and after 20000 iterations we obtained more than 1000 efficient samples for all the parameters in all traits. As with most traits measured so far for these spider species (Chapter III), the results of the half-sib design showed that maternal effects explained a substantially larger proportion of the phenotypic variance relative to additive genetic effects, which showed substantially lower, almost negligible variation.

Table 4. 1 - Heritability (h^2) and maternal effects (m^2) for the focal traits. Values were estimated from the mode of the posterior distributions obtained in the statistical model; the low (LCI) and high (HCI) Bayesian credible intervals are presented. N – sample size., **GR** – growth rate, **AE** – assimilation efficiency, **PPSR** – prey-predator body size ratio, **DT** – development time.

trait	h^2	LCI	HCI	m^2	LCI	HCI	N
GR	0.184	0	0.405	0.289	0	0.58	483
AE	0.154	0.015	0.318	0.256	0.012	0.493	489
PPSR	0.138	0.011	0.27	0.203	0.016	0.402	512
DT	0.293	0.001	0.592	0.46	0.01	0.86	520

We therefore used the information on the dam families to calculate the ecological degrees of freedom and to manipulate MIFD. From the above models we obtained BLUPs (Best Linear Unbiased Predictors) for each dam family and then assessed the ecological degrees of freedom by running a PCA on the 4 BLUP variables (one for each trait) with "varimax" rotation, which allows to increase the spread of trait variation across the orthogonal axes (Tabachnick and Fidell 2014). This procedure maximizes the evenness of the variance explained by each trait-PC combination while maintaining the orthogonality (independence) of the PCs, thus purposely used here to increase the potential for complementarity in effect traits and to avoid the effects of some of the previously documented maternal correlations (Chapter III). We used Levin's index (L) to calculate the degrees of freedom of the resulting PCA (Schluter 2000):

$$L = \frac{1}{\sum_i^k p_i^2} \qquad \text{(eq1)}$$

where p_i is the proportion of variance explained by PC i, and k is the total number of PCs in the analysis. L ranges between 1 and k.

The use of BLUPs for statistical analysis has been shown to be flawed in many circumstances (Hadfield et al. 2010). However, here we just used them not to report statistical associations but, on the contrary, to load each trait on separate orthogonal axes. However, for comparison, we show here the results of the PCA using BLUPs and the PCA calculated directly from the variance-covariance matrix of maternal effects (M matrix) obtained from bivariate MCMCglmm on standardized (0,1) traits (as in Chapter III). The results are substantially similar (Table 4.2 and Table 4.3).

Table 4. 2 - Principal components (rotated) analysis of the best linear unbiased predictors (BLUPs) estimated for the focal traits. **GR** – growth rate, **AE** – assimilation efficiency, **PPSR** – prey-predator body size ratio, **DT** – development time.

TRAIT	PC1	PC2	PC3	PC4
GR	0.1	0.03	-0.32	0.94
AE	0.99	-0.03	-0.09	0.09
PPSR	-0.03	1	0	0.03
DT	-0.1	0	0.94	-0.32

Table 4. 3 - Principal components analysis (rotated) of the variance-covariance matrix for the maternal effects (M matrix) estimated for the focal traits **GR** – growth rate, **AE** – assimilation efficiency, **PPSR** – prey-predator body size ratio, **DT** – development time.

TRAIT	**PC1**	**PC2**	**PC3**	**PC4**
GR	0.18	-0.13	-0.26	0.94
AE	0.97	-0.08	-0.14	0.17
PPSR	-0.08	0.99	0.07	-0.11
DT	-0.15	0.07	0.95	-0.25

Since regardless of the method use (BLUPs or M matrix) each PC explained ¼ of the variance, the resulting Levin's index was exactly 4 (the maximum). Using the scores of the above PCA for each dam family, maternal distances were calculated using weighed Euclidean distances, using as weights (*w*) the relative contribution of the maternal heritability m^2 for each of the traits that loaded with a value >0.9 in each principal component (e.g. that explained most of the variance in that PC). For each pair of dam families (*i,j*), the multidimensional maternal distance (MD) from trait 1 to *k* was calculated as follows:

$$MD_{ij} = \sqrt{w_1\left(d_{1i} - d_{1j}\right) + \cdots + w_k(d_{ki} - d_{kj})} \qquad \text{(eq2)}$$

Where d is the PC-score value for the dam family and for the trait $(1 \ldots k)$ loading on that PC, and the w are the weights:

$$w_{1 \ldots k} = \frac{m_{1 \ldots k}^2}{\sum_1^k m^2} \qquad \text{(eq3)}$$

Here, Euclidean distances were obtained using the maternal effects of each trait because we were interested in obtaining realistic maternal distances. Hence, traits with stronger maternal contributions had a higher weight in the distance equation and on the manipulation of MIFDs. That way the maternal contribution for that trait had in turn a more important contribution to the potential ecological effect of that trait. However, depending on the type of question, this methodology can also be used to manipulate the percentage of genetic variance explained by each PC, or when used across species, the relative abundance of each species, as usually done in functional diversity indices (Mouchet et al. 2010).

Bootstrap confidence intervals for MDs where obtained using the library *boot* in R (see the R code below). Figure 4.1 shows the plot with all the distances among families along with their CIs.

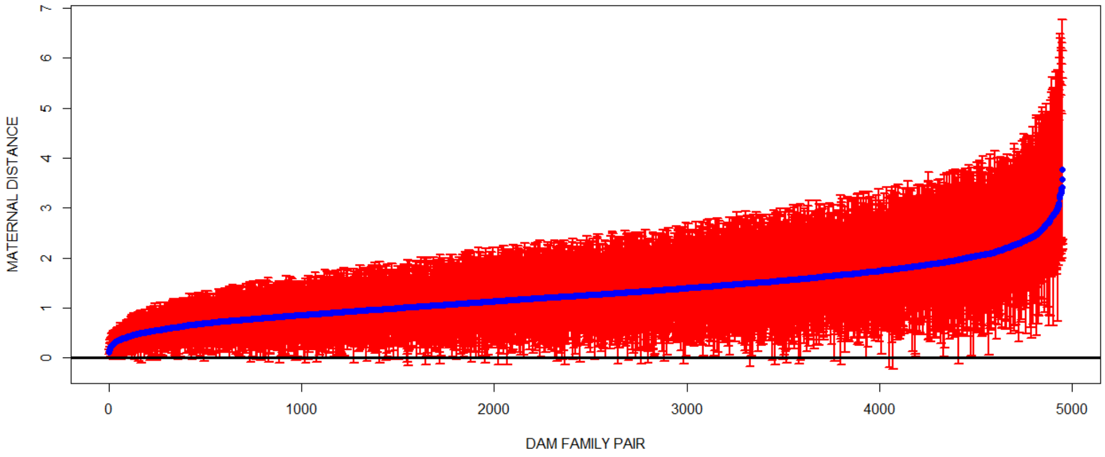

Figure 4. 1 - Multidimensional maternal distances (with bootstrap basic 95% CIs) between pairs of dam families.

In order to manipulate MIFDs inside each mesocosm, pairs of spiders coming from family pairs from the left part of the distribution in Figure 4.1 were randomly selected for the Low Maternal Diversity (LMD) treatments. Each week, we systematically used the family pairs with the lowest maternal distances from within the available stock, deciding at random which spiders to use from each family as long as they met the requirement for the feeding treatment of origin (see below). This was similarly done for family pairs from the right part of the distribution for High Maternal Diversity (HMD); i.e., choosing each week the family pairs with maximum MD. The spiders to use for assignment also conformed to the environmental treatment (e.g., poor environment, *PE*; rich environment, *RE*; or *DE*, diverse environment). Kin effects in the Low MD treatments were unlikely because the percentage of experimental replicates in which 2 half-sibs were present (i.e., 2 spiders coming from the same sire) was only 3%, being the number of full-sibs obviously even smaller. We ran sensitivity analyses for the distances of the spiders chosen for the experiment using different priors and prior structures as well as including day of birth in the models or not (Chapter III). We also ran the same procedure using dam family means instead of BLUPs. Using mesocosm as the unit of replication, all of the above analyses resulted in distance correlations above 0.7 with the original distances and in all cases the HMD treatment had significantly higher distances than the LMD treatment (not shown).

Statistical analyses

The consumed area was used to estimate a Trophic Cascade Index (TCI) for each quadrant in each experimental box containing spiders. We constructed an index following a combination between the trophic cascade index of (Lensing and Wise 2006) and the difference/sum ratio index in (Armas et al. 2004). The latter adjustment improved statistical accuracy due to the demonstrated stable statistical properties of a difference/sum ratio of two quantities measured in similar units:

$$TCI_{E_i} = \frac{\overline{\Delta l_C} - \Delta l_{E_i}}{\overline{\Delta l_C} + \Delta l_{E_i}} \qquad \text{(eq4)}$$

Where TCI_{E_i} stands for “Trophic Cascade Index” for replicate *i* in experimental treatment E, $\overline{\Delta l_C}$ is average lettuce consumption across Control replicates (Mean ± SE, 2.32 ± 0.27 cm^2, n = 22), which was obtained by first averaging the lettuce consumption among days within each of the 4 quadrants within a box, then taking the mean among quadrants within a box and finally averaging the latter among blocks. Δl_{E_i} is lettuce consumption in replicate *i* of experimental treatment *E*, which was obtained by averaging lettuce consumption among quadrants per day and then among the 4 days of observation, considering that each day the lettuce disk was replaced by a fresh one. Note that a higher TCI means a stronger trophic cascade since it involves that less lettuce is consumed in the treatment with predators, and that the index ranges between 0 and 1.

When random factors needed to be included in models with continuous (normal) dependent variables, we used Generalized Linear Mixed Models (GLMM) using the function *lmer* in library *lme4* (Bates et al. 2014) of R version 3.5.3 (R Core Team 2019). If random factors did not need to be fitted, we used the function GLM in R. To test planned-comparisons by orthogonal contrasts we used the function *glht* within the library *multcomp* (Hothorn et al. 2008). We used the function *ranova* within the library *lmerTest* (Kuznetsova et al. 2017) to test whether random factors needed to be included in the final models. Block was used as a random effect when necessary. To test whether a trophic cascade was occurring in the experiment, we compared with an orthogonal contrast the mean percentage of lettuce consumption in mesocosms without spiders against that in the mesocosms of the other four treatments with spiders (data log-transformed). To test if the trophic cascade resulted from consumptive effects, we compared cricket mortality using the same procedure. The differential mortality of small *vs.* large crickets was tested by including a difference-sum ratio of the mortality of each type of cricket, as in TCI above. Anti-predator behavior was tested by including the number crickets that were in the lettuce as a response variable and the log of the actual number of crickets alive at each visit as a covariate in a GLMM with Poisson distribution, and testing the same contrast as above. It is expected that crickets displaying anti-predator will look to conceal its presence from predators and avoid the lettuce, where these will be more conspicuous. Mesocosm was additionally added as a random factor. Differences in the number of cannibalism events inside each mesocosm were also tested with a Poisson GLMM. Predicted means from models were extracted using the library “emmeans” (Lenth 2018).

Results

Lettuce consumption was ca. 1.2X higher in mesocosms without spiders as compared to the rest of mesocosms in the other 4 treatments (GLMM, *Contrast Estimate* = 1.9 ; Z = 7.3; $P < 0.0001$; Figure 4.2), indicating the existence of a trophic cascade in the mesocosms. This trophic cascade was due to cricket mortality, which was 4.2X higher in mesocosmos with spiders as compared to mesocosms without spiders (GLM, *Contrast Estimate* = -18.14 ; Z = -21.7; $P < 0.0001$; Figure 4.2). No differences were found in the mortality of large *vs.* small crickets in treatments with or without spiders (GLM, *Contrast Estimate* = -0.05 ; *Z* = -1.6; *P* = 0.114). The occurrence of crickets in lettuce patches was not affected by the presence of spiders (GLMM, *Contrast Estimate* = -0.61; *Z* = -0.59; *P* = 0.553).

The magnitude of the trophic cascade, as revealed by a trophic cascade index (TCI), differed among the 4 treatments with spiders (GLMM, χ^2_3 = 10.02; *P* = 0.018, Figure 4.4). Planned comparisons showed that higher MIFD led to 1.22X stronger trophic cascades (HMD_PE *vs*. LMD_PE, *Contrast Estimate* = 0.07; *Z* = 2.37; *P* = 0.018; Hedge's d = 0.57), but that neither carryover effects from the rearing environment (HMD_RE *vs*. HMD_PE, *Contrast Estimate* = 0.04; *Z* = 1.43; *P* = 0.154), nor environmental diversity (LMD_PE *vs*. LMD_DE, *Contrast Estimate* = 0.02; *Z* = 0.61; *P* = 0.540) affected the strength of the trophic cascade. As expected, cannibalistic rates differed among the 4 treatments with spiders (GLMM, χ^2_3 = 15.6; *P* = 0.001; Figure 4.5). However, cannibalistic rates did not differ between high and low MIFD treatments (HMD_PE *vs*. LMD_PE, *Contrast Estimate* = -0.32; *Z* = -1.0; *P* = 0.320), nor between treatments with different carryover effects either (HMD_RE *vs*. HMD_PE, *Contrast Estimate* = 0.38; *Z* = 0.6; *P* = 0.547). Although we failed to find an effect of environmental diversity on the strenght of the trophic cascade, cannibalistic rates were 2.2X higher in mesocosms containing spiders reared in diverse environments compared to those coming from the same environment (LMD_PE *vs*. LMD_DE, *Contrast Estimate* = -0.79; *Z* = -2.06; *P* = 0.040).

The differences found for the strength of the trophic cascade in high *vs* low MIFD treatments were likely due to changes in cricket behavior, as we failed to find differences in cricket mortality between those two treatments (GLMM, HMD_PE *vs*. LMD_PE, χ^2_1 = 0.27; *P* = 0.610). However, we did not find differences in cricket behavior on their use of lettuce patches across all treatments (see above), Possibly, other antripredator behavioral differences that we were unable to document led to lower lettuce consumption in treatments with high MIFD.

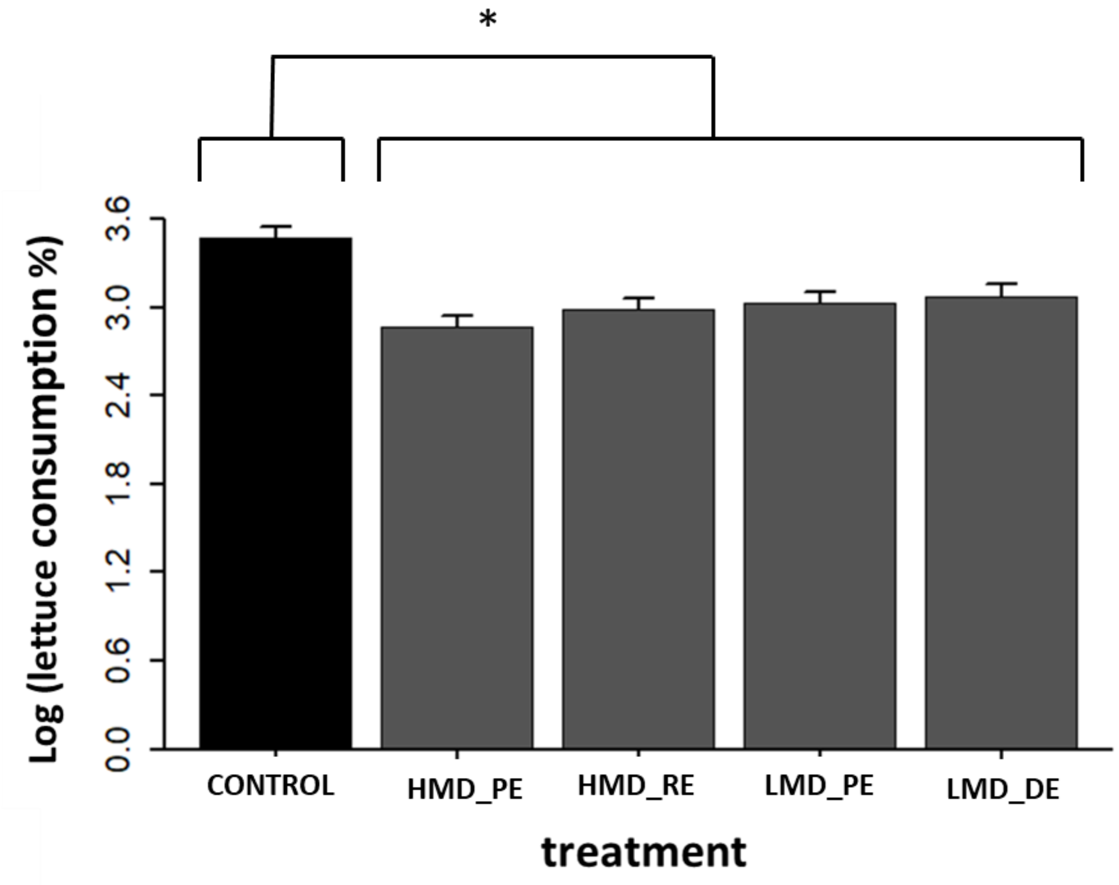

Figure 4. 2 – Percent of lettuce consumption (mean values ± standard error) on the experimental treatments. **Control** – control treatment; **HMD_PE** – high maternal diversity and poor environment; **HMD_RE** – high maternal diversity and rich environment; **LMD_PE** - low maternal diversity and poor environment; **LMD_DE** – low maternal diversity and diverse environment. * denotes statistically significant comparisons.

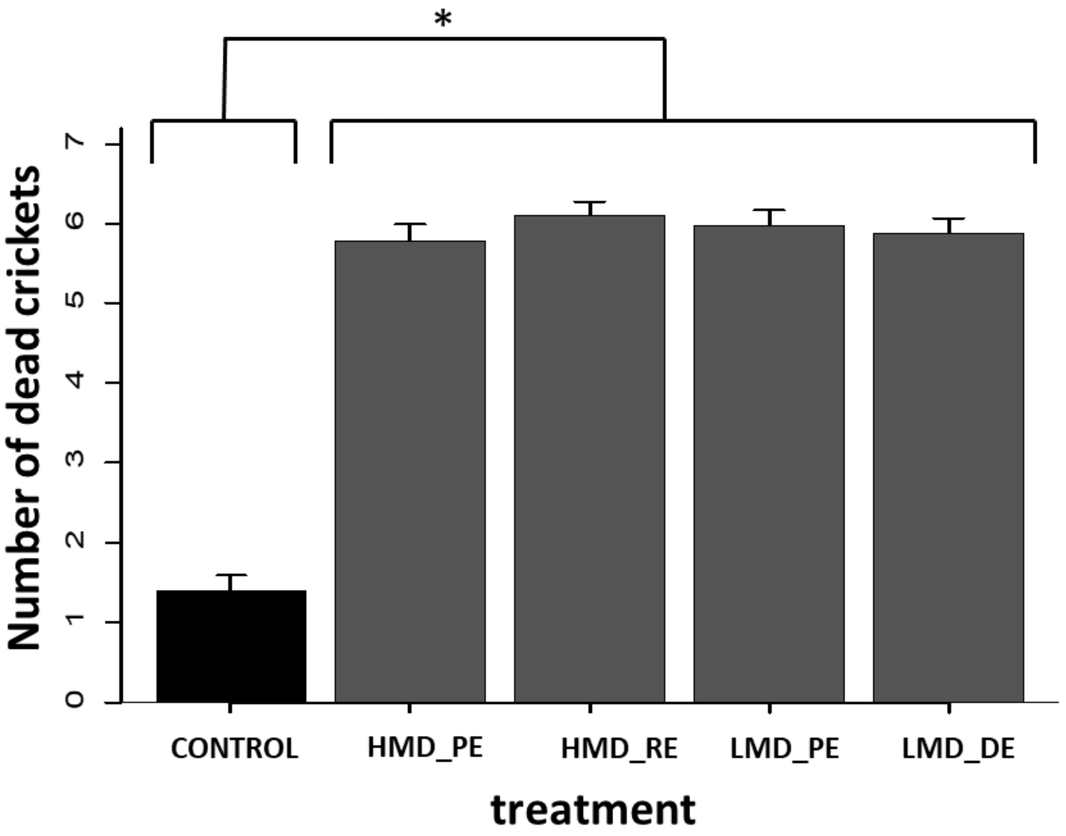

Figure 4. 3 – Cricket mortality in each experimental treatment expressed as the mean number of dead individuals. **Control** – control treatment; **HMD_PE** – high maternal diversity and poor environment; **HMD_RE** – high maternal diversity and rich environment; **LMD_PE** - low maternal diversity and poor environment; **LMD_DE** – low maternal diversity and diverse environment. * denotes statistically significant comparisons.

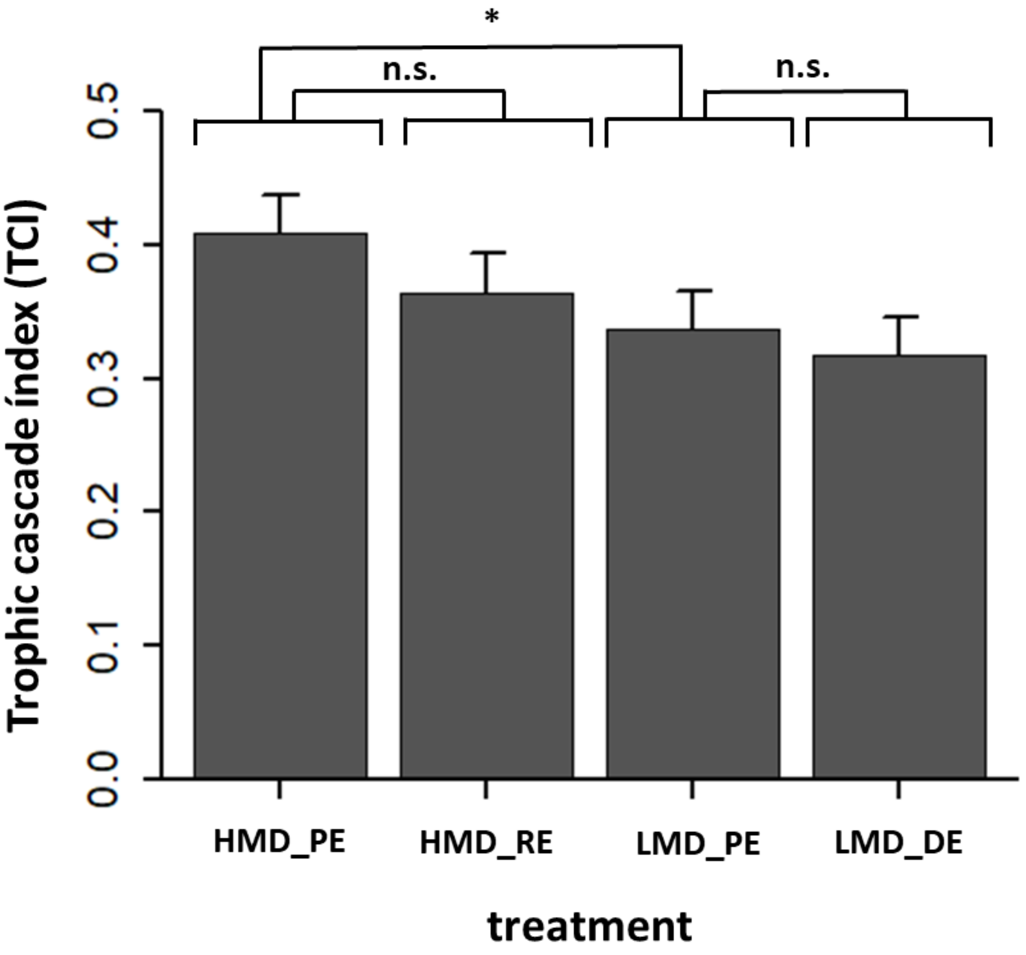

Figure 4. 4 – Trophic cascade index (TCI) calculated in each experimental treatment. **HMD_PE** – high maternal diversity and poor environment; **HMD_RE** – high maternal diversity and rich environment; **LMD_PE** - low maternal diversity and poor environment; **LMD_DE** – low maternal diversity and diverse environment. * denotes statistically significant comparisons

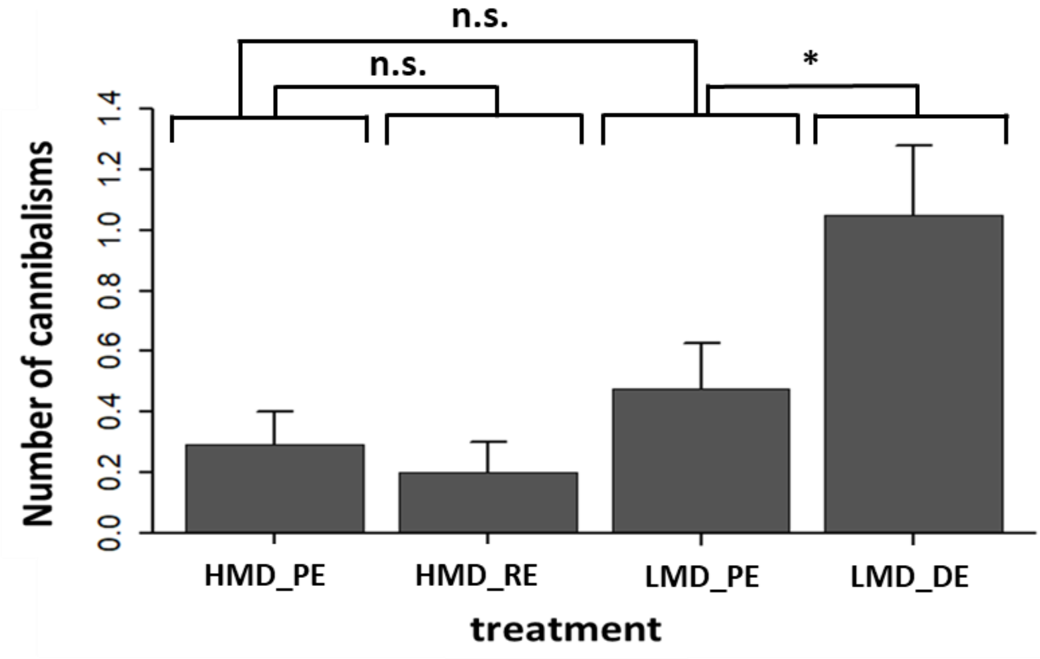

Figure 4. 5– Mean number of cannibalized individuals in each experimental treatment. **HMD_PE** – high maternal diversity and poor environment; **HMD_RE** – high maternal diversity and rich environment; **LMD_PE** - low maternal diversity and poor environment; **LMD_DE** – low maternal diversity and diverse environment. * denotes statistically significant comparisons.

Discussion

In this study, we show that in a trophic chain composed of spiders, crickets and lettuce, maternally-driven intraspecific diversity in the predator population, strongly affected the strength of trophic interactions. Importantly, mesoscosms with generalist predators with higher MIFD exerted stronger top-down control than those with predators of low MIFD. These results provide support for the complementarity hypothesis of predator diversity on top-down control (Finke and Denno 2004; Ives et al. 2005; Steffan and Snyder 2010; reviewed in Griffin et al. 2013), but with the particularity that here we experimentally manipulated multidimensional intraspecific functional diversity for the first time. Thus, intraspecific diversity did not lead to higher rates of cannibalism and as a consequence they did not dampen the trophic cascade (Finke and Denno 2004; Moya-Laraño 2011), instead, they strengthened it. These results recapitulate those found for interspecific diversity, as when intraguild predation (the interspecific equivalent of cannibalism) is weak, complementarity effects and stronger top-down control are expected (Finke and Denno 2005).

Although the demonstration of the trophic cascade by removing spiders in the Control treatment mesocosms was mostly due to cricket mortality (Density-Dependent Indirect Interaction, DDII), the differences in the strength of the trophic cascade between treatments with high or low MIFD was probably originated from differences in cricket behavior (Trait-Mediated Indirect Interaction, TMII). This is because we were unable to find differences in cricket mortality between high and low MIFD treatments that could explain the differences in lettuce consumption. However, the differences in behavior responsible for the stronger trophic cascade in high MIFD treatments were likely more subtle than our behavioral assessment allowed to detect. In addition, how the 4 trait dimensions actually led to complementarity effects on a behavioral trophic cascade is not a simple matter. Previous research has found that predator activity is positively correlated with the strength of a trophic cascade (Keiser et al. 2015), but none of the traits used to obtain our MIFD distances could be unambiguously assigned to activity. The most parsimonious explanation for our result is that several other unmeasured spider traits are affecting cricket behavior, and that these traits are maternally correlated to one of the 4 dimensions used in our index, being the former the direct cause of the complementarity effect.

The environment from which the offspring of the spiders came from did not result in carryover effects affecting the magnitude of the trophic cascade or in the rate of cannibalism, in absence of environmental variation. However, when we combined offspring reared in contrasting environments, with manifest differences in body size (Figure C1), we found, as expected, that the rate of cannibalism increased. However, this increase in cannibalism was not sufficient to dampen the trophic cascade in our laboratory mesocosms.

Since our approach of manipulating MIFD is a feasible and straightforward way to manipulate multidimensional trait distances, we envision a future of experiments manipulating MIFD as both response and effect traits. Furthermore, since we used simple weighted Euclidean distances, this multidimensional distance index allows weighting according to the variance component we are more interested in, e.g. maternal, genetic or environmental. Furthermore, if used to manipulate distances across species, weighting by the relative abundance of each species can also be incorporated, as in several conventional functional diversity indices currently being used by researchers (e.g. Mouchet et al. 2010). If weighting by species, then the abundances of species in the experiment should follow the same proportions as those used for weighting. Furthermore, although in this study we used a more simplified version of environmental diversity, it would be interesting, in future studies, to test whether multidimensionality in more complex rearing environments (e.g., nutrient diversity, diversity of predatory threats) could also lead to complementarity effects in trophic cascades from multidimensional carryover effects.

Previous studies have assessed or directly manipulated intraspecific trait variation in a predator species and to assess its impacts upon community structure and cascading trophic interactions (Post et al. 2008; Ingram et al. 2011; Keiser et al. 2015).These studies concerned the effect of single traits, whereas it is clear that variation is multidimensional. To our knowledge, this is the first study to consider the ecological effects of multidimensional intraspecific functional diversity. Additionally, we show that such effects are due to maternal variance. Also, few studies have considered the ecological effects of maternal effects before. Indeed, maternal effects are known to affect the stability of population dynamics, both theoretically (Benton et al. 2001; Inchausti and Ginzburg 2009) and empirically (Benton et al. 2005, 2008; Plaistow and Benton 2009), the range expansion and species turnover in birds (Duckworth 2009, Duckworth et al. 2015), and the stability of predator-prey dynamics (Garbutt et al. 2015). Our study adds to these studies focusing on the ecological role of maternal effects affecting three trophic levels, and points to the central role that maternal effects may have in ecosystems. Therefore, researchers likely need to account for this important proportion of trait variation in ecological studies.

The fact that we found 4 independent axis of maternal variation that act complementarily, strengthening a trophic cascade, may have important consequences for eco-evolutionary dynamics as in the wild these induced changes in primary productivity from top-down control could in turn affect the evolution of these maternally induced traits. Importantly, if we assume a genetic basis for the maternal traits that we included in this study, there would be 4 maternal genetic degrees of freedom *sensu* (Schluter 2000). Here, we have used the term "ecological degree of freedom", defined as the evenness in the shared amount of variability explained by each of the orthogonal axes of trait variation that may affect an ecosystem function. Similarly, to the "genetic degrees of freedom", the Levin's index (Methods) can be used to estimate the ecological degrees of freedom, which in our case were maximal (4). Whether maternal or purely additive, this approach can set the basis to include multidimensionality in eco-evolutionary dynamics in traits that would act as both effect (ecology) and response (evolution) traits.

Modern biological pest control strategies advice practitioners to incorporate measures to increase natural enemy diversity to reduce herbivory (Letourneau et al. 2009). However, the control of the most destructive and difficult-to-manage pest species (spider mites, aphids, thrips and whiteflies) is usually pursued with strategies that consider single, but highly efficient, predators (Van Lenteren 2012). Our results add novel information of central importance in pest control, as breeders of biological control agents could maximize the complementarity of traits and improve the efficiency of their predators by using the approach we propose here.

Thus, experimentally manipulating multidimensional functional diversity opens a wide range of possibilities for future studies addressing questions in Biodiversity Ecosystem Function (BEF), eco-evolutionary dynamics across trophic levels, and their applications, as it is biological pest control.

Acknowledgments

This study was supported by a PhD grant (PD/BD/106059/2015) attributed to Jorge Henriques by the Portuguese Science and Technology foundation (FCT), by the grant P12-RMN-1521 from the Andalusian government and by the grant CGL2015-66192-R from the Spanish Ministry of Economy and Competitiveness, both partially funded by the European Regional Development Found, both attributed to Jordi Moya-Laraño and to the FPU scholarship (FPU13/04933) from the Spanish Ministerio de Educacion, Cultura y Deporte to Dolores Ruiz-Lupión.

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CHAPTER V

Conclusions and Perspectives

This thesis contributes for the improvement of our understanding of the sources of intraspecific variation in foraging traits in a cannibalistic wolf-spider *Lycosa fasciiventris* and its effects on a trophic cascade with this species as a top predator.

We first showed that defense against cannibalism in spiders follows a continuum of variation that can be broadly classified in two strategies: (a) reduced activity and (b) avoidance of patches with cannibals. Which of these strategies is displayed depends on weight loss of the residents, which probably correlates with the quantity of cues released. These results suggest that the social environment, in a cannibalistic species, allows the maintenance of phenotypic variation within populations. Moreover, we showed that maternal effects, an overlooked source of phenotypic variation, predominated over additive genetic effects in determining the phenotypic variation of foraging traits. This variance component also displayed interactions with the environment, leading to maternal-by-environment interactions (Vega-Trejo et al. 2018). Finally, we showed that environmental variation in the spider-rearing environment promoted higher cannibalism among them but did not affect their predation on crickets. In contrast, variation in maternal effects strongly impacts food web structure through trophic cascades. Indeed, by manipulating the maternally-driven multidimensional intraspecific functional diversity of spiders placed in a food web, we observed stronger trophic cascades when it was high.

Our findings highlight the need to study neglected sources of phenotypic variation such as maternal and indirect effects, which may be key to understanding the mechanisms behind the maintenance of intraspecific variation and its impacts upon ecological processes.

Here, I provide a brief summary of the key results from this thesis and a brief discussion concerning potential future directions.

1. Key results

1.1. Anti-cannibalism behavior is not determined by genetic or maternal effects.

We tested the sources of variation in traits associated to avoidance of cannibalism in a solitary wolf spider, namely boldness, cautiousness and exploratory behavior. We did not find any evidence for maternal or genetic variance in behavioral patterns associated to cannibalism; rather, trait variation was dependent on the social environment.

1.1.1. Social environment as an indirect genetic effect is a source of phenotypic variation

Intraspecific variation in behavioral traits associated with avoidance of cannibalism was explained by weight loss of the individuals releasing conspecific cues (Table 2.3), which is likely correlated with cue production (i.e. the social environment). Specifically, weight loss from the elicitors had a significant effect on the expression of boldness and exploratory behavior, with more weight loss leading to less exploratory behavior but more boldness. Weight loss showed to be genetically determined (Table 2.1) and thus, variation in the social environment may allow for the maintenance of different strategies to cope with cannibals via indirect genetic effects.

1.1.2. Anti-cannibal behavior represents a behavioral syndrome within a continuum of strategies to cope with predation risk

The behavioral traits assessed in response to conspecific cues were correlated, suggesting the existence of a behavioral syndrome. Indeed, bolder individuals were more cautious and less exploratory (Figure 2.3). This complex behavioral phenotype implies that individuals venturing in the conspecific patch tend to behave more cautiously by displaying decreased velocity and activity in the presence of conspecific cues. As wolf-spiders hunt by sight, decreased movement is expected to reduce conspicuousness towards conspecifics. In contrast, individuals that avoid patches with conspecifics explored sites without conspecifics more actively. Possibly, such higher activity may allow them to explore this environment better (e.g., in search for food), and compensate for leaving the environment with conspecifics relatively unexplored. Therefore, this variation observed in the behavioral traits suggest a continuum of strategies to cope with cannibals with two extremes: avoidance, i.e. moving away from patches with conspecific cues, or stealthiness, i.e., reducing conspicuousness by moving more slowly and less often but a higher residence time in patches with conspecific cues.

1.2. Intraspecific variation for foraging traits

Another focus of this thesis was to assess the sources of variation in foraging traits in our model species. To this aim, we performed a variance partition analysis for these traits and assessed additive genetic, maternal and environmental effects. We also studied how traits covaried among them and identified the sources of such covariation.

1.2.1. Maternal effects were the predominant source of intraspecific variation

Through variance partitioning analysis based on a half-sib design, we found that maternal variance predominated over additive genetic variance in determining phenotypic variance for foraging traits in wolf spiders. Maternal effects were especially high in the traits measured early in life, body size and body condition, then waned throughout the individual life-cycle as observed for the estimates calculated for foraging traits measured in later stages. Also, maternal effects estimates were higher in early life stages and waned throughout development.

1.2.2. Maternal effects interacted with the environment

The two different food regimes, which spiderlings were exposed to, significantly affected their growth rates and assimilation efficiency and interacted with the maternal variance component (Figure B.2). Thus, we found evidence of maternal-by-environment interactions (MxE), denoting that this variance component is contingent upon the environment in which it is measured. This raises awareness towards the importance of context when assessing maternal variance. These environmental interactions may denote different plasticity of the maternal genotypes towards different environmental conditions as illustrated by the reaction norms for these traits (Figure B.2).

1.2.3. Maternal traits partially explained maternal variance in early life traits

To identify which aspects of the maternal phenotype could be contributing to the maternal effects variance, we attempted to disentangle the effect of specific maternal traits on offspring traits (Sinervo and Doughty 1996; Noble et al. 2014). Accounting for maternal traits (body size, body condition and clutch size) produced a reduction in the maternal variance in offspring body size and condition by 7% and 18%, respectively (Table 3.2), not providing a strong evidence that maternal effects can be attributed to any of the maternal traits tested.

Nonetheless, we found a negative relation between clutch size (number of offspring born) and offspring body size, denoting a trade-off between offspring size and number, as expected from life-history theory (Fox and Czesak 2000). We also found a positive relation between mother body condition and offspring size, suggesting that females in better condition are able to better provision their offspring, resulting in bigger hatchlings. In contrast, mother size showed a negative effect on offspring body condition, suggesting that smaller mothers produced better provisioned offspring at birth.

These results indicate that the mother physiological state and size can regulate the offspring number-size trade-off, as shown in other studies (Uller and Olsson 2005; Gagliano and McCormick 2007).

1.2.4. Maternal effects explained correlations among traits

Correlations among traits were also partially explained by maternal effects, with no evidence supporting the occurrence of genetic correlations among traits. Indeed, a negative maternal correlation between body size and PPSR (Figure 3.1) indicates that if females provisioned offspring to be born with bigger bodies, spiderlings preferred prey with smaller size (relative to their own size). Moreover, a positive and significant maternal correlation was found between assimilation efficiency and growth rate (Figure 3.1). Indeed, some females provision resources to their offspring such that these have high assimilation efficiency and growth rates, whereas others have offspring with low assimilation efficiency and growth rates.

1.3. Intraspecific variation impacts ecological processes

Despite the recognized importance of intraspecific variation in modulating ecological processes and ecosystem functioning, most studies do not unravel the origin of such variation. In this thesis, we assessed the drivers of such variation and evaluated its impact. To this aim, we created groups of spiders with either high or low multidimensional intraspecific functional diversity and either high or low environmental variance (being raised in a common or different environments). We then placed these groups of spiders in a mesocosm with crickets and lettuce and measured the magnitude of trophic cascades.

1.3.1. Assessing multidimensional maternal intraspecific variation

In this study, we adopted a novel approach to assess multidimensional intraspecific functional diversity. We did this by first obtaining the BLUPS (best linear unbiased predictors) of each trait, then calculating the ecological degrees of freedom among the BLUPS via PCA (ensuring orthogonality among axes of variation) and finally calculating maternal multidimensional distances.

We found that this multidimensional variation was ruled by 4 independent axes. The Levin’s index, another form of calculating ecological degrees of freedom, gave similar results. This approach can set the basis to include multidimensionality in eco-evolutionary dynamics. These results also highlight the role of maternal effects in eco-evolutionary dynamics which have been mostly ignored to date.

1.3.2. Multidimensional intraspecific maternal functional diversity strengthens a trophic cascade

Within a trophic chain, composed of spiders, crickets and lettuce, maternally-driven multidimensional intraspecific diversity, in the predator population, strongly affected the strength of trophic interactions. Indeed, higher predator diversity exerted stronger top-down control when compared with low predator intraspecific diversity (Figure 4.4), which runs counter theoretical predictions (Finke and Denno 2004; Moya-Laraño 2011). As no differences were found in cricket mortality, between high and low intraspecific variation treatments (Figure 4.3), differences in lettuce consumption was probably due to differences in cricket behavior induced by the presence of the predator (trait-mediated Indirect Interactions, TMII).

1.3.3. The role of environmental heterogeneity in defining trophic cascades

The environmental background (poor or rich rearing environment) did not affect the magnitude of the trophic cascade or the rate of cannibalism. However, in the treatment with environmental variation, the rate of cannibalism increased (Figure 4.5). This is probably because spiders originating from different environments differ in size, and this variation favors cannibalism. However, this increase in cannibalism did not result in a dampening of the trophic cascade as observed (Figure 4.4).

2. Main perspectives

This thesis opens new exciting directions for future research concerning the role of indirect genetic effects in driving intraspecific variation, its ecological implications and further, its evolutionary potential. Here, I discuss some unanswered and relevant question which may contribute to the development of the research topic explored in this thesis.

2.1. Is the behavior towards response to conspecifics cues a foraging strategy or rather a defense mechanism?

In this thesis, we assessed the behavioral patterns towards the response to conspecific cues. The syndrome found underlies a behavioral response towards these cues. In cannibalistic species, social interactions are very complex as individuals within a population can be both prey and predators of their conspecifics. As wolf-spiders hunt by sight, this behavior leads to reduced conspicuousness of prey. However, being cryptic may also be favored in predators. Performing experiments with natural predators and prey would allow a better understanding of this behavior. Using a similar experimental setup but replacing conspecific cues by kairomones, we could test how this species changes its behavioral patterns when exposed unequivocally to predation risk. In this way, we could test if those responses are correlated and if the patterns observed concern anti-predator behavior, a hunting strategy, or both, according to risk of cannibalism and individual personality.

2.2. Is there an underlying pace-of-life syndrome associated to the behavioral traits assessed?

Pace-of-life-syndromes (POLS, Ricklefs and WIlkelski 2002) are suits of correlated behavioral, physiological and life-history traits arrayed on a continuum from slow to fast lifestyles. In this study, although we explore genetic and maternal correlations among traits, we did not find any relationship that could support this theoretical framework. However, our results show high variation in personality and development across individuals.

Our experimental design did not allow any state-behavior feedback (Sih et al. 2015) and therefore variation in personality traits could not affect other traits, such as size or body condition. For example, bolder individuals were not allowed to forage *ad libitum*, producing a higher difference among offspring growth rates, due to the limited food supply that was provided to spiderlings. Thus, rearing offspring in less strict environments (such as feeding individuals *ad libitum*) may allow the occurrence of such feedbacks and perhaps these syndromes would emerge. Also, individuals could be placed in an experimental setup where these could freely choose among prey types. For instance, a source of highly nutritive prey during development would be conspecifics. So not only quantity, but the quality of the prey should be considered.

Future studies measuring life-history traits which were omitted from this study such as size at maturity and life span could also be measured. Assessing genetic covariance between such life-history traits and (e.g.) behavioral traits related to risk aversion (e.g. boldness) may allow testing for genetic tradeoffs as hypothesized by POLS theory (slow vs fast lifestyles) as an explanation for the maintenance of individual behavioral variation in wild populations.

2.3. Are maternal effects genetically or environmentally determined?

Due to our breeding design we could not disentangle maternal effects assessed into dominance, genetic effects and maternal environment. By performing maternal half-sibs, it would be possible to isolate dominance effects, while rearing females in different environments would allow to disentangle the remaining variance components from the maternal environment, giving us a clearer picture of the forces generating intraspecific variation via maternal effects. Maternal half-sibs, however, cannot be performed with *L. fasciiventris*, as this species breeds only once. Other species of spiders, which breed more than once (Marshall and Gittleman 1994) could be more suitable for this sort of experiment.

2.4. Is there any transgenerational plasticity related to food scarcity?

In this study, we observed that offspring originated from different females showed different reaction norms, namely in growth rates and assimilation efficiency. Transgenerational plasticity occurs when the environment experienced by parents influences offspring reaction norms.

As females used in this experiment came from the field, we were unable to control their rearing environment and therefore, properly assess their environmental experiences. Controlling for the female rearing environment, namely for food provisioning would allow to determine the importance of the mothers environmental experience and verify if, in this case, we have environment-dependent maternal effects (Räsänen and Kruuk 2007).

Ideally, the parental generation would be reared in poor vs rich environment and then we would determine the reaction norms of offspring also reared in poor and rich environments, recurring to a split brood design, as applied in this study.

2.5. What are the mechanisms driving maternal effects?

In this study, we attempted to assess the mechanism responsible for the maternal variance observed for offspring traits, especially for those early in life where variance was exceptionally high. In this study we attempted to identify such mechanisms by fitting mother body size and condition. Other maternal traits could have been assessed, such as egg investment (egg size, egg weight or egg density) which could have given us a more direct measure of resource allocation by mothers (Johnson et al. 2014). Egg provisioning is as key aspect of an organism’s environment because it constitutes the entire energy budget of the offspring for embryogenesis and maintenance (Rossiter 1991). Moreover, early provisioning may affect offspring survival, development and behavior (Sinervo and McEdward 1988). Thus, quantifying and determining the magnitude of such effects allows pinpointing the mechanisms underlying maternal effects and their effects upon population ecology and character evolution (Kirkpatrick and Lande 1989).

2.6. How can environmental heterogeneity shape intraspecific variation?

In this study, we manipulated, in a simplistic way, environmental diversity by selecting individuals reared in common environments vs different environmental backgrounds. However, it would be more realistic if we considered the multidimensionality also in rearing environments (e.g., nutrient diversity, diversity of predatory threats) and their complementary effects in trophic cascades. For that we would need to collect individuals from the field and do an exhaustive habitat characterization or we would need to rear individuals in the lab with more complex rearing environments. By collecting or rearing individuals in these complex environments we would have a better understanding of how environmental diversity can shape intraspecific variation.

2.7. How does intraspecific variation interact with environmental changes?

In this study, we showed that intraspecific variation can impact ecological processes, namely through trophic cascades. It would be interesting to test whether those impacts are context dependent. For example, we could test different treatments concerning multivariate intraspecific variation (as we tested in this study) but in different environmental settings such as different temperature regimes. In this way, we could assess if intraspecific variation would still strengthen trophic cascades, as observed, would now dampen trophic cascades due to (e.g.) increased rates of cannibalism which are shown to increase with temperature (e.g. Stoner et al. 2010).

2.8. Is predation shifting vulnerability traits towards new fitness optimum values?

In this study, we focused mainly on foraging traits in predator species, although we disregarded the importance of intraspecific variation in the prey which can modulate the outcome of predator-prey interactions. Traits that determine the ability of prey to avoid being preyed upon are called “vulnerability” traits. Vulnerability traits may include body size, body shape, defense (physical or chemical protection), ability to avoid detection, mobility, ability to detect predators, and evasive or escape behavior.

After being exposed to predation, as in our mesocosms experiment, certain vulnerability traits will be selected and mean trait values in the prey populations will certainly shift towards new fitness optimum. These changes may impact ecological processes such as trophic cascades. However, the evolutionary impact of such selection depends on the degree of transmission of such traits through generations. Therefore, it would be of most interest to assess what traits are being selected by predation and what are the effects of predation in shifting trait values towards new fitness optimum values.

As these traits are key to predict the outcome of predator-prey interactions it is crucial to understand what drives such variation. Therefore, it would be of most interest to identify candidate traits in the prey species (e.g. body size and activity) and assess if these are genetically based and therefore possess evolutionary potential. For that, similarly to the predator, we could apply a breeding design (e.g. half-sib) to explore the genetic architecture of such traits. Knowing that, we could assess its evolutionary potential but also predict the outcome of predator-prey interactions within an eco-evolutionary framework.

2.9. How does intraspecific variation in predators affects prey evolution?

In this study, we assessed the ecological impacts of intraspecific variation, namely how such variation modulates trophic cascades. However, we did not assess what are the evolutionary impacts of such variation. The fact that trait variation in the predator modified the strength of the trophic cascade implies that the selection pressure exerted on prey also differed among treatments. Therefore, it is likely that trait variation in predators will affect prey evolution.

This could be tested by placing prey populations in the presence of populations of predators with more or less intraspecific variation and studying selection gradients, and, if feasible during several generations to document evolutionary responses. Note that care should be taken so that some prey survives to form the next generation. Another constrain in performing this experiment is that it is as yet unclear how the prey responds to the different treatments. That is, we know that the strength of the trophic cascade changes in response to predator variation, but we have not identified the traits in the prey that lead to such changes. It is thus not clear which traits in the prey are likely to be the target of selection.

2.10. How does intraspecific variation in prey affect the strength of trophic cascades?

In this thesis, we did not experimentally test the effects of intraspecific variation in the prey. By manipulation intraspecific variation in both predators and prey, we could test how critical is intraspecific variation in determining the stability of ecological systems.

Additionally, through manipulation of intraspecific variation in both combined treatments of both predators and prey, we could set up initial condition on both species (high or low variation) and let the experimental system to evolve for several generations. At the end of the experiment it would be possible to see if populations collapse or remain stable around an equilibrium. The data from this experiment could allow to develop theoretical models for prediction of the stability of food webs and consequently community and ecosystem resilience. If the models produced could be generalized, this could provide important tools for management of wild populations with conservation purposes. Due to the difficulties of performing these procedures in our current model species, we could perform these experiments in other model species. In this case, it would be easier to establish a trophic chain with soil mites and collembolans, which due to their easiness in rearing and short generation time would allow to perform these experiments.

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Appendix A

Supplementary materials Chapter II

Appendix A

Table A. 1 – Model comparison of additive genetic and maternal effects on the ability to elicit behavioral traits on focal individuals. Δ DIC is the difference between DIC values against the null model. **V_{sire}** – variance among sire families, **V_{dam}** – variance among dam families. **elct_EXP** – eliciting exploratory behavior, **elct_BOLD** – eliciting boldness; **elct_CAUT** – eliciting cautiousness, **elct_ACT** – eliciting activity.

TRAIT	model	DIC	Δ DIC
elct_EXP	null	-373.3504	0
	V_{sire}	-367.4591	5.8913
	V_{dam}	-368.0191	5.3313
	V_{sire} + V_{dam}	-364.2174	9.133
elct_BOLD	null	733.3643	0
	V_{sire}	737.5492	4.1849
	V_{dam}	736.7291	3.3648
	V_{sire} + V_{dam}	739.7408	6.3765
elct_CAUT	null	-648.0447	0
	V_{sire}	-641.1212	6.9235
	V_{dam}	-643.4546	4.5901
	V_{sire} + V_{dam}	-638.8537	9.191
elct_ACT	null	-480.5217	0
	V_{sire}	-477.3999	3.1218
	V_{dam}	-477.4743	3.0474
	V_{sire} + V_{dam}	-475.1554	5.3663

Appendix B

Supplementary materials Chapter III

Appendix B

Table B. 1 – Summary results from models fitting additive genetic effects, maternal effects and their environmental interaction variance components. ΔDIC is the difference between DIC values against the null model (lowest DIC). Models in bold represent the best candidate models which were 2 DIC distant of the next best model. Traits: **BS** – body size; **BC** – body condition, **AE** – assimilation efficiency, **GR** – growth rate; **PPSR** – predator-prey size ratio. **VCs** - variance components: **V_A** - additive genetic variance, **V_M** – maternal variance, $V_{\mathbf{GxE}}$ - genotype-by-environment interaction, **V_{MxE}** - maternal-by-environment interaction. **V_{sire}** – variance among sire families, **V_{dam}** – variance among dam families, **$V_{sire\ x\ treatment}$** – variance for the interaction between the sire component and food treatment, ***$V_{dam\ x\ treatment}$*** – variance for the interaction between the maternal component and food treatment, **V_B**– variance among blocks, **V_R** – residual variance.

TRAIT	VCs	DIC	Δ DIC	V_{sire}	V_{dam}	V_{GxE}	V_{MxE}	V_B	V_R
BS	null	-4610.139	0						1.341x10-3 (1.253x10-3 to 1.460x10-3)
BS	V_A	-4821.503	-211.364	2.517x10-4 (1.615x10-4 to 4.164x10-4)					1.083x10-3 (1.012x10-3 to 1.186x10-3)
BS	**V_M**	-5219.999	-609.86		5.943x10-4 (4.332x10-4 to 7.985x10-4)				7.573x10-4 (7.004x10-4 to 8.192x10-4)
BS	**$V_A + V_M$**	-5219.748	-609.609	3.573x10-5 (7.883x10-6 to 1.672x10-4)	4.862x10-4 (3.825x10-4 to 7.591x10-4)				7.650x10-4 (6.969x10-4 to 8.188x10-4)
BC	null	-3647.447	0						2.898x10-3 (2.724x10-3 to 3.194x10-3)
BC	V_A	-3787.569	-140.122	3.776x10-4 (2.488x10-5 to 6.612x10-4)					2.513x10-3 (2.342x10-3 to 2.740x10-3)
BC	**V_M**	-3927.489	-280.042		7.796x10-4 (5.3797x10-4to 1.049x10-4)				2.194x10-3 (2.030x10-3 to 2.395x10-3)
BC	**$V_A + V_M$**	-3927.323	-279.876	1.140x10-4 (2.671x10-5 to 3.394x10-4)	6.361x10-4 (3.939x10-4 to 9.425x10-4)				2.242x10-3 (2.025x10-3 to 2.379x10-3)
AE	null	-733.6822	0					1.297x10-3 (3.755x10-4 to 2.885x10-3)	2.352x10-2 (2.120x10-2 to 2.565x10-2)
AE	V_A	-747.588	-13.9058	9.557x10-4 (3.765x10-6 to 2.176x10-3)				9.996x10-4 (2.557x10-4 to 2.663x10-3)	2.224x10-2 (2.008x10-2 to 2.465x10-2)
AE	V_M	-770.9354	-37.2532		2.256x10-3 (1.226x10-3 to 4.096x10-3)			8.662x10-4 (2.879x10-4 to 2.603x10-3)	2.209x10-2 (1.980x10-2 to 2.442x10-2)
AE	$V_A + V_M$	-769.5987	-35.9165	5.691x10-4 (1.499x10-4 to 1.776374x10-3)	1.674x10-3 (5.912x10-4 to 3.422x10-3)			9.837x10-4 (3.814x10-4 to 2.635x10-3)	2.089x10-2 (1.885x10-2 to 2.318x10-2)

Appendix B

AE	$V_A + V_{GxE}$	-743.69	-10.0078	1.404x10-3 (7.031x10-4 to 2.713x10-3)		5.411x10-4 (2.041x10-4 to 1.262x10-3)		1.845x10-3 (7.324x10-4 to 3.827x10-3)	2.183x10-2 (1.960x10-2 to 2.386x10-2)
AE	**$V_M + V_{MxE}$**	-778.1531	-44.4709		1.682x10-3 (7.659x10-4 to 3.030x10-3)		1.039x10-3 (3.015x10-4 to 2.103x10-3)	1.431x10-3 (7.901x10-4 to 3.549x10-3)	1.993x10-2 (1.788x10-2 to 2.221x10-2)
AE	$V_A + V_M + V_{GxE}$	-762.9469	-29.2647	1.279x10-3 (6.267x10-4 to 2.634x10-3)	1.689x10-3 (9.650x10-4 to 3.676x10-3)	4.304x10-4 (2.294x10-4 to 1.234x10-3)		1.578x10-3 (8.390x10-4 to 3.709x10-3)	2.017x10-2 (1.843x10-2 to 2.258x10-2)
AE	**$V_A + V_M + V_{MXE}$**	-776.2958	-42.6136	1.392x10-3 (6.802x10-4 to 2.726x10-3)	1.253x10-3 (4.315x10-4 to 2.401x10-3)		9.747x10-4 (3.412x10-4 to 2.046x10-3)	1.424x10-3 (5.699x10-4 to 3.135x10-3)	1.990x10-2 (1.762x10-2 to 2.184x10-2)
AE	$V_A + V_M + V_{GxE} + V_{MxE}$	-772.182	-38.4998	1.104x10-3 (5.207x10-4 to 2.355x10-3)	1.201x10-3 (5.429x10-4 to 2.393x10-3)	3.543x10-4 (1.633x10-4 to 1.139x10-3)	4.857x10-4 (2.563x10-4 to 1.805x10-3)	1.589x10-3 (8.899x10-4 to 3.866x10-3)	2.023x10-2 (1.754x10-2 to 2.183x10-2)
GR	null	-4393.981	0						2.652x10-4 (2.430x10-4 to 2.932x10-4)
GR	V_A	-4428.987	-35.006	1.905x10-5 (9.004x10-6 to 3.905x10-5)					2.415x10-4 (2.257x10-4 to 2.749x10-4)
GR	V_M	-4431.28	-37.299		2.461x10-5 (1.155x10-5 to 4.377x10-5)				2.431x10-4 (2.175x10-4 to 2.664x10-4)
GR	$V_A + V_M$	-4433.933	-39.952	1.176x10-5 (3.858x10-6 to 3.055x10-5)	1.230x10-5 (3.849x10-6 to 2.872x10-5)				2.399x10-4 (2.154x10-4 to 2.652x10-4)
GR	$V_A + V_{GxE}$	-4494.573	-100.592	1.315x10-5 (7.392x10-6 to 2.762x10-5)		2.654x10-5 (1.725x10-5 to 5.444x10-5)			2.204x10-4 (1.962x10-4 to 2.409x10-4)
GR	**$V_M + V_{MxE}$**	-4566.944	-172.963		1.389x10-5 (7.334x10-6 to 2.561x10-5)		5.727x10-5 (3.607x10-5 to 8.460x10-5)		1.909x10-4 (1.680x10-4 to 2.079x10-4)
GR	$V_A + V_M + V_{GxE}$	-4497.336	-103.355	1.503x10-5 (8.141x10-6 to 3.081x10-5)		2.787x10-5 (1.526x10-5 to 5.354x10-5)			2.144x10-4 (1.893x10-4 to 2.326x10-4)
GR	$V_A + V_M + V_{MXE}$	-4561.492	-167.511	1.495x10-5 (8.557x10-6 to 2.814x10-5)	1.260x10-5 (7.205x10-6 to 2.435x10-5)		5.546x10-5 (3.321x10-5 to 8.154x10-5)		1.864x10-4 (1.670x10-4 to 2.081x10-4)
GR	$V_A + V_M + V_{GxE} + V_{MxE}$	-4556.704	-162.723	1.374x10-5 (6.846x10-6 to 2.600x10-5)	1.201x10-5 (6.010x10-6 to 2.264x10-5)	1.008x10-5 (3.853x10-6 to 3.225x10-5)	4.217x10-5 (2.067x10-5 to 7.049x10-5)		1.868x10-4 (1.686x10-4 to 2.087x10-4)
PPSR	null	1578.645	0					6.445x10-2 (2.614x10-2 to 1.469x10-1)	7.898x10-1 (7.068x10-1 to 8.951x10-1)
PPSR	V_A	1576.977	-1.668	2.431x10-2 (8.971x10-3 to 6.770x10-2)				5.107x10-2 (2.493x10-2 to 1.374x10-1)	7.709x10-1 (6.814x10-1 to 8.742x10-1)
PPSR	**V_M**	1570.481	-8.164		3.846x10-2 (1.187x10-2 to 9.836x10-2)			6.850x10-2 (2.249x10-2 to 1.409x10-1)	7.286x10-1 (6.637x10-1 to 8.507x10-1)
PPSR	$V_A + V_M$	1572.817	-5.828	1.359x10-2 (6.176x10-3 to 5.562x10-2)	3.353x10-2 (6.790x10-3 to 7.958x10-2)			5.286x10-2 (2.219x10-2 to 1.380x10-1)	7.592x10-1 (6.597x10-1 to 8.473x10-1)
PPSR	$V_A + V_{GxE}$	1590.351	11.706	4.141x10-2 (2.157x10-2 to 8.830x10-2)		1.733x10-2 (6.818x10-3 to 4.279x10-2)		6.719x10-2 (3.640x10-2 to 1.561x10-1)	7.596x10-1 (6.759x10-1 to 8.613x10-1)
PPSR	$V_M + V_{MxE}$	1576.368	-2.277		4.983x10-2 (2.568x10-2 to 9.666x10-2)		2.278x10-2 (7.799x10-3 to 5.631x10-2)	0.08016142 (3.886x10-2 to1.623x10-1)	7.027x10-1 (6.301x10-1 to 8.068x10-1)
PPSR	$V_A + V_M + V_{GxE}$	1587.511	8.866	4.015x10-2 (2.226x10-2 to 9.372x10-2)	4.601x10-2 (2.586x10-2 to 1.048x10-1)	1.639x10-2 (6.360x10-4 to 4.396x10-2)		7.319x10-2 (4.211x10-2 to 1.619x10-1)	7.369x10-1 (6.430x10-1 to 8.286x10-1)
PPSR	$V_A + V_M + V_{MXE}$	1582.073	3.428	4.291x10-2 (2.347x10-2 to 9.185x10-2)	5.154x10-2 (2.424x10-2 to 9.526x10-2)		1.968x10-2 (7.943x10-3 to 5.726x10-2)	6.602x10-2 (3.967x10-2 to 1.565x10-1)	7.190x10-1 (6.284x10-1 to 8.112x10-1)
PPSR	$V_A + V_M + V_{GxE} + V_{MxE}$	1590.264	11.619	3.839x10-2 (1.887x10-2 to 8.439x10-2)	3.608x10-2 (1.893x10-2 to 8.480x10-2)	1.593x10-2 (5.793x10-3 to 4.061x10-2)	1.916x10-2 (6.248x10-3 to 5.197x10-2)	8.699x10-2 (3.510x10-2 to 1.474x10-1)	7.145x10-1 (6.254x10-1 to 8.079x10-1)

Table B. 2– Parameter estimates (posterior mean and credible interval) for the fixed effects considered (food treatment and age) for the analysis of variance components from the basic model ($V_{sire} + V_{dam} + V_{residual}$). Body condition (measured during the experimental trials for PPSR) was additionally used to control for individual state during PPSR trials. **BS** – body size; **BC** – body condition, **AE** – assimilation efficiency, **GR** – growth rate; **PPSR** – predation-prey ratio.

	Parameter estimates		
TRAIT	**Food treatment**	**Age**	**Body condition**
BS	-	0.017 (-0.135 to 0.155)	-
BC	-	0.007 (-0.099 to 0.125)	-
GR	0.946 (0.817 to 1.080) ***	-0.249 (-0.328 to -0.176) ***	-
AE	0.152 (0.012 to -0.297) *	0.155 (0.095 to 0.227) ***	-
PPSR	0.073 (-0.113 to 0.237)	-0.014 -0.100 to 0.057)	0.138 (0.060 to 0.221) **

Significance of parameter estimates is given as *p< 0.05; ** p < 0.01; *** p < 0.001.

Table B. 3 – Genetic (r_A) and maternal (r_M) correlations among traits. Genetic correlations are presented in the lower diagonal while maternal correlations are presented in the upper diagonal. Between parenthesis are shown the 95% Bayesian credible intervals. Significant estimates are represented in bold. **BS** – body size; **BC** – body condition, **AE** – assimilation efficiency, **GR** – growth rate; **PPSR** – predation-prey ratio. **NT** – not tested genetic correlation due to absence of additive genetic effects on one or both traits considered.

	BS	**BC**	**AE**	**GR**	**PPSR**
BS	-	-0.229 (-0.502 to 0.028)	0.152 (-0.286 to 0.533)	0.166 (-0.066 to 0.518)	**-0.407 (-0.692 to -0.064)**
BC	0.165 (-0.337 to 0.584)	-	0.147 (-0.305 to 0.525)	0.221 (-0.219 to 0.636)	0.081 (-0.439 to 0.491)
AE	0.231 (-0.397 to 0.551)	0.029 (-0.366 to 0.561)	-	**0.596 (0.195 to 0.787)**	-0.153 (-0.488 to 0.331)
GR	NT	NT	NT	-	-0.072 (-0.418 to 0.416)
PPSR	-0.157 (-0.548 to 0.348)	0.238 (-0.305 to 0.568)	-0.041 (-0.449 to 0.398)	NT	-

Appendix B

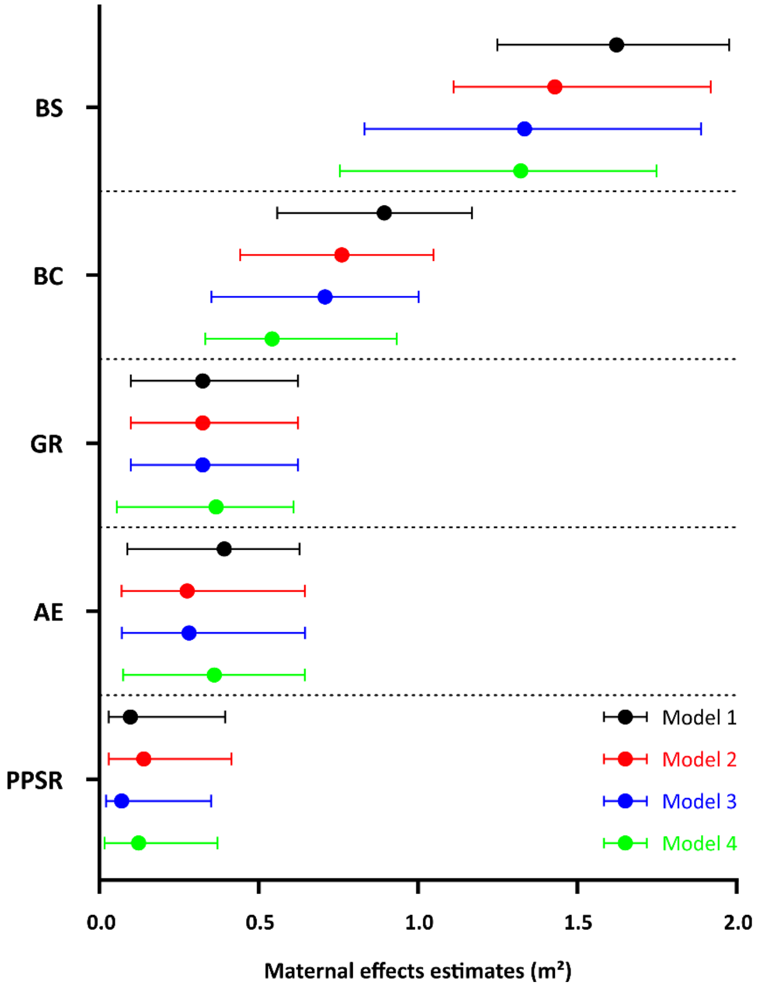

Figure B. 1 – Comparison of maternal effects estimates (m^2) among the traits measured in this study for models including maternal traits and site of collections. Points represent the posterior mode for the estimates measured and the intervals represent Bayesian credible intervals (95%). Estimates are significantly different if these do not overlap. Model 1: model without maternal traits and site; Model 2: with maternal traits; Model 3: with site; Model 4: with maternal traits and site. **BS** – body size, **BC** – body condition, **GR** - growth rate, **AE** - assimilation efficiency, **PPSR**- predator-prey size ratio.

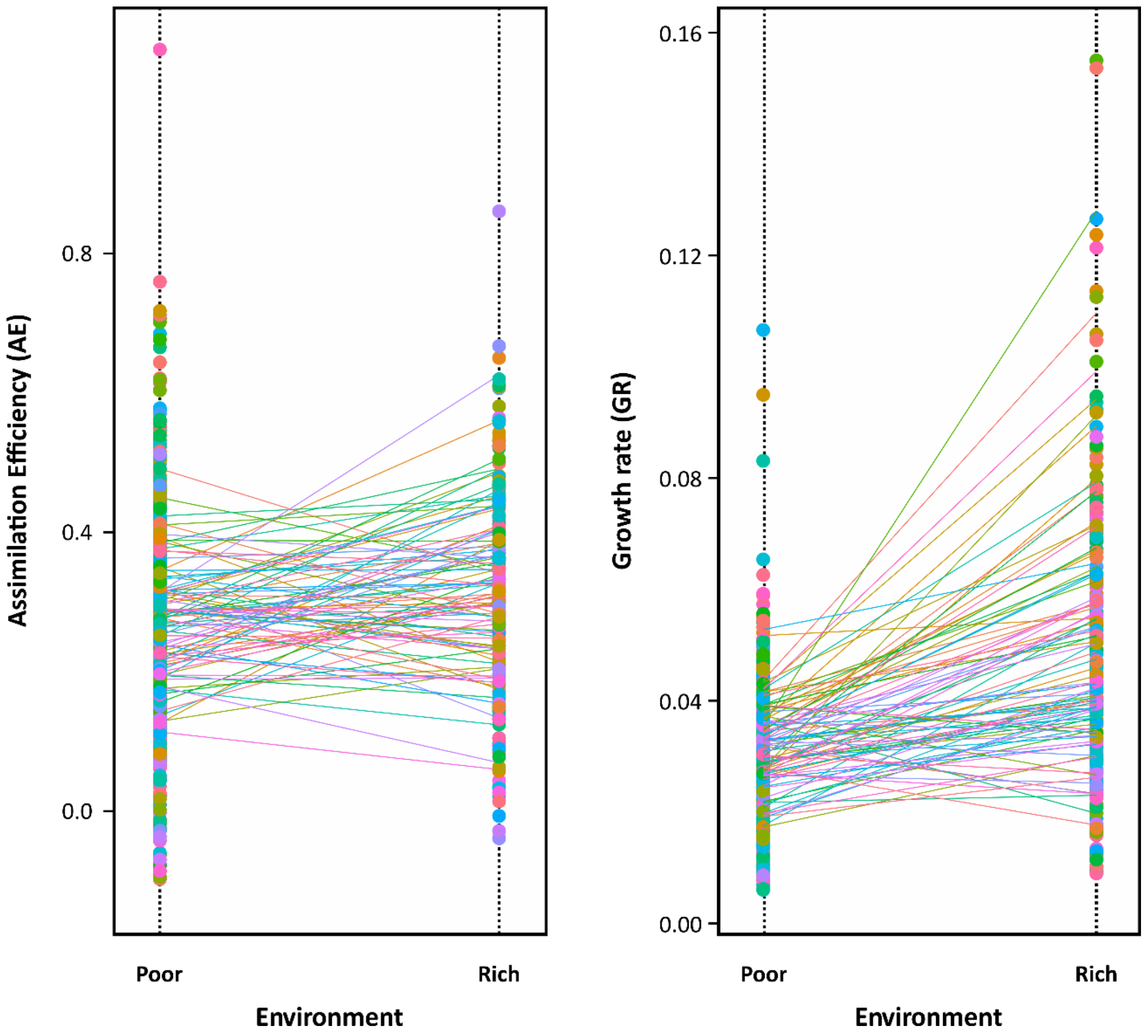

Figure B. 2 – Reaction norm plot illustrating how dam families respond to environmental variation for assimilation efficiency (AE) and growth rates (GR). Different colors correspond to different families. Mean family values were calculated in each environment and reaction norms were constructed by the lines linking the mean family values across environments (in Rich environment dam family was fed three times the amount of food than Poor environment).

Appendix B

Appendix C

Supplementary materials Chapter IV

Appendix C

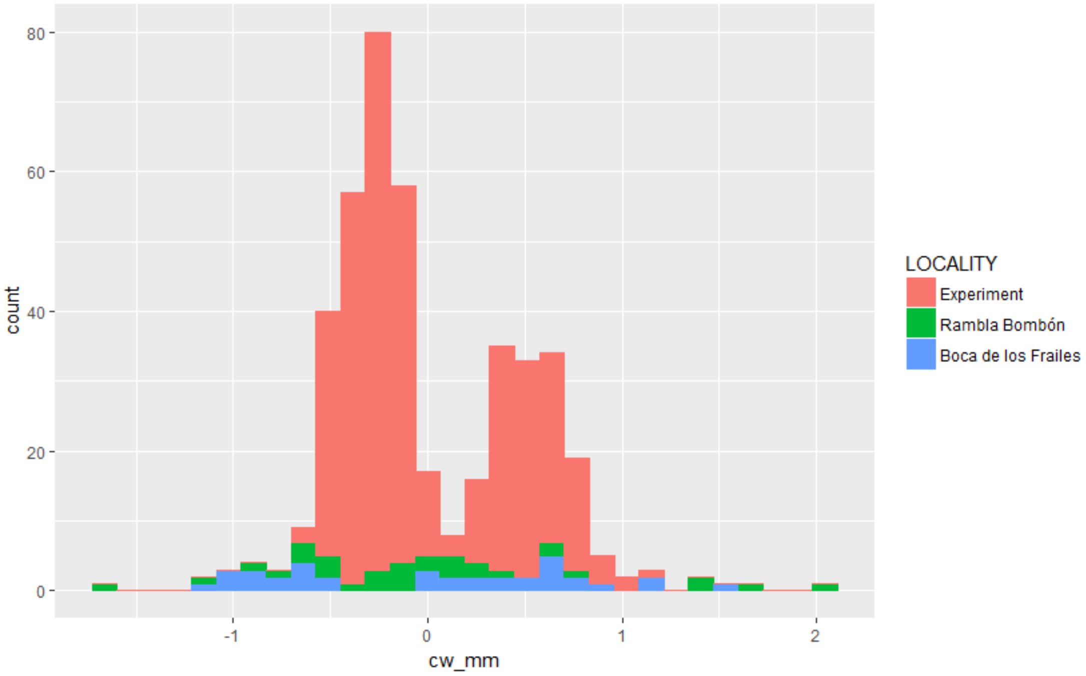

Figure C. 1 - Variability in body size (carapace width in mm after centering) in the spiders in the experiment and those collected from two localities in the wild. The 70 spiders from two localities were of approximately the same age as those that we had in the laboratory. We found that the variation in body sizes in the laboratory was within the range found in nature. The two peaks correspond to the two rearing environments (poor on the left and rich on the right, showing clear differences in body size). The experimental range in body sizes fits well within that in the wild.