A phylogeographic, ecological and genomic analysis of the recent range expansion of the wasp spider *Argiope bruennichi*

Dissertation

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Summary of the thesis

Rapid, poleward range expansions are observed for an increasing number of species in the past decades. These distributional changes are commonly attributed to global environmental change. Recent research, however, indicates that genetic adaptation might also play an important role in explaining the success of range expansions. Considering the fast pace of many range expansions, such contemporary evolutionary processes are unlikely to rely on the emergence of new mutations. Instead, standing genetic variation acts as important resource to fuel adaptation. This variation can be present in a population´s gene pool or it is introduced by secondary contact and admixture of formerly isolated genetic lineages. In the past years, ample evidence has been compiled for an association of admixture, adaptation and range expansions for numerous plant and animal species.

Here, I present an analysis of the recent range expansion of the European wasp spider *Argiope bruennichi*. Originally, this species inhabited the Mediterranean region and warm oceanic climates in France and South-Western Germany. From around 1930 onwards, the spider started expanding its range into increasingly continental climates and can now be found as far north as Finland. This thesis aims to disentangle environmental and genetic factors, involved in the species´ range expansion. In particular, I analyze the interconnection of genetic admixture and invasion success. I approach these questions using population genetic and phylogeographic methods, morphological analyses, ecological experiments and finally whole genome- and transcriptome sequencing.

In chapter one, I conduct a detailed genetic and ecological analysis of the spider´s range expansion. I base this study on a dense sampling of more than 2.000 contemporary specimens. In addition, I include about 500 historical spiders from natural history collections. I present genetic and morphological data, as well as several ecological experiments on thermal tolerance and preference and a reciprocal transplant study. My results indicate that the spider´s range expansion is associated with admixture of formerly isolated genetic lineages from around 1930 onwards. The ecological experiments indicate that invasive spider populations have simultaneously adapted to colder temperatures by shifting their thermal preference and tolerance.

Like many other spider species, *Argiope bruennichi* has a wide ranging Palearctic distribution. In chapter two, I conduct a phylogeographic survey over the species´ whole range, from the Macaronesian islands over Europe to East Asia. Next to *Argiope bruennichi*, I include a second widely distributed spider species, the nursery web spider *Pisaura mirabilis*. The study is based on mitochondrial and nuclear genetic markers. I highlight the importance of outer-European glacial refugia for the wasp spider. I then show the effects of secondary contact in shaping the postglacial genetic structure of the two species. The analysis identifies several instances of incongruent phylogenetic patterns for mitochondrial and nuclear DNA markers, possibly due to recurrent selection on mitochondria.

DNA from natural history collections provides a valuable resource to trace historical genetic changes during range expansions. For that reason, I present an analysis of DNA sequencing and microsatellite genotyping success in historical spider specimens in chapter three. In addition, I exemplarily illustrate the utility of historical specimens to trace historical genetic changes in populations.

In the above chapters, I have presented evidence for admixture leading to differential adaptation in spider populations. However, the functional basis of this adaptation remains unknown. For that reason I embark towards unraveling its genomic architecture in chapter four. Initially, I generate the first available draft genome sequence of a spider species. Based on this data, I analyze genome-wide differences of native and invasive wasp spider populations across an environmental gradient.

Gene regulatory evolution is a possible mechanism to provide the means for rapid contemporary adaptation to environmental stress. For this reason, I conduct a genome-wide gene expression analysis of native and invasive wasp spiders, which have been exposed to temperature stress in chapter five. I discuss the gene expression divergence between Northern and Southern European spiders in relation to the possibility of recent contemporary adaptation.

Zusammenfassung der Dissertation

In den letzten Jahrzehnten weiten immer mehr Tier- und Pflanzenarten ihr Areal in Richtung der Pole aus. Für gewöhnlich werden diese Expansionen auf den globalen Wandel zurückgeführt. Neue Forschungsergebnisse deuten allerdings darauf hin, dass auch genetische Anpassungen einen bedeutenden Anteil am Erfolg derartiger Arealausweitungen haben. Die hohe Geschwindigkeit vieler Invasionen macht eine Beteiligung neu auftretender Mutationen an solchen, rezenten evolutionären Prozessen unwahrscheinlich. Stattdessen könnte existierende genetische Variation als eine wichtige Quelle für Anpassungen dienen. Die nötige Variation kann im Genpool einer Population bereits vorhanden sein oder durch sekundären Kontakt vormals isolierter Linien eingebracht werden. Einige Beispiele für eine Assoziation von genetischer Durchmischung, evolutionären Anpassungen und Arealausweitungen bei diversen Pflanzen- und Tierarten sind bereits bekannt.

Die vorliegende Arbeit präsentiert eine Analyse der rezenten Arealausweitung der Europäischen Wespenspinne *Argiope bruennichi*. Ursprünglich war die Art vor allem im Mittelmeerraum und einigen warmen Regionen in Frankreich und Südwestdeutschland verbreitet. Um 1930 begann die Spinne ihr Areal in zunehmend kontinentale Klimaregionen auszuweiten und wird heute sogar in Finnland gefunden. In meiner Dissertation versuche ich sowohl Umwelteinflüsse als auch genetische Ursachen zu identifizieren, die an der Arealausweitung der Wespenspinne beteiligt sind. Im Besonderen untersuche ich die Verbindung von genetischer Durchmischung und dem Invasionserfolg. Zur Identifikation dieser Faktoren nutze ich populationsgenetische und phylogeographische Methoden, morphologische Analysen, ökologische Experimente und schließlich Gesamtgenom- und Transkriptomsequenzierungen.

Im ersten Kapitel präsentiere ich eine detaillierte Untersuchung der Arealausweitung der Wespenspinne. Die Studie basiert auf einer flächendeckenden Besammlung von mehr als 2.000 rezenten Spinnen. Zusätzlich untersuche ich ca. 500 historische Proben aus naturhistorischen Museen. Neben genetischen und morphologischen Daten präsentiere ich ökologische Versuche über die Temperaturtoleranz und –präferenz sowie ein reziprokes Transplantationsexperiment. Meine Ergebnisse zeigen, dass die Arealausweitung der Spinne mit der Durchmischung genetischer Linien ungefähr seit

den 1930er Jahren assoziiert ist. Die ökologischen Versuche deuten an, dass invasive Spinnenpopulationen sich über eine Verschiebung ihrer Temperaturtoleranz und -präferenz an kühlere Temperaturen angepasst haben.

Argiope bruennichi ist eine weit verbreitete, paläarktische Art. In Kapitel zwei führe ich eine phylogeographische Untersuchung über das gesamte Areal der Art durch, von den Makaronesischen Inseln über Europa bis nach Ostasien. Neben *Argiope bruennichi*, untersuche ich eine zweite, weit verbreitete Spinnenart, die Raubspinne *Pisaura mirabilis*. Die Studie basiert auf mitochondrialen und nukleären Markern. Ich identifiziere mehrere, außereuropäische Glazialrefugien für *Argiope bruennichi*. Außerdem zeige ich die Bedeutung von sekundärem Kontakt um die postglaziale, genetische Struktur der beiden Arten zu erklären. Meine Analyse zeigt mehrere Fälle von inkongruenten phylogenetischen Mustern für mitochondriale und nukleäre Marker, möglicherweise aufgrund von wiederholter Selektion von Mitochondrien.

DNA aus Naturhistorischen Sammlungen stellt eine wertvolle Ressource dar, um historische genetische Veränderungen während Arealausweitungen zu verfolgen. Aus diesem Grund präsentiere ich in Kapitel drei eine Analyse des Genotypisierungserfolges bei historischen Spinnenproben. Zusätzlich zeige ich anhand einiger Beispiele den Nutzen alter DNA, um historische, genetische Veränderungen in Populationen zu verfolgen.

In den vorhergehenden Kapiteln habe ich gezeigt, dass genetische Durchmischung mit differentieller Anpassung von Spinnenpopulationen einhergeht. Allerdings bleibt die funktionelle Basis dieser Anpassungen unbekannt. Aus diesem Grund unternehme ich in Kapitel vier einen ersten Schritt, um die genetische Architektur dieser Adaptionen zu entschlüsseln. Zunächst generiere ich die bislang erste, verfügbare Rohfassung der Genomsequenz einer Spinnenart. Darauf basierend analysiere ich genomweite Sequenzunterschiede zwischen nativen und invasiven Wespenspinnenpopulationen über einen Umweltgradienten.

Genregulatorische Evolution ist ein möglicher Mechanismus, um schnelle Anpassung an Umweltstress zu ermöglichen. Daher untersuche ich in Kapitel fünf genomweite Genexpressionsunterschiede nativer und invasiver Wespenspinnen, die Temperaturstress ausgesetzt waren. Ich diskutiere die Rolle von Genexpressionsdifferenzierung zwischen

Nord- und Südeuropäischen Populationen im Zusammenhang mit der Möglichkeit rasanter Anpassung.

General introduction

Species ranges, their limits and expansion in a changing world

"No species is truly cosmopolitan in its distribution. Most are confined to rather small areas, and all have limits to their geographic ranges beyond which they are not found" (Gaston, 2009). At first glance, this is a straightforward and obvious statement. But on closer examination, the underlying causes of limited ranges turn out to be highly complex and are matter of intense, ongoing research (Sexton et al, 2009).

In the simplest case, geographic limits to dispersal can prevent species from expanding their range, e.g. an ocean or a mountain chain. But apart from such physical barriers, countless other biotic and abiotic factors contribute to shape and extent of a range (Geber, 2011). A species depends on climatic variables (e.g. temperature and humidity). Its occurrence is limited by the availability of nutrients or the presence of competitors, predators and parasites (Slatkin, 1987). Hybridization with neighboring taxa can result in fitness loss at the range edge and limit a geographic distribution (Sexton et al, 2009). All these factors act in concert and determine where a species can sustain a positive population growth (Alexander & Edwards, 2010).

Species´ ranges are not static, and changing environmental conditions can allow their expansion, e.g., the extinction of a predator or warming climate (Alexander & Edwards, 2010; Crozier, 2004). A classical example is provided by postglacial recolonizations. Countless plant and animal species followed the warming climate by shifting or expanding their range at the end of the Pleistocene (Hewitt, 1999; Taberlet et al, 2002). Today, global change confronts organisms with similar condition of warming climate and habitat changes (Bridle & Vines, 2007). Faced with a rapidly changing environment, organisms can respond in manifold ways. **1.** Dispersal limited species will be hindered in following the environmental change, possibly leading to mass extinctions in the coming decades (Thomas et al, 2004). **2.** More dispersive organisms will be able to trace environmental change and shift or expand their ranges towards the poles (Chen et al, 2011; Thomas et al, 2001). **3.** Species with a broad stress tolerance might endure the novel conditions (Pörtner & Farrell, 2008). **4.** And last, some taxa could be able to cope with the new environmental settings by evolutionary change (Parmesan, 2006; Hoffmann & Sgro, 2011). Such adaptation could even allow organisms to benefit from

environmental change and lead to range expansions (Franks & Hoffmann, 2012; Thomas et al, 2001). The frequency of such rapid adaptation is matter of much research and discussion in recent years.

Adaptations and range expansions - Impediments and possibilities

From an evolutionary point of view, limited geographic ranges are a paradox (Bridle & Vines, 2007). Actually, organisms should constantly adapt to new conditions at their distributional edge, enabling repeated range expansions. However, adaptation at range margins can be constrained by several processes. First, populations at range edges are often genetically depauperate due to founder effects and small and scattered distributions (Eckert et al, 2008). This reduced variation can render them less responsive to selection (Pujol & Pannel, 2008). Moreover, migration of locally adapted alleles from central populations into marginal gene pools can lead to maladaptation in this new environment (Kirkpatrick & Barton, 1997; Gaston, 2009). In addition, a range expansion often requires simultaneous adaptation to opposing environmental factors or traits, e.g. temperature and humidity, or small body size and high fecundity (Colautti et al, 2010). Despite these theoretical impediments, recent research suggests that adaptation at range edges is possible. This generally holds true if the effect of genetic drift is mitigated by a large effective population size (Sexton et al, 2009) and selection not antagonized by high gene flow (Freeland, 2005). Gene flow does not even necessarily swamp adaptation, but might be a precondition for adaptation by introducing adaptive genetic variation into a population (Davis & Shaw, 2001; Kremer et al, 2012; Bridle et al, 2010). When dispersal is limited to juveniles, poorly adapted specimens may die before they reach maturity and thus before they can swamp a marginal population's gene pool. Premature dispersal can thus fully unfold the positive effects of gene flow (Kawecki, 2008). Moreover, a reduced pressure from parasites and predators is often observed at range margins. Such relaxed conditions can allow a population to reach high densities and respond to other selective pressures (Phillips et al, 2010).

In recent years, many examples have been compiled for adaptation at range edges. Potentially adaptive traits during a range expansion can be manifold. Initially, high

dispersal ability (Thomas et al, 2001; Hill et al, 2011; Phillips et al, 2006) or fecundity (Burton et al, 2010; Phillips et al, 2010) evolve in order to allow expanding populations to reach high densities. Later evolutionary responses include host switching and traits related to competitiveness (Thomas et al, 2001; Parmesan, 2006). Moreover, the evolution of phenotypic plasticity could be of major importance to cope with different ecological conditions (Richards et al, 2006; Sexton et al, 2009). A change of body size is often observed along geographic clines and could be of great evolutionary importance, as most life history traits scale with size (Gardner et al, 2011; Millien et al, 2006). As climate is an important determinant of species ranges (Parmesan, 2006; Gaston, 2003), another key adaptation does relate to climate tolerance (Hoffmann & Willi, 2008; Davis et al, 2005; Rehfeldt et al, 1999). An expansion towards the pole could require an increased cold tolerance or improved overwintering capabilities, e.g. the evolution of a diapausing phenotype (Igantowicz & Helle, 1986; Tanaka, 1997).

Local adaptation allows the establishment of several ecotypes along an environmental cline (Davis & Shaw, 2001). It is maintained via different selective regimes, acting e.g., along a climatic gradient (Davis & Shaw, 2001). A linkage of ecologically relevant alleles, with those contributing to reproductive isolation or pleiotropic effects, can potentially even lead to reproductive isolation (Rundle & Nosil, 2005). Unlike allopatric speciation by geographic isolation, divergence of lineages during such ecological speciation is initiated by adaptation to different environmental regimes (Schluter, 2009).

Hybridization – Destructive or creative force?

A traditional view on molecular adaptation involves the emergence of novel mutations, on which selection can act (Orr, 2005). New mutations probably emerge too slow for providing the common means of adaptation in a rapidly changing environment (Prentis et al, 2008). But potentially adaptive mutations do not necessarily have to arise newly. Instead, they can already be present in a population in the form of standing genetic variation (Hermisson & Pennings, 2005; Barrett & Schluter, 2007). Another important possibility for the rapid introduction of potentially adaptive variants is the admixture of formerly isolated genetic lineages (Arnold, 1999 & 2006). Originally, such secondary

contact has been viewed as maladaptive, and authors have highlighted the negative effects of swamping or hybrid incompatibilities (Mayr, 1992; Rhymer & Symberloff, 1996). Recent research has compiled compelling evidence for the association of admixture, adaptations and range expansions in many plant and animal species (Kolbe et al. 2004; Gaskin et al. 2009; Keller & Taylor 2010; Lucek et al. 2010; Turgeon et al. 2011; Krehenwinkel & Tautz, 2013). The positive effects of population admixture range from a simple release of inbreeding depression (Hogg et al, 2006; Madsen et al, 1999) over the introduction of adaptive alleles into a gene pool (Whitney et al, 2010; Verhoeven et al, 2011), to the establishment of evolutionary novelty by recombination (Ellstrand, & Schierenbeck, 2000). The phenotypic consequences of this novel adaptive variation can manifest in several ways. First, hybrid offspring can show intermediate phenotypes between parental populations (Hermansen et al, 2011). Secondly, an introgression of adaptive traits from one population to the other is possible (Whitney et al, 2010; Song et al, 2011; Pardo Diaz, 2012). And last, novel combinations of alleles can lead to extreme phenotypes (Schwarzbach et al, 2001). This phenomenon is known as transgressive segregation and allows selection to act on a widened character space (Lexer et al, 2003). The hybridization of different species can even lead to the emergence of a differentially adapted lineage with species-like characteristics (Mallet, 2007; Nolte & Tautz, 2010). The resulting "hybrid species" can colonize a habitat, in which neither of its parental species could exist. Several authors have provided evidence, for such hybrid speciation scenarios (Rieseberg et al, 2003; Mavarez et al, 2006; Nolte et al, 2005; Larsen et al, 2010; Hermansen et al, 2011). The incidence of secondary contact between formerly isolated lineages is expected to rise dramatically in the near future. Global change has tremendous impact on ecosystems and species distributions worldwide (Crispo et al, 2011). Species are transported into new habitats, distributional barriers break down due to human activities, and global warming has contributed to range expansions of various species. This has caused numerous biological invasions and hybridization of formerly isolated species (Garroway et al, 2010; Mooney & Hobbs, 2000; Dukes & Mooney, 1999). Considering this background, admixture has to be taken into account in studies on contemporary adaptation during global change (Nolte & Tautz, 2010).

Phylogenetics and phylogeography - Detecting genetic structure and hybridization

Adaptation due to secondary contact is a straightforward concept. But the identification of the according evolutionary lineages and their hybrid progeny is much less simple. The emerging technologies of molecular genetics have offered a new perspective on taxonomy and phylogeny in the past decades. By comparing DNA sequences of different taxa or populations, diagnostic mutations can be identified, which allow distinguishing genetic lineages or to reconstruct their relationship (Field et al, 1988; Whelan et al, 2001; Tautz et al, 2003; Hebert et al, 2004). Different regions of the genome accumulate mutations at different rates. Accordingly, genetic markers are available for analyses on any level of the tree of life, from deeply split lineages down to recently diverged conspecific populations (Pace et al, 1986; Harrison, 1989; Sunnucks, 2000). In this regard, the discipline of phylogeography is well suited to unravel the genetic and geographic history of species. Phylogeography aims to explain geographic structure of lineages within and among species, with a focus on their biogeographical past (Avise, 2001). The Pleistocene history of European species provides classical examples for phylogeographic work. Pleistocene glaciations trapped many European taxa in glacial refugia, most importantly Iberia, Italy and the Balkan Peninsula. The resulting isolation led to considerable genetic divergence between these regions and fueled allopatric speciation. During their postglacial recolonization, many of these lineages met and formed areas of secondary contact in Central Europe, so called hybrid zones (Hewitt, 1999 & 2000; Taberlet et al, 1998). By analyzing fast evolving DNA sequences, e.g. mitochondrial genes or nuclear microsatellites, glacial lineages and their hybrid zones can be identified (Avise, 2001; Schmitt, 2007). An inclusion of DNA from natural history collections into a phylogeographic analysis does even allow tracing the movement of genetic lineages in real time (Wandeler et al, 2007).

Detecting the phenotypic consequences of adaptation

Genetic adaptation should usually manifest in an advantageous phenotype (Futuyma, 2005). Accordingly, an initial screen for adaptation can involve the comparison of phenotypic characters. Adaptive characters can be manifold, ranging from morphological

(e.g. bodysize) (Gardner et al, 2011) over behavioral (e.g. dispersal) (Thomas et al, 2001; Hill et al, 2011; Phillips et al, 2006) and physiological traits (e.g. temperature tolerance) (Huey & Kingsolver, 1989; Douglas & Grula, 1978). While such traits are assessed studying whole organisms, phenotypic differences can be quantified on the molecular level as well. These involve protein structure (Wüthrich, 1989), metabolite profiles (Ding et al, 2010) or RNA expression levels of genes (Brawand et al, 2011).

Phenotypes can be analyzed on contemporary samples, but another appealing approach involves the assessment of historical phenotypic change (Suarez & Tsutsui, 2004). In this regard, natural history museums present a promising source for historical phenotypic data, especially for morphological studies (Babin-Fenske et al, 2008; Ozgo & Schilthuizen, 2011). However, phenotypic differentiation of populations does not necessarily indicate adaptive divergence. The same genotype often produces divergent phenotypes under different environmental conditions, e.g. climate- or feeding regimes. Such environment-dependent plasticity is well known to contribute to the expression of different phenotypes (Richards et al, 2006). Even the environment, a parent was exposed to, can influence the expression of offspring phenotypes (Mousseau & Fox, 1998). Considering these sources of error, phenotypic analyses should be based on F_1 or better F_2 individuals, bred and raised under identical conditions (Crabbe et al, 1999). This idea of assessing phenotypes in common environmental conditions is underlying two classical approaches to detect local adaptation: common garden -and reciprocal transplant experiments. In a common garden, different ecotypes are kept under the same environmental conditions. Such an experimental design allows ruling out environmental effects on phenotypic expression (Townsend et al, 2009). In a reciprocal transplant, ecotypes are switched between their respective habitats. A fitness loss under transplanted conditions indicates local adaptation (Freeland, 2005).

Genomics of adaptation - Detecting its functional signatures and architecture

Phenotypic studies have compiled examples of local adaptation in various plant and animal species (Futuyma, 2005). However, these numerous findings in support of local adaptation do not clarify its genetic architecture, which can be highly complex.

Adaptation could result from a single mutation of major effect (Maynard-Smith & Haigh, 1974) or due to the contribution of multiple loci (Pritchard & Di Rienzo, 2010). These genes could interact additively, each independently adding to a phenotype. On the other hand, phenotypic effects of different loci could be dependent from each other due to epistatic interactions (Wolf et al, 2000). An adaptive mutation could be translated into a functional amino acid change (Lang et al, 2012; Cavalli Sforza & Bodmer, 1971), or be located in non-coding, regulatory DNA (Chan et al, 2010). Adaptive regulatory mutations can alter gene expression, acting from within the sequence of a gene (e.g. an enhancer or promoter) (Chan et al, 2010) or as an external signal (e.g. a transcription factor) (Mandel et al, 2009; Romero et al, 2012). At the same time, gene expression could vary due to different gene copy numbers, which allow for simultaneous transcription (Perry et al, 2007). Evidence exists for all these evolutionary scenarios. But their relative role in adaptation is still discussed and not satisfactorily understood. The emerging techniques of next generation sequencing offer increasing possibilities to examine genetic structure even across whole genomes (Hudson, 2008). Such technical advances make a large variety of genome wide tests for adaptation feasible.

Several genetic approaches allow identifying loci, associated with phenotypic trait of interest, or directly test for signatures of adaptation in the genomic sequence. The first category comprises methods, which associate a phenotype to variation in the genome. The underlying assumption is that individuals, who share a certain phenotype, will also share the alleles, which contribute to its expression. These alleles in turn are linked to neutral genetic variants, which can easily be scored. Methods to link phenotypic traits to genomic variation include quantitative trait locus (QTL) mapping (Connor & Hartl, 2004), bulked segregant analysis (Michelmore et al, 1991), genome-wide association studies (GWAS) (Manolio, 2010) or mutant screens (Nolan et al, 2000). An analysis of several parallel evolved selection lines can also reveal alleles, involved in phenotypic change (Chan et al, 2012).

Phenotype independent tests for adaptation involve screening the genome for signatures of selection. Balancing selection maintains adaptive polymorphisms, leading to genomic regions of high variability. Positive selection on newly arisen or introduced variants will quickly shift adaptive mutations to high frequency. And negative selection is constantly

removing maladaptive variants (Biswas & Akey, 2006; Nielsen, 2005, Mead et al, 2003; Aguilar et al, 2004; Pagel & Pomiankowski, 2008). The corresponding genomic regions can be analyzed using neutral markers. Several tests have been developed to identify footprints of selection in genomic data. One of these methods is based on the ratio of non-synonymous (dN) and synonymous (dS) mutations per non-synonymous and synonymous site in a particular gene (Pagel & Pomiankowski, 2008). Assuming that an amino acid changing (non-synonymous) mutation is adaptive and selected for, a high dN/dS ratio indicates positive selection. However, this test is only feasible, when a considerable number of substitutions have already accumulated. It is thus not well suited to study contemporary adaptation.

A genome wide screen for selective sweeps is a popular test for tracing more recent adaptation (Maynard-Smith & Haigh, 1974; Nielsen; 2005). The underlying theory assumes that positive selection leads to a wide window of reduced variation around an adaptive mutation in the genome, a selective sweep. In recent years, screens for genomic islands of divergence are gaining increasing popularity to identify genomic regions of ecological importance. Local adaptation in the face of gene flow will be initiated by divergent selection on small genomic regions between populations, so called islands of divergence (Via, 2012).

Another widely used approach to detect adaptation in the genome involves the screening of allele frequency changes over environmental gradients (Freeland, 2005). An allele, involved in adaptation to certain ecological conditions, will occur in highest frequency in the respective environment.

Apart from strong selection on single genes, adaptation could be mediated by many mutations of minor effect and in different loci (Kawecki, 2000; Pritchard & Di Rienzo, 2010). As a consequence, subtle allele frequency shifts of many loci could be observed as a signal of adaptation. Such signatures would be hard to detect and distinguish from demographic effects (Pritchard & Di Rienzo, 2010). A parallel study on independent populations under similar selective pressures might help identifying loci, actually contributing to such polygenic adaptation (Pritchard & Di Rienzo, 2010).

During the past years, scientists searching for genomic signatures of selection have gleaned a substantial amount of candidate loci, potentially involved in recent local adaptation. However, the identification of a candidate locus is far from being the end of the scientific enterprise. To confirm a gene's adaptive function, it has to be subjected to further downstream analysis. For example, gene knockout experiments can help to clarify the actual phenotypic effect of a locus (Austin et al, 2004). So far, only few studies arrived at this final stage and functionally confirmed ecologically important candidate loci. Well characterized examples include pelvic spine reduction and lateral plate development in sticklebacks (Chan et al, 2010; Colosimo et al, 2005), host switching in fruit flies (Lang et al, 2012) or bacteria (Mandel et al, 2009) and coat color polymorphisms in rodents (Hoekstra et al, 2006; Linnen et al, 2009). The functional genetic responses to recent global change are still largely unknown (Franks & Hoffmann, 2012). Consequently, research into this topic is a worthwhile endeavor.

The wasp spider *Argiope bruennichi* - A new model species for studying adaptation in the face of global change

Here, I present a new model species for studying the evolutionary consequences of range expansions during global change, the European wasp spider *Argiope bruennichi*.

Argiope spp. are orb-weaving spiders (family Araneidae). Due to its highly complex and diverse mating system, the genus has received considerable attention by behavioral ecologists (Huber, 2005). The genus *Argiope* consists of nearly 100 species and is distributed worldwide, predominantly in the tropics and subtropics (Platnick, 2013). Only few species reach the temperate climate zone. Of these, the Nearctic garden spider *Argiope aurantia* and its sister species, the Palearctic wasp spider *Argiope bruennichi*, show the northernmost distributions (Platnick, 2013. *Argiope bruennichi* is found from Japan over China and Central Asia throughout Europe, North Africa to Madeira and the Azores (Gutmann, 1979). It usually occurs on grasslands and fallows, where it constructs a horizontal web between grass stalks close to the ground. The spiders mature and mate in summer. The female will then produce several eggsacs until autumn and die before the winter. The offspring hatches a few weeks after oviposition, but remains in the protective

silk case of the eggsac for the coming winter. Around May, the little spiderlings leave the eggsac and reach maturity within the next weeks (Koehler & Schaller, 1987).

Argiope bruennichi is considered to be a thermophilic species (Guttmann, 1979). Its European distribution was historically largely limited to the Mediterranean and some warm oceanic climate regions in France and Southwestern Germany. Apart from an isolated occurrence around Berlin, it was absent in Northern Europe (Guttmann, 1979). From approximately 1930 onwards, the species started to slowly expand its European range towards northern latitudes. The range shift accelerated in the past decades. Today, *Argiope bruennich*i has spread over most European countries and can be found as far north as Norway, Sweden, Estonia and Finland (Guttmann, 1979; Kumschick et al, 2011; Tervihuo et al, 2011). Consequently, the species expanded its range by more than 1000 km in less than 100 generations.

The wasp spider is a large and conspicuously colored species. Therefore, its range expansion is well documented (Guttmann, 1979; Kumschick et al, 2011; Tervihuo et al, 2011). In addition, wasp spiders usually occur in large population densities (pers. obs.). These features make wasp spiders easy to find and collect in large quantities. Furthermore, *Argiope bruennichi* is easy to keep under laboratory conditions. All these characteristics make them an attractive model to study the biology of contemporary range expansions.

Several authors have examined the spider's spread across Europe, and different explanations have been brought forward. These include a tracking of warming climate, the increase of colonizeable fallows and historical dispersal limitation by long stretches of unsuitable habitat (Guttmann, 1979; Kumschick et al, 2011). However, the spider invaded significantly colder regions in the past 50 years, and suitable fallows were available long before it started expanding its range (Kumschick et al, 2011; Geiser, 1997). Furthermore, wasp spiders are excellent long distance dispersers, capable of migrating huge distances by wind mediated dispersal, so called ballooning (Foelix, 2011; Follner & Klarenberg, 1995). A dispersal limitation is thus questionable. Instead, it has been speculated that genetic adaptation, e.g. to cooler temperatures, could have enabled the current range expansion (Kumschick et al, 2011; Geiser, 1997).

Argiope bruennichi fulfills several criteria for possible evolutionary adaptation at range edge. Its expansion is characterized by very high population densities, even at the range front (Krehenwinkel & Tautz, 2013). The spider's passive wind mediated dispersal also gives reason to expect little genetic structure within large geographic regions. In combination with a highly monogynous mating system and obligate sexual cannibalism (Welke & Schneider, 2010), one can expect very large effective population sizes for the species. *Argiope bruennichi* is thus probably not very susceptible to random drift, which could counteract selection or impoverish genetic variation at the range edge. Moreover, its dispersal is limited to first instar offspring (Walter et al, 2005; Follner & Klarenberg, 1995). Maladapted dispersers will probably be removed by selection, before they can contribute to the local gene pool. In addition, a secondary contact of formerly isolated wasp spider populations is likely due to the species´ dispersal ability. *Argiope bruennichi* is thus an interesting candidate species to study the evolutionary consequences of hybridization during global change.

Summary

In this PhD thesis, I establish the wasp spider *Argiope bruennichi* as a new model species for studying the genetics of adaptation during contemporary range expansions. In particular, I investigate the interplay of environmental and genetic factors and their role in determining a specie´s range expansion success.

First, I provide a general framework, highlighting population genetic and phenotypic changes during the species´ range expansion. Secondly, I analyze the genomic signatures of adaptation during the range expansion, using next generation sequencing technology.

I include a detailed analysis of the wasp spider's contemporary European range expansion in chapter one. The analysis is based on genetic markers, several ecological experiments and morphological data. Using a dense sampling of contemporary spiders and historical museum material, I trace genetic changes during the range expansion in real-time and show that adaptation at the range edge seems to be indeed involved in the spider's invasion success.

Based on a dense population sampling all over the species´ range, I introduce a broader phylogeographic framework for *Argiope bruennichi* in chapter two. The study is based on mitochondrial and nuclear DNA-markers. Moreover, I conduct a phylogeographic study on a second widely distributed spider species, the nursery web spider *Pisaura mirabilis*. A focus of these studies lies on the location of glacial refugia for highly dispersive Palearctic species and the potential role of selection in preventing mitochondrial gene flow.

In order to trace temporal genetic changes during a range expansion, access to historical DNA material is desirable. However, old DNA is often degraded and not easy to work with. In Chapter three, I present a PCR-based screen for historical DNA amplification success of nuclear and mitochondrial DNA of distinct fragment sizes. I include samples of different age, starting from 1820 onwards and from two large natural history collections. The results provide a general framework to estimate the feasibility of historical DNA analyses in spiders and other wet preserved arthropods.

Based on data from the phylogeographic studies and ecological experiments, I conduct a next generation sequencing-based approach to identify genome-wide signatures of adaptation. The results are presented in chapter four. The study comprises the establishment of a first draft genome sequence of a spider species. In order to identify signatures of adaptation, I then analyze whole genome data from population pools along two geographic clines through the spider's range. The genomic analysis is extended by a screen of allele frequencies for several candidate loci over the whole geographic range of the wasp spider.

In chapter five, the genomic analysis is complemented by a genome wide expression study, using RNA sequencing technology. For this study, lab bred first instar spiderlings from different populations in southern Portugal, Latvia and Estonia were subjected to heat and cold stress. Subsequently, gene expression profiles for Northern and Southern European spiders were evaluated. These experiments simulated hot or cold autumn days in the spider's native and invaded range respectively. I discuss gene expression divergence in association with the results of a thermal tolerance experiment.

Chapter 1: Northern range expansion of European populations of the wasp spider *Argiope bruennichi* is associated with global-warming-correlated genetic admixture and population specific temperature adaptations

1.1 Introduction

The past decades are distinguished by unprecedented global change, which is altering ecosystems to a not yet predictable degree. In particular, ongoing global warming affects biodiversity worldwide (Gitay et al, 2002; Willis & Bhagwat, 2009; Walther et al, 2002; Hickling et al, 2006; Chen et al, 2011). Many taxa are contracting their ranges or even facing extinction (Thuiller et al, 2011). Others adapt to the new environmental conditions (Bradshaw & Holzapfel, 2006; Hill et al, 2011), or escape the warmth by shifting their distributions to higher latitudes (Parmesan & Yohe, 2003; Walther et al, 2002; Chen et al, 2011). Yet other species benefit and expand their ranges, apparently in response to global warming (Parmesan, 2006). However, the contribution of contemporary adaptive genetic changes to the success of such range expansions still needs to be fully evaluated.

Considering the fast pace of global climate change, evolutionary responses must happen in very short timeframes, which in turn makes the accumulation of new adaptive mutations unlikely. Instead, selection on standing genetic variation is expected to provide the mechanism for fast adaptations (Barrett & Schluter, 2007). Adaptive alleles can already be present in a population's gene pool or can be introduced by interpopulation admixture (de Carvalho et al, 2010; Dowling & Secor, 1997). In today's globalized world, the secondary contact of formerly isolated lineages is increasingly likely (Crispo et al, 2011). Such admixture is well known to contribute to adaptive potential and has been linked to invasiveness and invasion success in many plant and animal species (Kolbe et al, 2004; Gaskin et al, 2009; Keller & Taylor, 2010; Lucek et al, 2010; Turgeon et al, 2011). Hybridization between different taxa can even lead to the emergence of differentially adapted hybrid lineages. The emerging "hybrid species" distinguishes itself by a set of characters, which allows the species to colonize a new habitat that was unsuitable for both parental species (Rieseberg et al, 2003; Mallet, 2008; Nolte & Tautz,

2010). Climate-change-induced hybridization could thus contribute to the expansion of species into new climatic regimes (Hoffmann & Sgro, 2011).

Here, we analyze the recent range expansion of the European wasp spider *Argiope bruennichi* (Scopoli 1772), a well-studied model organism in behavioral ecology (e.g., Welke & Schneider, 2010). The species´ original European range comprised the whole Mediterranean and warm Oceanic climate regions (Figure 1.1). Apart from a single isolated occurrence around Berlin, it was absent from the Northern Continental climate region of Europe. From approximately 1930 onwards, the species started slowly expanding its range into increasingly Continental climate regions in, e.g., Western Poland. In the past decades, this range shift has accelerated and the spider is now found as far north as Norway, Sweden and Finland (Guttmann, 1979; Terhivuo et al, 2011). Compared to their original range, the spiders have moved into significantly colder habitats since the middle of the 20th century (Kumschick et al, 2011). Within a few decades, they have now reached latitudes, in which their persistence is hard to explain solely by global warming (Geiser, 1997; Kumschick et al, 2011).

Wasp spiders are efficient long-distance dispersers. By wind-mediated transport, so called ballooning, spiderlings can cover distances of many kilometers (Follner & Klarenberg, 1995). Evidence from other spider species indicates that they are even capable of ballooning several hundred km (Foelix, 2011). This should allow them to track warming climate quickly, but also increases the likelihood of secondary contact between long separated populations. Consequently, wasp spiders are promising candidates for studying the evolutionary consequences of climate-change-induced admixture.

Our study was set up to answer two general questions: First, we reconstructed the historical origin of populations that have invaded the Continental climate zone of Northern Europe and tested whether they show signs of recent admixture. To address this question, we analyzed approximately 2,000 contemporary and 500 historical museum specimens, using mitochondrial sequences as well as nuclear microsatellite and SNP markers. Our large sampling of museum specimens allowed tracing genetic changes in invading wasp spider populations over the past 100 years to narrow down the historical onset of the expansion and admixture of the spider populations. Second, we asked whether invading populations show signs of new adaptations, such as better cold

tolerance or differences in morphology. To address this question, we conducted a reciprocal transplant experiment between a Northern and a Southern population in the field to test overwintering ability of egg sacs. In addition, we tested thermal preference and tolerance of first instar spiderlings in the laboratory. Then, we assessed morphological size features of the different populations.

Our results allow us to infer a historical scenario that involves an initial passive range expansion, as well as a secondary admixture of old lineages, possibly due to global warming. Our temperature adaptation experiments suggest that the Northern populations have indeed different temperature preferences, as well as a changed morphology, suggesting new adaptations. Since these adaptations correlate with the admixture of lineages, we speculate that hybridization of gene pools may have played a causal role in this.

1.2 Material and methods

Sample collection and morphological analysis

About 2,000 wasp spiders from nearly 300 localities across the Palearctic were collected by hand or sweep net in 2010 and 2011, or were acquired from private collections. All these specimens are stored in 70% Ethanol in the collection of the Max Planck Institute for Evolutionary Biology in Plön, Germany. Additionally, about 500 samples were obtained from the arachnological collections of the Senckenberg Museum in Frankfurt, the Naturkundemuseum in Berlin, the Zoological Museum Alexander König in Bonn and the Institute of Zoology at the Chinese Academy of Sciences in Beijing. One leg of each specimen was removed with heat sterilized forceps for DNA extractions and stored in 100% ethanol. A complete list of sampling localities and museum specimen identifiers is provided in supplementary table 1.1. Subsequently, we will distinguish historical and contemporary samples. Historical samples refer to spiders collected between the 19th century and 1960. This period comprises the majority of our museum samples and represents the time before and at the early phase of the spider´s range expansion. Contemporary samples were collected after 1960, with a focus on 2010 and 2011. This time represents the ongoing, rapid invasion of Northern Europe.

We distinguish six different geographic locations across the Palearctic (see Figure 1.1 & supplementary table 1.1). East Asian samples originate from Japan and Eastern China, while the Central Asian group comes from the dry steppe regions of Western China, Uzbekistan and Southern Iran. Russian samples come from Continental Western Russian steppe and Eastern Ukrainian steppe. Southern Caucasian samples come from the regions south of the Caucasus Mountains and along the Southern Caspian Sea. Within Europe, we distinguish native and invasive areas. The native areas correspond to regions that have been historically inhabited by the species. This is largely equivalent to Oceanic climates in, e.g., France and Southwestern Germany, and the Mediterranean region (based on genetic similarity, we include North-African spiders into this category). The invasive areas comprise Northern Continental European climate zones, which, apart from an isolated occurrence around Berlin, have been colonized approximately since the 1930´s.

This distinction of native and invasive spiders is blurred at the species´ former distributional range limit in Southwestern Germany and Southern Austria. We thus fine-scaled the geographic division, based on differences in our mitochondrial and nuclear genetic data (see Figure 1.2-1.5 & 1.7), as well as climatic differences between each region (see Peel et al, 2007). Consequently, we included populations from Western Germany and the Benelux states into the native group. Although they have been established during the species´ range shift, these populations are genetically coherent with other native European ones. In addition, they have been established in regions of rather mild Oceanic climate and not in the colder Continental parts of Europe.

Selected specimens were examined under a Leica MZ95 binocular. A set of morphological measurements was generated, using a Leica measuring eyepiece or a Leica MRC Axiocam in combination with the Axiovision measuring software (Leica, Wetzlar, Germany). We chose adult female's body size (prosoma width, as measured at the widest part) for our measurements.

Molecular analysis

Contemporary and historical samples were processed in different rooms and two separate sets of tools and laboratory equipment were used. Extractions were carried out with leg

tissue and the 5 PRIME Archivpure DNA Kit, according to the manufacturer's protocol (5 PRIME, Hamburg, Germany). Slight changes were implemented for the historical samples: a negative control extraction was included, 1.5 µL Glycogen (20mg/mL) was added to the precipitation reaction and the amount of resuspension solution reduced to 10-30 µL.

PCR primers were designed using the Primer3Plus software (Untergasser et al, 2007). A 1,200 bp fragment of the mitochondrial COI gene was amplified for contemporary samples. 10 µL PCRs were run in ABI verity fast thermal cyclers (Applied Biosystems, Foster City, US), using the Qiagen Multiplex PCR kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol. A published partial mitochondrial genome sequence of *Argiope bruennichi* (Hassanin et al, 2005) served as the template for primer design. 5 µL PCR product was purified in an 8 µL reaction by adding 0.12 µL Exonuclease I (20,000 units/ml) (NEB, Ipswitch, US), 0.45 µL Shrimp Alkaline Phosphatase (1U/µL) (Fermentas, St. Leon-Rot, Germany) and 2.33 µL water. This reaction was incubated for 20 min. at 37°C and then heat inactivated for 20 min. at 80°C. Cycle sequencing was performed using the ABI Big Dye Kit and samples subsequently cleaned up using the X-Terminator purification kit, according to manufacturer's protocols. Sequencing was carried out on an ABI 3730 DNA Analyzer. Sequences were edited using the Codon Code Aligner software (Codon Code Corporation, Dedham, US) and then aligned with MEGA (Tamura et al, 2007) under default parameters. Based on sequence variation, we designed PCR primers for a short 95 bp COI fragment, which contained diagnostic SNPs to distinguish all major phylogeographic groups within the wasp spider. These primers resulted in successful PCR with most historical samples. In addition, we designed four primer pairs targeting DNA sequences, covering the whole 1200 bp COI fragment. These primers could be amplified in a Multiplex PCR and proved useful for a large fraction of the old DNA samples.

To derive polymorphic nuclear markers for *Argiope bruennichi* (microsatellites and SNPs) we performed a single lane 454 shotgun sequencing run (454 Life Sciences, Branford, US) of genomic DNA. This yielded over 80.000 fragments, of which nearly 1,000 included microsatellites. Microsatellite primers were designed for 16 polymorphic

loci, which were typed for a selection of contemporary populations. Since the typing of Asian population suggested the presence of null alleles for the microsatellites screened, we derived also diagnostic SNP markers. To obtain these markers, we sequenced random nuclear DNA fragments of approximately 400 bp (derived from the 454 reads) for a selection of Asian and European samples. In this way we identified a set of seven loci, apparently reciprocally fixed for Asia and Europe respectively, which were combined in a Multiplex SNP genotyping assay. This assay targeted a fragment length between 66-278 bp, such that it could be utilized also for a wide range of historical samples. SNPs were typed using the ABI SnapShot Multiplex Kit, according to the manufacturer's protocol. Polymorphisms for microsatellites and SNPs were called and edited using the ABI Genemapper software.

Phylogeographic and population genetic analysis

Sampling locality maps were created with GenGIS (Parks et al, 2009). Median joining haplotype networks were constructed for contemporary and historical samples using the software Network (Fluxus Technology Ltd, Suffolk, England). In order to reduce their complexity, the networks were preprocessed using one round of star contraction, with a maximum star radius of five. Estimates of nucleotide diversity for the mitochondrial sequences were generated by DnaSP (Librado & Rozas, 2009). Microsatellite analyzer (MSA) (Dieringer & Schlötterer, 2003) was used to generate estimates of heterozygosity for the analyzed populations and population-wise distance matrices (Nei´s standard genetic distance). The Phylip package served to construct a neighbor joining tree (Felsenstein, 1989), based on the distance matrix. In addition to the distance-based approach, we analyzed our data using the R package Geneland (Guillot et al, 2005). The Geneland analysis was carried out assuming a maximum of 10 populations, with 100.000 iterations and thinning at every 100^{th} iteration. A non-correlated allele frequency model with enabled spatial model was used. The individual ancestry for the SNP dataset was calculated using STRUCTURE (Pritchard et al, 2000; Falush et al, 2003). STRUCTURE was run with a burnin period length of 50.000 and 10.000 MCMC replications after burnin.

Thermal preference tests and reciprocal transplant experiment

Argiope bruennichi is an annual species that matures in summer, produces egg sacs until early autumn and then dies with the first frost. The spiderlings hatch a few weeks after oviposition, but overwinter in the protective silk envelope of the egg sac. Around May they leave their egg sac and reach maturity within about three months (Köhler & Schäller, 1987). This prolonged diapause constitutes a considerable part of the spider´s whole life span and includes the climatically most severe winter season. An adaptation that enables the spiders to colonize a new and possibly colder habitat will thus be likely expressed in the first instar´s phenotype. This life stage is therefore ideally suited for studying thermal adaptation and overwintering capabilities and was the stage chosen for our experiments.

Mated adult females were collected in August 2011 in Northern Europe (Northern Germany, Poland, the Baltic States, Sweden and Denmark, in total 94 females from 24 populations) and in early September 2011 in the Western Mediterranean (Portugal, Spain and Southern France; in total 76 females from 13 populations). The spiders were kept in 200 mL plastic cups at room temperature, fed with house flies and their webs sprayed with water every day. Between September and early November 2011, they each constructed 1-2 egg sacs.

To assess thermal preference parameters, 60 egg sacs were kept under laboratory conditions until early December. By this time the spiderlings had hatched and were forced to emerge from the egg sac by splitting the silken wall. Thermal preference was tested for four spiderlings from each egg sac in a temperature gradient, generated in an ABI verity fast cycler (Applied Biosystems, Foster City, US). This PCR cycler can be set to a gradient of six different temperatures, spanning a maximum temperature difference of 25°C. We set up a gradient from 4°C to 29°C and covered the PCR machine's plate inlay with a layer of tissue paper. This in turn was covered with a black Makrolon plastic plate (128.5 x 86.5 x 11.75 mm). The plate contained eight channels (each 104 x 5.5 mm), which each spanned the temperature gradient. We allowed the gradient to establish for 30 minutes before transferring one spiderling to each channel and adding a

transparent plastic cover plate. After initially walking around in the gradient, the spiderlings came to rest after about 30 minutes. We thus noted their position after 30 minutes and repeated this two more times after 15 min intervals. The same setup, but with the gradient switched off, served as a control.

The upper temperature tolerance was tested by putting single spiderlings (four from each egg sac) into a 1.5 mL Eppendorf tube and gradually heating them up. Preliminary tests showed that all spiderlings survived temperatures of up to 39°C. We thus started at 40°C and increased the temperature by 2°C every ten minutes. Confronted with raised temperatures the spiderlings increase their activity until they reach their tolerance limit and fall into rigor. After each round of heating, the spiders in rigor were identified and the temperature noted. We also tried to test lower temperature tolerance, but since all spiders reduce their movements at colder temperatures, a clear test could not be devised.

To test for overwintering capacity, we set up a reciprocal transplant experiment with 228 egg sacs to compare overwintering performance of Northern and Southern European populations under outdoor conditions. Each egg sac carries a silken collar on its upper side, through which twine was sewn. This twine was then attached to 20 cm bamboo poles with adhesive tape. These poles were placed into prepared transparent plastic tanks (79 x 57 x 42 cm). In order to create natural conditions, each tank contained a large piece of grass sod from a wasp spider habitat. Both sides, as well as the cover of each container, had large ventilation holes, covered with a fine mesh. This allowed for circulation, but prevented predators and parasites from entering. Each container was equipped with an equal number of egg sacs derived from Northern and Southern European spiders. Two containers were set up on the estate of the Max Planck Institute in Plön, Germany (54.16°N, 10.42°E, Dec. 3rd, 2011) and two in a Garden near Santa Eulalia on the Spanish island of Ibiza (38.99°N, 1.53°E, Dec. 1st, 2011). Egg sacs for the Northern European treatment were gradually adapted to the cooler climate by decreasing their ambient temperature from 20°C to 6°C over a 14 day period. The egg sacs were then overwintered on their respective sites until March 1st, 2012 (Ibiza) and March 3th, 2012 (Plön). Weather conditions in Plön included weeks with snow coverage and several nights below -10°C. In contrast, subzero temperature was not recorded in Ibiza in the respective period. At the end of the experiments, each egg sac was opened and the

spider's survival rate was estimated. We did not include egg sacs with completely dried eggs, as wasp spider offspring hatch within four weeks after oviposition (Welke & Schneider, 2012). Dried eggs were thus already damaged under laboratory conditions, before the experiment was set up. Most egg sacs contain several hundred spiderlings entangled in a dense network of silk, which makes exact counting difficult. Hence, we applied an estimate by classifying the egg sacs into two categories: clearly above or below 50% survival rate. Exact counting of dead versus live animals was done only for egg sacs that could not be clearly assigned to one of the two categories.

1.3 Results

To address the question of the historical origin of populations that expanded into Northern Europe, we conducted an extensive phylogeographic study encompassing also Asian populations. We analyzed mitochondrial sequences, as well as nuclear markers. The results will be presented in the following.

Mitochondrial data

1,200 bp of the mitochondrial COI gene was sequenced from 1,966 contemporary and 181 historical specimens from all across the species´ range. In addition, we sequenced a short COI fragment of 95 bp of 187 historical samples, in which DNA was too degraded for longer PCR. As this DNA fragment enabled the scoring of all major haplotype groups, we included these samples in the historical haplotype network. Thus, this network does not cover derived haplotypes, but just represents frequencies of haplotype groups. Due to the different sample size, we present haplotype networks for contemporary and historical samples separately (Figure 1.2).

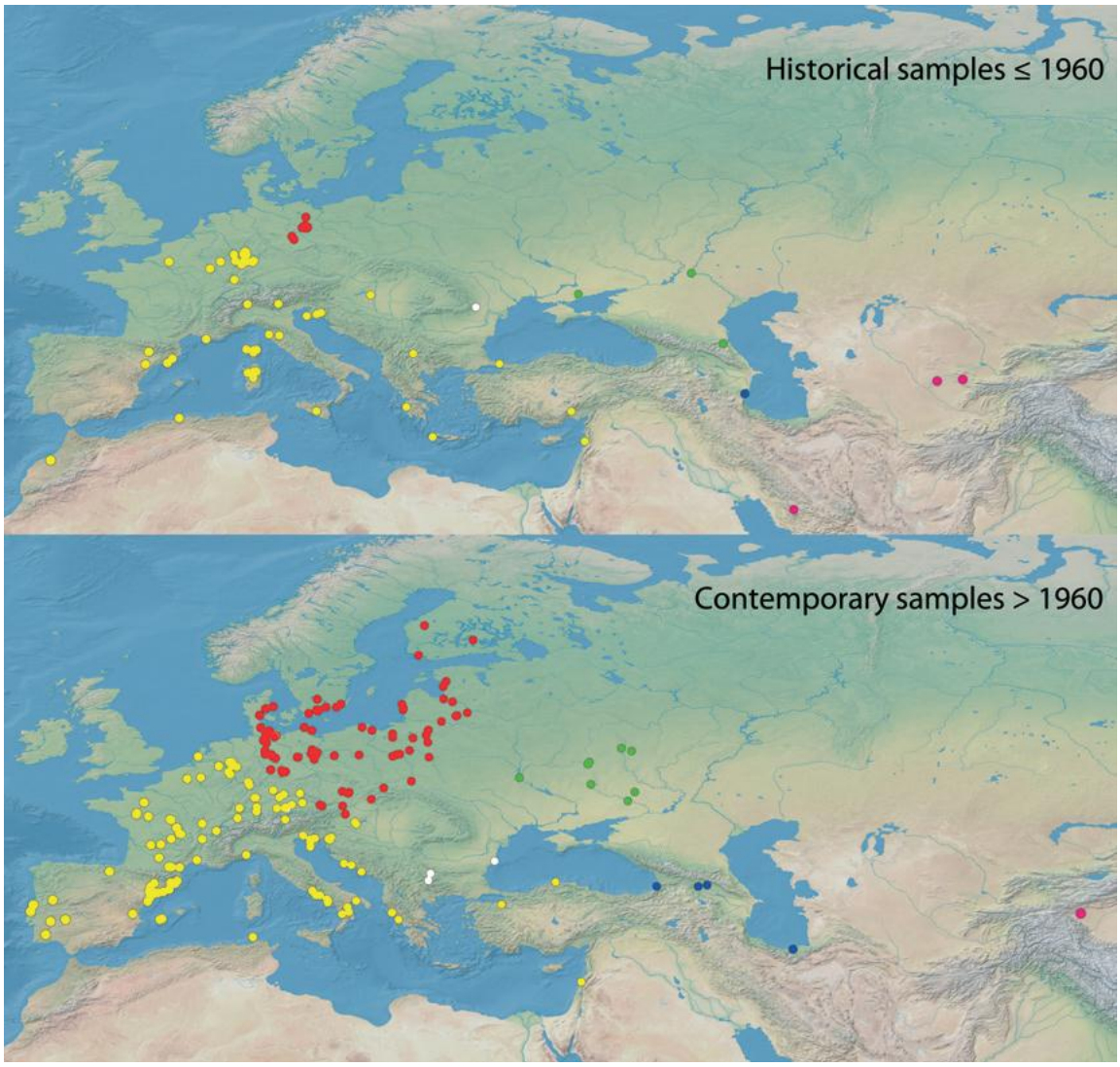

Figure 1.1 Sampling locations for historical (upper panel) and contemporary (lower panel) specimens. The colors represent geographic regions distinguished in the haplotype network in Figure 1.2. Historical specimens refer to samples until 1960, the time before and at the early phase of the range expansion. Contemporary samples represent spiders during the more massive range shift in the past decades. For an overview of far Eastern Asian sampling sites, see supplementary table 1.1. The maps give an impression of the spiders range shift from its native Mediterranean and Oceanic climate range (yellow dots) into increasingly Continental climates in Northeastern Europe (red dots).

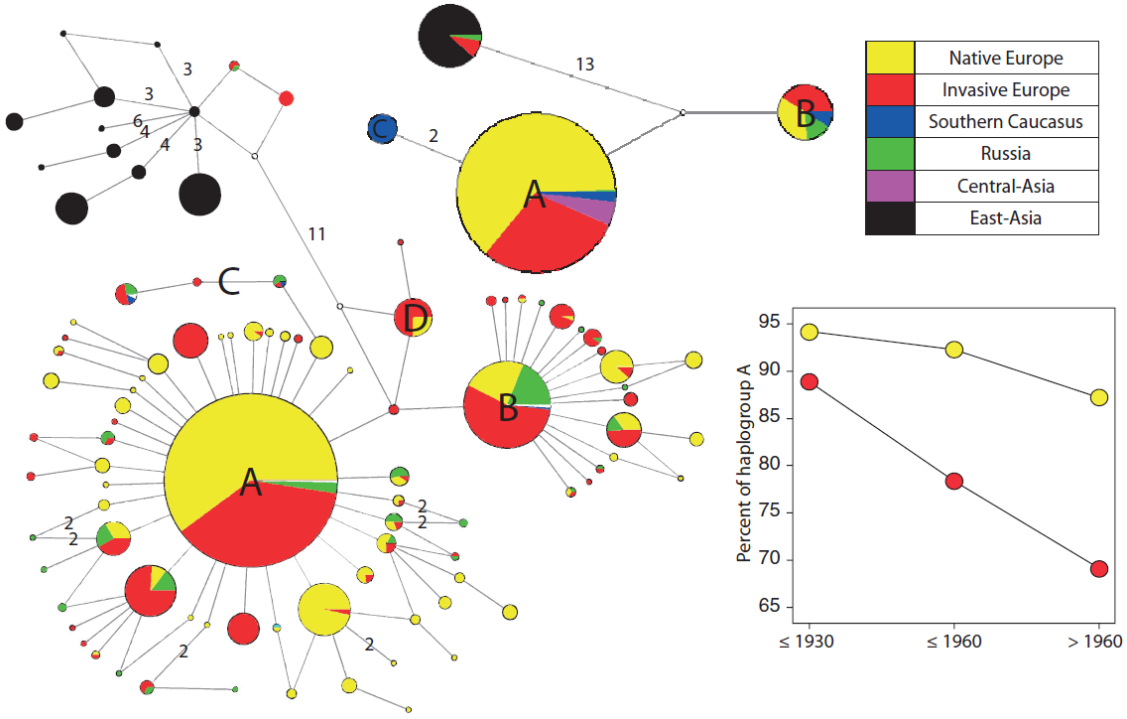

Figure 1.2 Median joining networks of mitochondrial sequences from Palearctic wasp spiders. The lower left network shows contemporary samples (N=1,966), based on 1,200 bp of the COI gene. The simplified upper right network is based on historical samples (N=368) from before 1960. Colors represent the geographic origin of sampled specimens (corresponding to the colors in Figure 1.1). The respective location designations (inset upper right) are used throughout the text. The white fractions in haplogroup A, B and C refer to 7 samples from Bulgaria and Romania which we could not unequivocally assign to a geographic region. Branches represent single mutational steps, if not indicated otherwise by numbers. Major haplotype groups discussed in the text are labeled with capital letters. White dots in the network represent hypothetical intermediates. Inset lower right: frequencies of haplogroup A in native (upper line) and invasive (lower line) European spider populations for the three time periods indicated on the x-axis.

The contemporary network was pruned by one round of star contraction, reducing the actual number of haplotypes from 273 to 96. The networks show a dumbbell pattern between Eastern- and Western Palearctic populations (Avise, 2001). Both groups are distinct by 13 mutations, corresponding to 1.1% sequence divergence. The connection between Eastern and Western lineages is not completely resolved, with two possible Western Palearctic haplotypes associated with the Eastern group.

The Eastern Palearctic is distinguished by comparatively deep divergence of up to 6 mutations between haplotypes, while the network of Western Palearctic populations shows rather shallow splits of only one or two mutations. Two major star-like radiations (Avise, 2001) are apparent (Figure 1.2), accounting for the majority of haplotypes observed in the region. We refer to these radiations as haplogroup A and B. Both groups are distinct by two mutational steps and connected by three specimens carrying an intermediate haplotype. With around 75% prevalence, haplogroup A is the most dominant Western haplogroup. It occurs in nearly every population from Portugal to central Asia. Aside from haplogroup A and B, only two additional independent groups can be found. Haplogroup D is distinct from both A and B by two mutations and only found in 45 contemporary samples. Haplogroup C is derived from haplogroup A and particularly prevalent in the Southern Caucasus region. The latter is the only location, in which haplogroups A, B and C have been present both historically and are still present. Contemporary populations from Russia display a similar haplotype distribution with all major groups present, except D. However, while haplogroup C amounts to the highest frequency in the Caucasus region, haplogroups A and B account for most of the Russian haplotypes. Russian populations also stand out by a high frequency of haplogroup B (nearly 50%). This makes their haplotype composition significantly different from European populations, which are distinguished by a lower frequency of haplogroup B (Chi-square test, d.f. = 2, Chi-square = 132.60, $p < 0.0001$). In addition, Russian populations harbor an Asian haplotype in low frequency (<1%). The respective haplotype is distinct from the closest Chinese and Japanese sequences by just a single mutation. We find this haplotype in one historical as well as one contemporary Russian sample.

Oceanic and Mediterranean European populations (native European range - compare Figure 1.1 & 1.3) show a considerable genetic homogeneity, with haplogroup A as the most dominant mitochondrial variant. Most derived haplotypes in the native European range originate from haplogroup A.

Invasive Continental European populations are distinguished by a very different haplotype composition. All major Western-Palearctic haplotypes are present in the spider's invasive range. Invasive populations carry a large frequency of haplogroup B (~25%), including several derived haplotypes, and account for a large fraction of

haplogroup C and D (10 out of 21 & 34 out of 45 sampled haplotypes). In addition, an Asian haplotype occurs in invasive populations from around 1930 onwards (4% of samples). The respective haplotype is derived from the one found in Russia by just one mutation. Although the frequency of Asian haplotypes is low (about 1% in contemporary Continental populations), it is present in several recently invaded regions of Europe. On the other hand, it is completely absent from the native Mediterranean and Oceanic European range, as well as the Southern Caucasus region. This suggests that admixture of several formerly isolated lineages has probably led to the haplotype composition of invasive populations. This results in significantly higher nucleotide diversity in invasive compared to native populations (π = 0.0011 (N = 56) vs. π = 0.0017 (N = 60) on average, U-test, U = 1041.5, Z = -3.48, $p < 0.0001$).

In order to narrow down the onset of this admixture, we conducted a more detailed analysis of historical haplotype frequency changes. Haplogroup A is most prevalent in native Western European populations. Hence, we chose frequency changes in this haplogroup as an indicator for admixture. We compared native and invasive European populations. The large number of short COI sequences allowed us to split our data into three categories (Figure 1.2, inset). The first one includes specimens sampled until 1930 and represents populations from before the range expansion. The second is based on samples collected after 1930 until 1960 and corresponds to populations at the early phase of the range shift. The last category comprises samples from after 1960, with a focus on 2010 and 2011. This analysis allowed us to draw a clear picture of historical genetic changes. Before the range expansion, native and invasive populations did not significantly differ in their haplotype composition (94% native vs. 89% invasive, Fisher's exact test, two tailed $p = 0.31$). Until 1960, an increase of new haplotypes is observed in the invasive range, but little change is evident in native populations. The difference between native and invasive populations between 1930 and 1960 is significant (92% native vs. 78% invasive, Fisher's exact test, two tailed $p = 0.025$). By today, an additional leap has shifted the haplotype frequency in native and especially invasive populations, leading to highly significant differences between those regions (87.2% native vs. 69.1% invasive, Fisher's exact test, two tailed $p < 0.0001$). These results indicate that a large

part of genetic changes is attributable to the period after 1960, while we can narrow down the onset of admixture to the time around 1930.

To summarize, invasive European populations are distinct from native ones by a higher degree of haplotypic admixture. This holds true for closely related lineages within Europe (haplogroups A, B, C & D), as well as introgression of distant Asian mitochondria. Moreover, the admixture seems to be a recent process and has been increasing in the past decades.

Microsatellite data

To obtain a better resolution at the population level, we genotyped 177 European specimens from 19 locations for 16 microsatellite loci. A Geneland analysis assigns these specimens to two genetic clusters (Figure 1.3). With an F_{ST} of just 0.03, these clusters show only slight differentiation. One cluster is distributed in the native Mediterranean and Oceanic climate range of the species. The other one covers the invasive populations in the Continental climate range of Europe. A phylogenetic analysis of the dataset, based on Nei´s genetic distance, confirms this clustering of populations into two different ecological zones (supplementary figure 1.1). The inclusion of 79 Russian samples into the microsatellite analysis shows these populations to be distantly related to European ones (supplementary figure 1.1). The closest relatives of invasive European spiders are hence found in the species´ native European range.

Similar to the mitochondrial data (see above), the microsatellite data from the European locations show a significantly increased genetic diversity in invasive populations compared to native ones (averaged expected heterozygosity 0.61 vs. 0.53, t-test, equal variances not assumed, t = 2.483, d.f. = 9.292, p = 0.034). This result is indicative of nuclear genetic admixture in the invasive range.

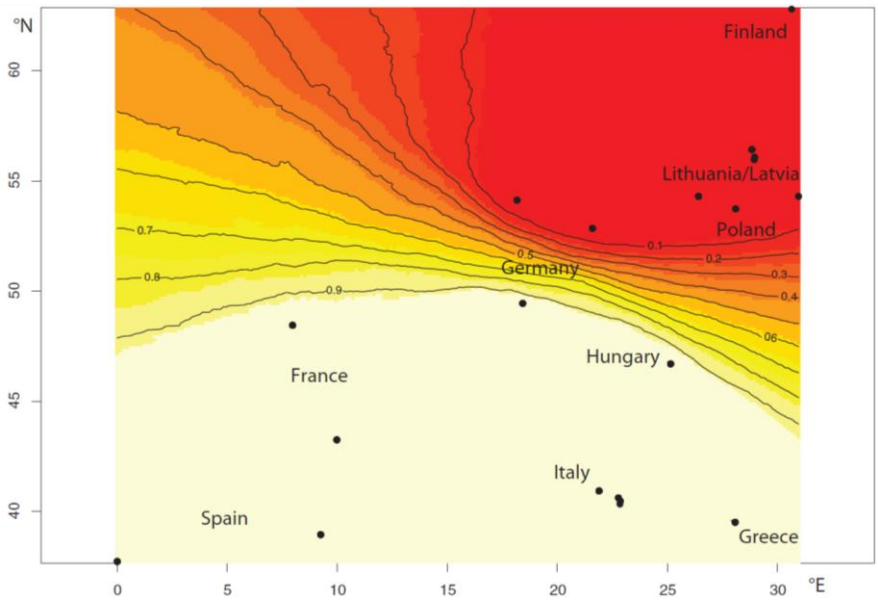

Figure 1.3 Geographic clustering of European wasp spider populations according to Geneland analysis. The map represents the corresponding sampling sites, indicated by black dots. Axes represent geographic coordinates. The longitudinal coordinates are shifted by 7.85° to the east. The Geneland map shows wasp spider populations forming a distinct genetic cluster in the invasive range in Continental Europe with a high posterior probability. The colored areas within each isocline represent posterior probabilities of belonging to the native European genetic cluster. The probability is highest in the white regions (0.9-1) and drops to 0.1 in the invasive range (red region).

SNP data

To better trace the origin and extent of the admixture observed in the mitochondrial data, we developed a set of seven diagnostic SNP loci that differentiate East-Asian and Western Palearctic populations. We genotyped 362 contemporary and 126 historical specimens for these loci. A STRUCTURE analysis of the SNP dataset confirmed the genetic distinctness between East Asian and Western Palearctic populations for these markers in the larger population sample (Figure 1.4). East Asian populations are largely fixed for their respective genotypes (99% Asian background on average, Figure 1.4). These clusters correspond also very well to the mitochondrial network (Figure 1.2).

This data allows assessing the degree of introgression of Asian alleles into Western Palearctic groups (Figure 1.5). We find that Central Asian populations carry 8% of Asian alleles on average. This is in contrast to the mitochondrial data, in which these spiders are completely fixed for the Western Haplogroup A. Russian populations show 5% Asian

introgression on average. Introgression into the Southern Caucasus region, on the other hand, is rather limited (1% Asian background on average), although they are located at similar longitudes compared to the Russian samples.

Invasive and native European spiders are also very different with respect to the introgressed amount of Asian alleles (U-test, U = 1929, Z = -3.053 $p < 0.0001$). Native populations are almost completely fixed for Western alleles, while invasive spiders carry significantly more Asian alleles (5% on average). Again the nuclear introgression is somewhat higher than the mitochondrial introgression from Asia (1% in invasive, not existent in native populations - see above). This is most evident for Swedish populations, where Asian mitochondria are completely absent, but they still carry an average of 3% of Asian SNP alleles with outliers of up to 17%.

European populations did not show much introgression before 1960 (0.7% Asian alleles on average). Few outliers are observed, but each of these belongs to samples from after 1930, a time when the species had already started its range expansion. Before the range expansion, Asian alleles were largely absent. A comparison of contemporary and historical samples shows a significant change of introgression for the invasive populations (U-test, U = 2734, Z = -3.147, $p < 0.0001$), but no change for native ones (U-test, U = 2510, Z = -0.64, $p = 0.522$). Thus, similar to mitochondrial admixture, the introgression of Asian nuclear alleles is of recent origin. In addition, the introgression is largely limited to invasive populations in Northern Europe.

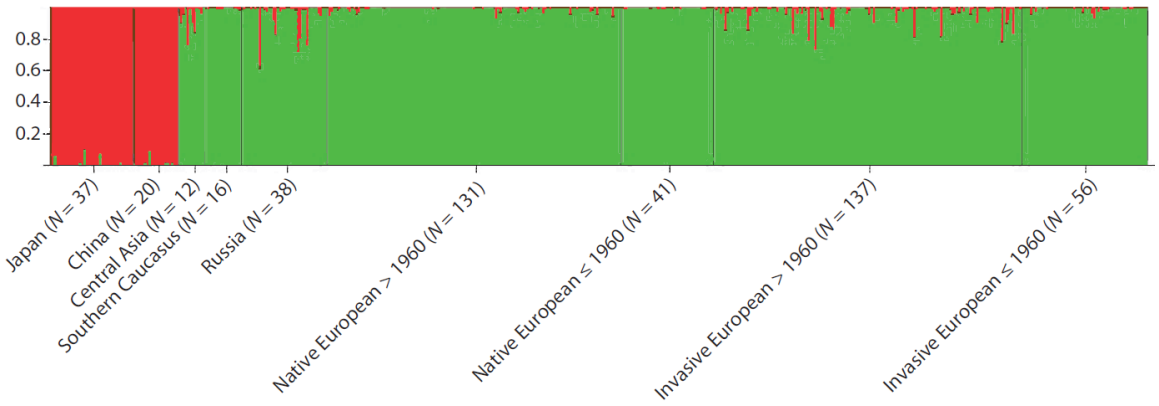

Figure 1.4 STRUCTURE analysis for the SNP dataset (k=2). Green bars correspond to Western Palearctic, red ones to East Asian genetic background. Bars represent single specimens from the regions depicted at the bottom.

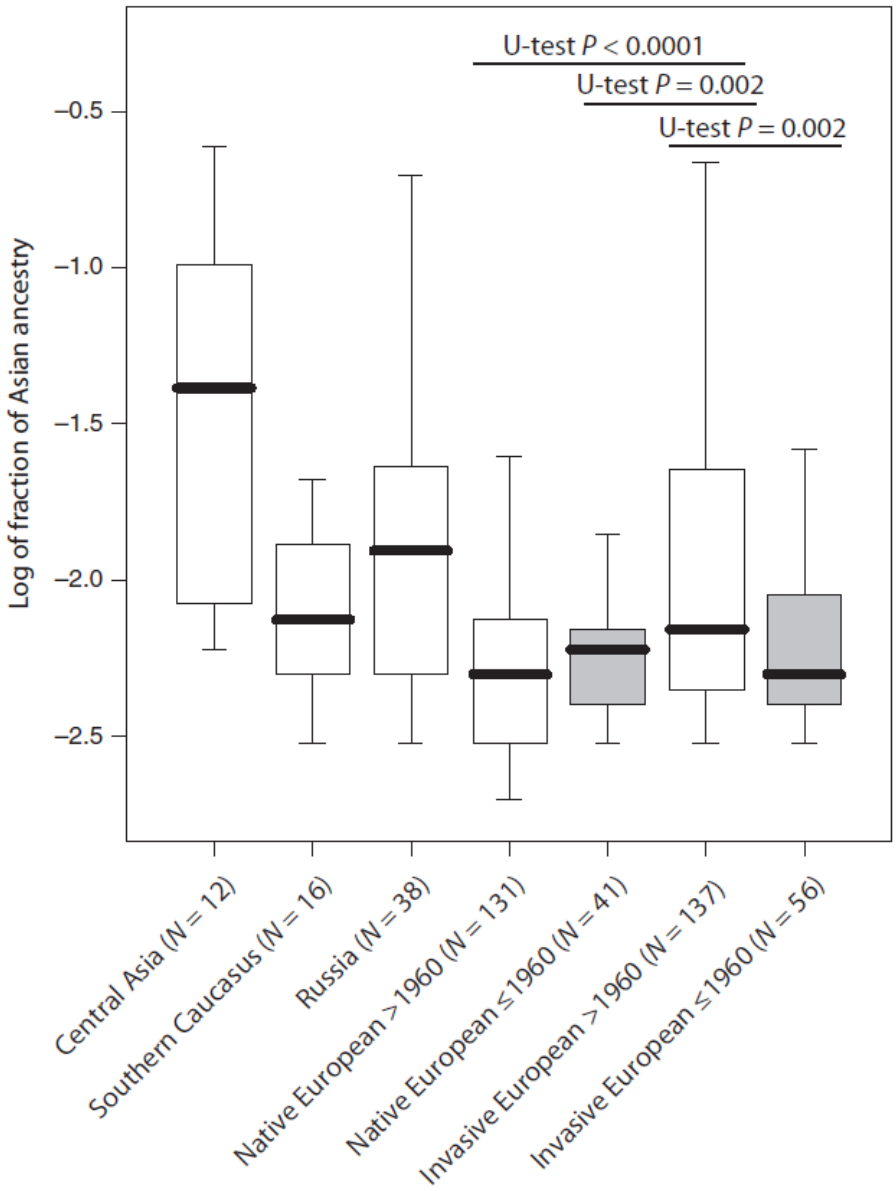

Figure 1.5 Box plots of the fraction of Asian ancestry in different Western Palearctic populations according to STRUCTURE (logarithmic scale). The boxes depict median (black bar) and upper and lower quartiles. Distinction of geographic regions is based upon the classification in Figure 1.1. Native and invasive European spiders were split into historical samples (≤ 1960, grey boxes) and contemporary ones (> 1960). Due to limited sample size, we lumped historical and contemporary data for Central Asian as well as Southern Caucasian spiders.

Body size

To assess whether size differences are evident between the different populations and the different collection times, we choose the measurement of prosoma width of female adult spiders for representing body size. This measure is more reliable than opisthosoma width, since the opisthosoma size is plastic and depends e.g. on the nutritional status. We find that Mediterranean spiders have a significantly larger prosoma width than the invasive ones from the Continental climate zones (Figure 1.6a). In addition, we find a significant size difference between Mediterranean and the more Northern Oceanic climate regions of the spider's native range. Spiders from the Oceanic climate regions in turn are significantly larger than invasive spiders from the Northern Continental climate regions. This holds true for historical, as well as contemporary samples (mean: 5.26 & 5.25 mm in historical and contemporary Mediterranean vs. 4.11 & 3.84 mm in Oceanic, vs. 3.44 & 3.38 mm in invasive material (ANOVA, $F = 214.613$, d.f. 895, $p < 0.0001$, Tamhane post hoc test shows Mediterranean, Oceanic and Continental invasive populations to form homogenous groups, all prosoma width distributions do not significantly deviate from normal, according to Kolmogorow-Smirnow test). Invasive as well as native spiders did not significantly change their mean body size within their respective groups in the past 100 years (Figure 1.6a). However, invasive spiders show a significant decrease of variance in this character (Levene´s test, $F = 23.18$, $p < 0.0001$) between historical and contemporary samples. Until 1960, the invasive spider's prosoma width varied from 1.58-6.10 mm and today from 2.22-4.68 mm, a variance change that is not seen in the other samples (Figure 1.6a).

In contrast to the adults, first instar spiders from the Mediterranean and Northern Europe did not show significant size differences (prosoma width, 0.55 vs. 0.56 mm on average, $N = 196$, t-test, equal variances assumed, $p = 0.85$, $t = 0.19$, d.f. = 194). The same holds true for egg sac sizes from the respective females (12.07 vs. 11.89 mm egg sac width on average, $N = 160$, t-test, equal variances not assumed, $p = 0.62$, $t = -0.50$, d.f. = 72). Hence, these populations are comparable with respect to these measures, which is of special relevance for the temperature tolerance experiments.

Thermal preference and overwintering

To study possible differences in thermal preferences between the populations, we determined in the laboratory upper thermal tolerance and thermal preference for spiderlings. Spiderlings from invasive Northern European egg sacs show indeed a significantly decreased thermal tolerance compared to Mediterranean ones (44.7°C vs. 48.3°C on average, N = 83, t-test, equal variances assumed, t = 6.99, d.f. = 81, p < 0.0001,) (Figure 1.6b) as well as lower preferred temperatures (10.4°C vs. 14.6°C on average, N = 61, t-test, equal variances assumed, t = 4.72, d.f. = 58, p < 0.0001) (Figure 1.6c).

To test overwintering capacity, we conducted a reciprocal transplant experiment using egg sacs of spiders from the Mediterranean and from the Continental climate regions (see Methods). We found a significant association between overwintering locality and survival frequency within egg sacs (Chi-square test, Chi-square = 8.22, d.f. = 3, P = 0.04) (Figure 1.6d). Interestingly, invasive and native populations are equally affected by reverted overwintering conditions. 45% of Mediterranean and 42% of the Continental egg sacs had less than 50% survival of spiderlings under reversed overwintering conditions, while only 27% of the Mediterranean and 20% of the Continental egg sacs had less than 50% surviving spiderlings at their native overwintering locality. The difference between native- and non-native overwintering locations is significant for invasive spiders (two tailed p = 0.023, N = 112, Fisher's exact test), but not for Mediterranean ones (two tailed p = 0.25, N = 51, Fisher's exact test), due to smaller sample size although the trend is the same. On the other hand, there is a significant difference between Continental and Mediterranean spiders overwintering in Continental climate (p = 0.024, N = 88, Fisher's exact test).

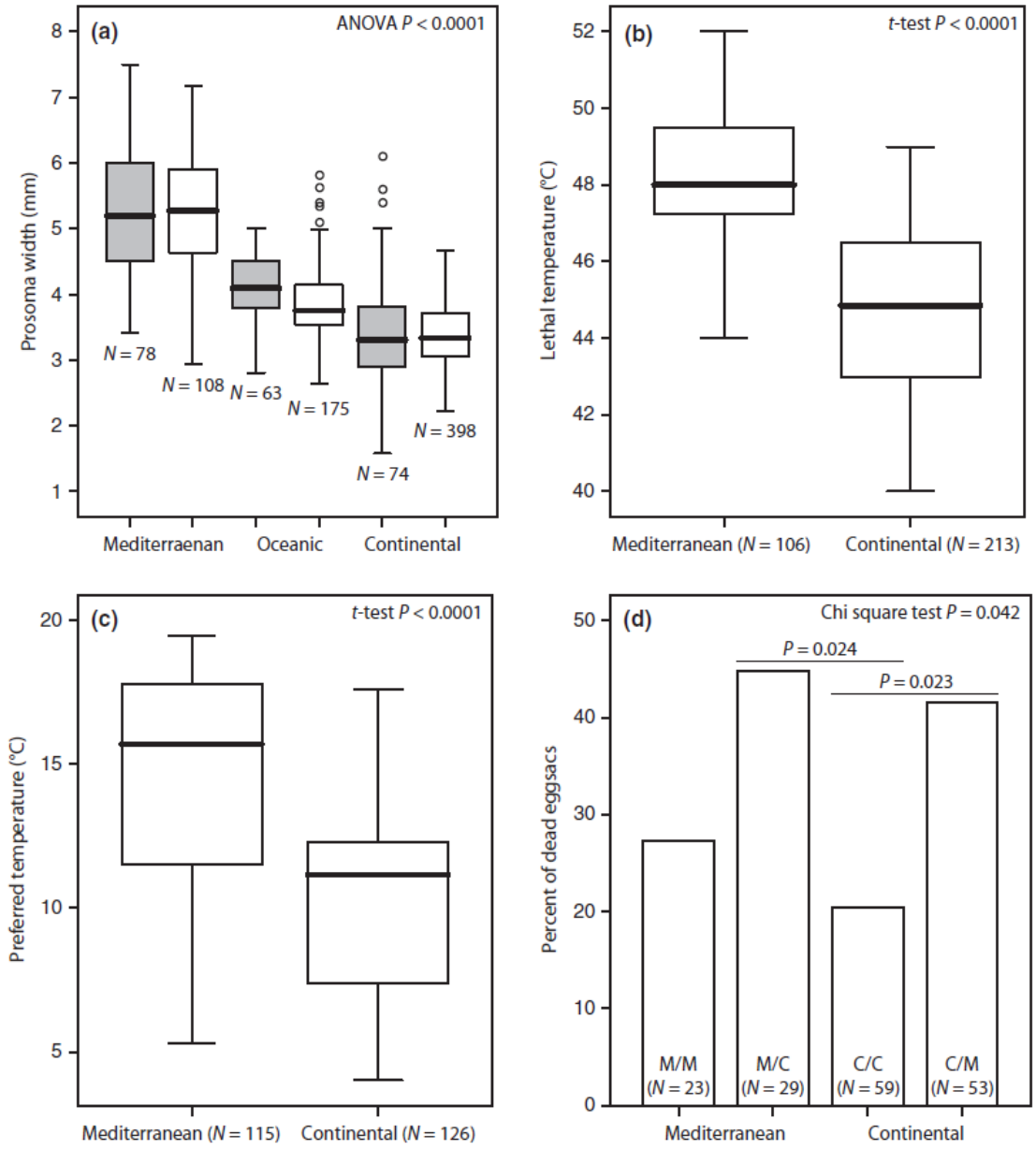

Figure 1.6 Morphological and physiological differences between native and invasive European populations. a) Prosoma width (representing body size) of adult females from native Mediterranean, native Oceanic climate and invaded Continental climate regions of Europe. Historical (≤ 1960, grey boxes) and contemporary samples (> 1960) are plotted separately. b) Upper lethal temperature, c) preferred ambient temperature of first instar spiderlings from Mediterranean and Continental areas. Box plots in figure 1.6a-1.6c show median (black bar), upper and lower quartiles and outliers. d) Fraction of dead eggsacs (< 50% surviving offspring) after reciprocal overwintering of Mediterranean -and Continental animals. The letters correspond to origin and overwintering location, e.g., C/C represent Continental population overwintered in Continental Climate.

1.4 Discussion

Our results indicate a correlation between historical admixture, contemporary adaptation and the successful colonization of a new habitat. Although such a correlation is no direct evidence for causation, this finding is in line with the notion of a contribution of hybridization to climate change triggered adaptation (Hoffmann and Sgro 2011). In the following, we will discuss these points in turn.

Phylogeography of the species

The mitochondrial and nuclear SNP data suggest two major divergent lineages, pointing to the existence of an Eastern and Western Palearctic Pleistocene glacial refugium for the species (see Figure 1.2 & 1.4). The genetic distance of 1.1% between these lineages corresponds to approximately 800,000 years of separation (Knowlton & Weigt, 1998). The comparatively low mitochondrial divergence within the Western Palearctic indicates a single refugium in the West. As the only region with all major European haplogroups (A, B, C) present, we suggest the Southern Caucasus as this refugial area. From here, a single Southwestern route could have led to the postglacial recolonization of the Mediterranean region. The Southwestern range shift was probably accompanied by a bottleneck and loss of mitochondrial lineages. An Eastern offshoot of the Mediterranean recolonization route possibly led into Central Asia. A common origin would explain the similar haplotype composition of Mediterranean and Central Asian populations. Apart from the Southern route, our data also support a Northern route over the Caucasus Mountains and along the Black Sea. This colonization event has been less affected by drift, as seen in the balanced haplotype distribution in, e.g., Russian populations. The star-like topologies of the haplotype networks indicate a recent recolonization from the species´ glacial refugium (see Figure 1.2). A more detailed treatment of the phylogeny and Palearctic-wide phylogeography of the species will be published elsewhere (Krehenwinkel et al, in preparation).

Introgression, admixture and range expansion

Native Western European wasp spiders occur in the mild Oceanic climate regions, including the Mediterranean region as well as France and Southwestern Germany (Figure 1.1). However, apart from an isolated satellite population in the Berlin area, they did not invade the Northern Continental climate regions of Europe in the past. Due to the spider's conspicuous coloration, it is easy to detect and has therefore been a focus of spider collectors since more than a century. Hence, its distributional pattern is very well documented (Kumschick et al, 2011). One reason for failing to expand further after the initial postglacial expansion could be a bottleneck, which may have exhausted the genetic potential of the wasp spider populations. This observation is in line with the general finding that the geographic ranges of many species are limited to a certain ecosystem and an invasion into new habitat is not easy (Bridle & Vines, 2007; Hoffmann and Sgro 2011). Accordingly, adaptation to a new ecological niche at the range margin can be of great evolutionary importance and enable range expansions (Peterson & Holt, 2003). But sufficient genetic variation may be a necessary precondition for such adaptation to occur. Many species possess ample genetic variation for niche parameters (Holt, 2009), leading to intraspecific niche differentiation over sometimes even small geographical scales (Rehfeldt et al, 1999; Castenholz, 1973). Populations with little variation however, will have difficulties to adapt to new niches or a changing environment (Kellermann et al, 2009). Such depleted variation has been shown to occur after range expansions, rendering populations less responsive to selection (Pujol & Pannell, 2008; Olivieri, 2009).

Nonetheless, from around 1930 onwards, the wasp spider started slowly expanding its range into increasingly Continental climate regions (Guttmann, 1979, Kumschick et al, 2011). Interestingly, we observe that this range shift was accompanied by growing genetic diversity in the species´ invasive range. This may initially have been caused by admixture with inner European lineages. The Mediterranean and the Northern Black sea recolonization routes met in Central Europe, leading to a haplotype composition similar to that of the glacial refugium in the Southern Caucasus region. The further expansion into Northern areas is then associated with increasing introgression of alleles from Asian source populations. Such a correlation between a recent range shift and admixture between formerly isolated genetic lineages has been suggested for several plant and

animal taxa (Kolbe et al, 2004; Nolte et al, 2005; Gaskin et al, 2009; Keller & Taylor, 2010; Lucek et al, 2010; Turgeon et al, 2011).

East Asian wasp spiders colonize a wide range of ecological regimes, from the tropical south of Japan and China to China´s cold Northeast and even South Eastern Siberia. Cold-tolerant Far-Eastern spiders are thus already present in climate zones that are currently being colonized by European populations. One may therefore speculate that an introgression of pre-adapted Asian alleles could have directly conferred adaptive traits, like cold resistance, to the introgressed populations.

Admixture is also known to have the potential to increase adaptive genetic variation, enabling quick responses to selection. A particularly interesting manifestation of such effects is provided by transgressive segregation (Rieseberg et al, 1999). This term refers to the occurrence of extreme phenotypes in hybrids far beyond each parental population. The degree of transgression is positively correlated with genetic distance between hybridizing lineages (Stellkens et al, 2009). This in turn implicates a particular evolutionary potential to the introduction of divergent Asian genetic material into the invasive European range. The possible evolutionary significance of Asian genetic material is additionally supported by its limited introgression into native European populations. East Asian alleles could have followed two routes into Europe, south and north of the Caspian Sea. However, significant introgression into the native Southern range is not observed. Asian mitochondria are completely absent from the Southern range and nuclear alleles appear to occur only at a very limited extent. A similar picture emerges for admixture of inner European lineages, which is largely limited to the spider's invasive range (see Figure 1.2, 1.4 & 1.5). This observation is well supported by recent results, showing that selection acts against invasion of maladapted alleles entering the range of locally adapted populations (Nolte et al, 2006; Verhoeven et al, 2011). In a previously unoccupied ecosystem, on the other hand, novel variation can be advantageous and admixture is not prevented.

The introgression of Eastern Palearctic alleles into Northern Europe has apparently occurred via Russian steppe populations. We detect the first Asian haplotype in a Russian specimen from 1902. Around 1930, Asian mitochondria appear in Northern European populations in Berlin for the first time. However, we do not observe a simple replacement

of Northern European populations by better adapted Russian ones, as has been argued for other recent evolutionary responses (Hansen et al, 2012). Our microsatellite analysis shows that the invasive European spiders are much more closely related to their Southern European relatives than to the Russian steppe populations (Figure 1.7).

The introgression of nuclear alleles from Asia appears to have been stronger than that of mitochondrial haplotypes. This is particularly evident in central Asian populations with no Asian mitochondria, but a high nuclear Asian background (see Figure 1.2 & 1.5). The observed bias towards reduced mitochondrial introgression might indicate a selective disadvantage of Asian mitochondria in a Western Palearctic nuclear background. Interspecific genetic incompatibilities are known to evolve between mitochondrial and nuclear genomes (Lee et al, 2008; Burton et al, 2006). This would lead to a tradeoff between adaptive introgression of nuclear alleles and selection against mitochondria. Another explanation for the disparate mitochondrial and nuclear introgression might be male biased gene flow (Croucher et al, 2011; Mao et al, 2010), although there is currently no evidence that the ballooning mode of dispersal could be sex-specific.

Adaptation and phenotypic responses

An invasion of the climatically distinct Northern Continental Europe likely required adaptation to the new environment. In this regard, the differences in thermal preference and tolerance of invasive and native wasp spiders are particularly interesting (see Figure 1.6 b & 1.6 c). These physiological changes indicate a shift in the ecological temperature niche in invasive spiders, possibly the result of adaptation to cooler Continental climate. If there would have been a purely environmentally induced range shift, such physiological changes would not have been observed. Similar intraspecific differences in thermal tolerance have been documented for a variety of taxa (Rehfeldt et al, 1999; Castenholz, 1973). A study on house spiders in Japan (Tanaka, 1996) showed a genetically determined latitudinal gradient in cold hardiness. This gradient must have been established recently and despite high gene flow in this global invasive species. Similar conditions probably apply to the wasp spider.

Our reciprocal transplant experiment shows a complementary association between survival and overwintering locality (see Figure 1.6d). The reduced survival of Mediterranean spiders in a Northern European environment might be caused by lower cold tolerance. The high mortality of Northern European spiders in the Mediterranean, on the other hand, could be indicative of a recently evolved obligate diapause. In spider mites, the occurrence of winter diapausing phenotypes seems to have a simple genetic basis and is determined by a single gene locus (Ignatowicz & Helle, 1986). Evolution of an obligate diapause might thus be an initial adaptation of spiders to cold climates. Young spiders may require frost for normal development, as has been established for Northern European laboratory populations of this species (Zimmer pers. communication). The dependence on cold overwintering conditions is well known from insects, for example the gall rod fly (Irwin & Lee, 2000). Another possible explanation for disturbed overwintering could be a shift in photoperiodic response of Northern European spiders. Such a shift has been shown to be the initial evolutionary response of pitcher plant mosquitoes during a northward range expansion (Bradshaw et al, 2000). As the poleward colonization of the wasp spider is a recent process, we suggest that these adaptations are of recent origin, probably less than 100 years old. In sticklebacks the evolution of thermal tolerance has been shown to evolve within a few generations, if sufficient standing genetic variation is provided (Barrett et al, 2011).

The successful colonization of a new habitat from the range edge is often associated with morphological changes. This is especially true for traits that influence dispersal capability, e.g., wing size in insects or leg length in cane toads (Thomas et al, 2001; Hill et al, 2011; Phillips et al, 2006). In addition to such dispersal-related characters, body size variation is a character of high ecological importance (Millien et al, 2006). Compared to their native Southern European range, invasive wasp spiders are confronted with a much shorter vegetation period. The spring is setting in later and the first autumn cold appears much earlier than in a Mediterranean habitat. The reduced body size of Northern European spiders may thus be a manifestation of a trade off in that Northern spiders have to mature earlier in order to be able to reproduce before the onset of autumn (see Figure 1.6 a). At the same time, Mediterranean spiders can reach much larger sizes and produce more offspring. We visited Northern and Southern European field sites extensively and

found living spiders in the Mediterranean region until early December. In Northern Europe the adult spiders disappear by late September. A reduction in body size seems to be an initial evolutionary response of many species to climate change (Babin-Fenske et al, 2008; Gardner et al, 2011).

Our comparisons with historical samples provide another interesting insight. In parallel with increased genetic variation, a high initial variance of body size is seen in the historical invasive specimens. This increased phenotypic variance could point to the initial formation of a hybrid swarm out of which new lineages have emerged, as it has been shown for hybrid speciation in *Cottus* fish (Stemshorn et al, 2011). Genetic admixture can lead to populations (hybrid swarms) with novel genetic combinations and consequently an increase in phenotypic variance. Within a few decades, a new, possibly optimized phenotype could then have arisen in the spider's invasive range. In fruit flies it was shown that a size gradient could evolve within 30 years (Huey et al, 2000).

It remains to be tested in how far plasticity is contributing to the observed phenotypic differences. Our common-garden set-up rules out environmentally-induced plastic responses for the thermal tolerance and preference tests. Such transplant experiments are generally considered to constitute the best test for genetic adaptation to climatic conditions (Hoffmann and Sgro 2011). But as we used offspring derived from wild-mated females, an influence of maternal effects cannot be excluded at present, although these would likely be genetic as well. A genetic component for the traits studied here has been shown to exist in various species. The genetics of body size differences as well as thermal preference and tolerance traits are well studied in insects (Edgar, 2006, Hoffmann et al, 2002, Hoffmann & Willi, 2008) and there is evidence for a genetic component of body size control in spiders (Higgins, 1992).

The role of other factors on the species´ invasion success

Global warming has enabled various animal and plant species to expand their ranges into higher latitudes. Such spreads, however, are usually limited to regions with suitable climates. In contrast, the wasp spider has expanded its range into a new climate zone and can be found in much colder areas than 100 years ago (Geiser, 1997; Kumschick et al,

2011). In addition, the population density did increase to a near complete coverage of the invaded range, with populations in almost every meadow (personal observation). The spider's spread is thus hard to explain by climate change alone. Apart from an adaptive genetic explanation it was also suggested (Guttmann, 1979; Kumschick et al, 2011) that the increase of fallow land could have opened dispersion corridors for the spider, which were previously blocked by unsuitable habitats like forests. But considering the high dispersal abilities of wasp spiders, this explanation seems less likely. Many spiders are efficient dispersers, capable of covering distances of up to several hundred km by aerial dispersal (Foelix, 2011). Aeronautic behavior has already been examined in the European wasp spider. Although it is not an obligate life history phase (Walter et al, 2005), it seems very common, enabling the spiders to travel several kilometers (Follner & Klarenberg, 1995). The species is present on all Mediterranean islands and shows no signs of genetic isolation between mainland and island populations. We find the same haplotypes on Mediterranean Islands as on the Mainland. Even the Macaronesian islands have been colonized by the spider (Schmidt, 1990). Despite being located several 100 km away from Europe, they show evidence for recent gene flow from the mainland (Krehenwinkel et al, in preparation). In addition, spiders tend to show increased dispersal activity in unstable habitats (Richter, 1970) and invasive wasp spiders primarily colonize fallows, a rather unpredictable habitat. This could also contribute to dispersal propensity. Furthermore, suitable corridors of fallow land were available in many parts of Europe, long before the spider colonized those regions (Geiser, 1997). On the other hand, the rapid and wide-ranging colonization of new habitats corresponds very well to the historical increase of genetic variation at the range edge. We thus postulate that genetic admixture was an important trigger that enabled range expansion.

The secondary contact of different wasp spider populations must have been initiated by significant changes to the environment. Admixture of formerly isolated lineages is associated with the onset of climate change for several species (Crispo et al, 2011). For example, climate change driven hybridization has recently been shown in an American squirrel species (Garroway et al, 2010). Such hybridization could act as an important driver of evolutionary processes (Hoffmann & Sgro, 2011). Interestingly, based on the museum samples, we can narrow down the initial admixture in the wasp spider to

approximately 1930 (see Figure 1.2 inset). This period approximates the initial onset of climate change (Delworth & Knutson, 2000). Additionally, we find a strong increase of this admixture after 1960, the approximate beginning of the currently ongoing global warming (Brönnimann, 2009).

The wasp spider's range expansion does not only fall into a period of increasing climate warming, but into a time of intense general global change. Several historic changes correlate with the initial introgression of Asian genetic material into Russia. By the end of the 19th century, Russia was on the verge of industrialization and traffic throughout the country increasing. By 1901, the Trans-Siberian Railway came into operation, leading from the far eastern Vladivostok to Moscow (Liliopoulou et al, 2005). This railway was intensively used in the Russo-Japanese war (1904-1905) and could have very well transported ballooning wasp spider offspring over considerable distances. In fact, global trade is known as important mediator of spider invasions (Kobelt & Nentwig, 2008).

1.5 Conclusion

We conclude that the northward expansion of the wasp spider is not a simple consequence of moving into increasingly warmer areas due to global climate change. Still, it may have been triggered by global warming, in combination with general human-induced changes that led to contact between long separated lineages. This resulted in a genetic admixture that may have facilitated the necessary adaptive changes to colonize areas that were previously not accessible by this species.

Chapter 2: Phylogeographic surveys of two widely distributed Palearctic spider species highlight the importance of extra-European glacial refugia and reveal a pronounced incongruence between nuclear - and mitochondrial markers

2.1 Introduction

Bear, hedgehog and grasshopper represent the major paradigms of European phylogeography and postglacial recolonizations. Countless studies confirmed three general patterns of genetic differentiation between glacial refugia, which are represented by these species (Hewitt, 1999 & 2000; Taberlet et al, 2002). Each species stands for certain Mediterranean centers of origin and postglacial recolonization routes into Central and Northern Europe. Brown bears recolonized Europe from an Iberian and an Eastern European refugium. Hedgehogs, moved into Central Europe from distinct refugia in Iberia, Italy and the Eastern Mediterranean region. And the grasshopper corresponds to a postglacial colonization from a single Balkan refugium. Iberian and Italian lineages were trapped by the Pyrenees and Alps, respectively (Hewitt, 1999; Schmitt, 2007). While recent work has contributed to a refined picture of European phylogeography (Habel et al, 2005; Provan & Bennett, 2008), the generality of the three paradigms is still widely accepted (Schmitt, 2007). A regional focus is common to most European phylogeographic studies, overlooking the Palearctic distribution of many taxa. In addition, much phylogeographic work solely relies on mitochondrial markers, whose information content can be blurred by demographic processes, selection or PCR artifacts. Paternal gene flow (Mao et al, 2010; Turmelle et al, 2011), infections with endosymbiontic bacteria (Hurst & Jiggins, 2005), selection (Bazin et al, 2006; Rand, 2001) or the amplification of nuclear copies of mitochondrial genes (Song et al, 2008; Bensasson et al, 2001) are known to lead to flawed divergence estimates for mitochondrial data. Consequently, the sole use of mitochondrial DNA as phylogenetic or taxonomic marker has been increasingly questioned in the past years (Ballard et al, 2002). The inclusion of nuclear DNA markers is recommended for a proper phylogeographic reconstruction (Toews & Brelsford, 2012, Ballard & Whittlock, 2003; Munoz et al, 2011).

Spiders are a phylogeographically particularly understudied group of animals. Most phylogeographic work on spiders has been limited to species with small geographic ranges or onto only a fraction of the particular species´ distribution (e.g. Bond et al, 2001; Cooper et al, 2011; Bidegaray Batista et al, 2007; Kuntner & Agnarson, 2011; Garb & Gillespie, 2009; Arnedo & Ferrandez, 2007). This is quite surprising, as more than 300 spider species are known to have a Palearctic or Eurasian distribution (Marusik, 1993).

Here, I present a detailed phylogeographic survey of the wasp spider *Argiope bruennichi*. It has a wide ranging, Palearctic distribution from the Azorean Archipelago and Madeira (Schmidt, 1990) over Europe, Northern Africa and Central Asia to Japan (Guttmann, 1979). The study comprises population samples from all over the species´ range. It is based on mitochondrial COI sequences, nuclear microsatellite markers and SNPs and two nuclear DNA sequences. In addition, I present an analysis of a second spider species, the nursery web spider *Pisaura mirabilis*, which has a similar distribution like *Argiope bruennichi*. I use mitochondrial COI and nuclear 28SrDNA sequences, as well as nuclear microsatellites and SNP markers in Europe-wide samples of the species. I then infer differences and parallels between the two species´ phylogeographic structures, in order to show general features of genetic structure.

The present study was set up for two reasons. First, I present a general phylogeographic framework, highlighting genetic structure, glacial refugia and centers of diversity for the two species. This part of the study highlights the consequences of Pleistocene isolation. Thereby, the studied spiders serve as phylogeographic models for widely distributed Palearctic species. Secondly, I analyze the consequences of recent secondary contact between formerly isolated glacial lineages. Many spiders are efficient dispersers. They are capable of quickly expanding their range by passive aerial migration (Su et al, 2007), possibly by several 100 km in a single generation (Foelix, 2011). This dispersal ability makes a secondary contact of spider lineages from distinct glacial refugia likely. Ongoing global change and human impact on ecosystems will probably even increase the likelihood of contact and hybridization (Crispo et al, 2011). I thus focus the second part of the analysis on contemporary gene flow between refugial areas.

My analysis highlights the importance of extra-European glacial refugia for the wasp spider, with a particular importance of East Asia and the Azores. Moreover, I can infer a

history of extensive gene flow between different glacial lineages in both spider species. Due to their high dispersal ability, secondary contact does not entail the establishment of narrow suture zones (Hewitt, 1999; Taberlet et al, 2002). Instead, different glacial lineages meet and nuclear gene pools are homogenized over huge geographic distances. At the same time, mitochondrial gene flow is severely restricted. Consequently, I find a pronounced incongruence between the phylogenetic signal of mitochondrial and nuclear markers. I speculate that the underlying cause of this incongruence might be strong selection against mitochondrial introgression or resilience of native populations towards immigration by females. Although the analysis shows that mitochondrial markers are very valuable to uncover historical population divisions, they are not sufficient to identify the actual contemporary genetic structure of spider populations.

2.2 Material and methods

Sample collection

This study is partly based on data from the study on a recent range expansion of the wasp spider in Europe (Chapter 1 - Krehenwinkel & Tautz, 2013). While the first study focused on genetic structure and recent adaptations in European populations, I widened the geographic focus here. For this reason, I include several hundred samples from the Macaronesian islands and East Asia and analyzed a new set of nuclear genetic markers for the studied spider populations. Wasp spiders were caught by hand or sweep net between 2010 and 2012. In addition, I include some museum samples from the collections of the Naturkundemuseum in Berlin, the Senckenberg Museum in Frankfurt, the Zoological Museum Alexander König in Bonn and the Chinese Academy of Sciences in Beijing. Overall, the sampling covers the whole distributional range of *Argiope bruennichi*. *Pisaura mirabilis* specimens were collected between May and August 2012 in Germany, Sweden, Lithuania, Latvia, Estonia and the Portuguese island Madeira. I acquired further European *Pisaura* samples from the Natural History Museum Berlin, the Senckenberg Museum Frankfurt and private collections. Sampling locations are plotted in Figures 2.1 and 2.7. All specimens were transferred to 70% ethanol and are currently

stored in the collection of the Max Planck Institute for Evolutionary Biology in Plön, Germany.

Molecular analysis

Sampling locality maps were created with GenGIS (Parks et al, 2009). Details on DNA methods are presented in chapter 1. I sequenced 1200 bp of the mitochondrial COI gene and two random nuclear fragments of ~350 bp for *Argiope bruennichi*. In addition, I genotyped a selection of wasp spider samples for 15 nuclear microsatellites and a set of five nuclear SNP loci (SNP markers SA16, 26, 23, 19 & 8 from Krehenwinkel & Tautz, 2013). A ~650 bp fragment of the mitochondrial COI gene was amplified for all *Pisaura mirabilis* specimens using standard barcoding primers (Folmer et al, 1994). Moreover, I sequenced an 850 bp fragment of the 28SrDNA (28Srd4.8a–28Srd7b1, Schwendinger & Giribet, 2005), four random nuclear DNA fragments of ~200 bp each and genotyped 13 nuclear microsatellite loci for the *Pisaura mirabilis* samples.

DNA sequences were edited using Codon Code Aligner (Codon Code Corporation, Dedham, US) and SNPs and microsatellite alleles were called using Genemapper (Applied Biosystems, Foster City, US). Sequences were aligned with MEGA (Tamura et al, 2007) under default alignment parameters. One further *Argiope aurantia* sequence was downloaded from Genbank and added to the COI alignment as outgroup for *Argiope bruennichi*. Neighbor joining trees were constructed in MEGA for the COI sequence alignments. Next to the phylogenetic trees, I generated median joining haplotype networks of the mitochondrial and nuclear sequences, using the software Network (Fluxus Technology Ltd, Suffolk, England). Nucleotide and haplotype diversity were estimated for the mitochondrial and nuclear sequences, using DnaSP (Librado & Rozas, 2009). I generated distance matrices for the microsatellite data (Nei´s genetic distance) using the software microsatellite analyzer (MSA) (Dieringer & Schlötterer, 2003). The PHYLIP package (Felsenstein, 1989) served to construct neighbor joining trees, based on the according matrices. SNP genotypes were edited by hand and allele frequencies for different populations calculated.

2.3 Results

In the following, I present the results for *Pisaura mirabilis* and *Argiope bruennichi* separately, beginning with the latter species.

Mitochondrial data

I sequenced 1200 bp of the mitochondrial COI gene for 786 *Argiope bruennichi* specimens. In addition, I included one Genbank sequence from the wasp spider's sister species, the Nearctic *Argiope aurantia*. I identified three distinct mitochondrial lineages within the Palearctic region, one in East Asia (Japan and Eastern China), one on the Azores and one in Europe, Northern Africa and Central Asia (referred to as European in the following text). Each of these lineages is divergent by roughly 1.6 % from the other and by 8% from the outgroup species *Argiope aurantia* (Figure 2.2). The mitochondrial data thus shows clearly defined ranges for East Asian, European and Azorean haplotypes. No mitochondrial introgression between these regions was found. In the following I describe phylogenetic networks and within lineage divergence for the three Palearctic wasp spider clades (Figure 2.2).

1. The Eastern Palearctic comprises several independent haplotype radiations, each divergent by five to seven mutations from the other. All sequences converge to a hypothetical ancestral sequence, which is giving rise to several haplotypes with distances of two to three substitutions. Several rare and distantly related haplotypes stand basal to most radiations.

2. The European region is distinct from East Asia by shallower phylogeographic splits. I identified four independent radiations, each just distinct by two nucleotide substitutions. Most derived haplotypes are distinct by just one substitution.

3. Madeiran populations exclusively harbor one single European haplotype. I only find the basal form of this haplotype without any derived sequences. Madeiran populations are thus completely monomorphic on the mitochondrial level.

4. The Azores are distinguished by a rather low phylogeographic divergence. I find two haplogroups (see Figure 2.2), separated by just one mutation. In comparison to Europe or East Asia, I generally find fewer derived haplotypes on the Azores.

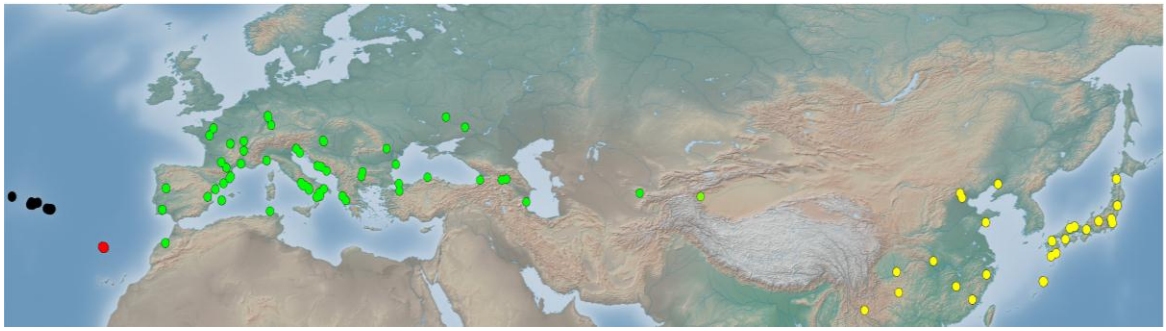

Figure 2.1 Sampling location for populations of the wasp spider *Argiope bruennichi*, which were used in this study. The colors correspond to genotypic clusters I identify, based on mitochondrial and nuclear genetic data.

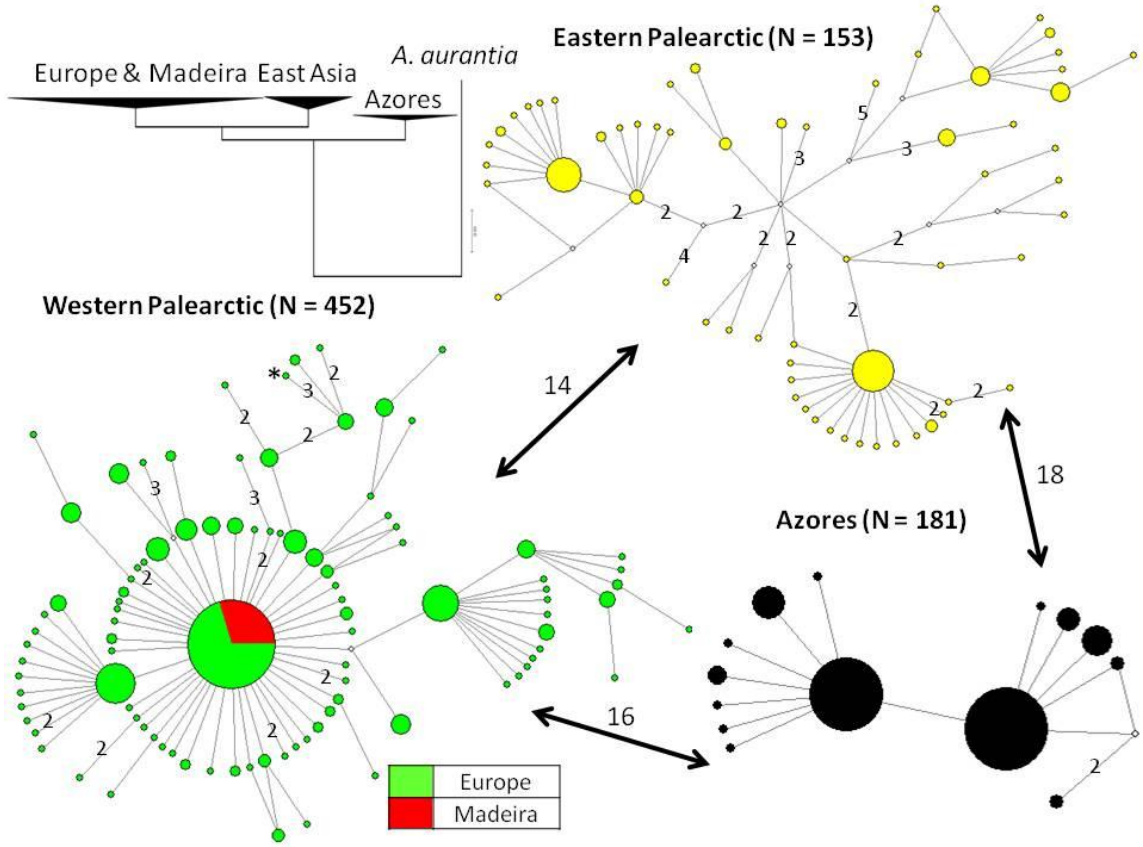

Figure 2.2 Mitochondrial haplotype structure for Palearctic wasp spider populations, based on ~1200 bp of the COI gene. In the upper left, I show a pruned neighbor joining phylogeny for all mitochondrial sequences, rooted using the wasp spider´s sister species *Argiope aurantia*. Each haplotype network shows genetic structure for one of the three major Palearctic mitochondrial clades. If not denoted by numbers, branches on the network signify a single substitution. Colors correspond to those on the sample map in Figure 2.1. Numbers on arrows correspond to the minimal number of mutation to connect networks. A black star on the upper part of the Western Palearctic network denotes the connection of the outgroup species *Argiope aurantia* to the *Argiope bruennichi* network.

Nuclear microsatellites - and SNP loci

I genotyped the Palearctic specimens for a set of 15 microsatellite loci. However, I encountered a large problem with allelic dropout for Eastern Palearctic specimens. Many markers did not even amplify in a PCR for these populations. Consequently, I limited the microsatellite analysis to populations from the Western Palearctic region and included only few properly genotyped East Asian samples as outgroup.

I generally find little differentiation over very large geographic regions. Only East Asian and European populations form largely separated clades. However, a few European specimens group into the East Asian cluster. In the whole Western Palearctic region, I find only two monophyletic populations, one on Madeira and one on the Azores, but both are paraphyletic within the European mainland populations (Figure 2.3). This close nuclear genetic relationship of Azorean and European spiders is quite contrary to the clear monophyly I observe in the mitochondrial dataset (see Figure 2.2).

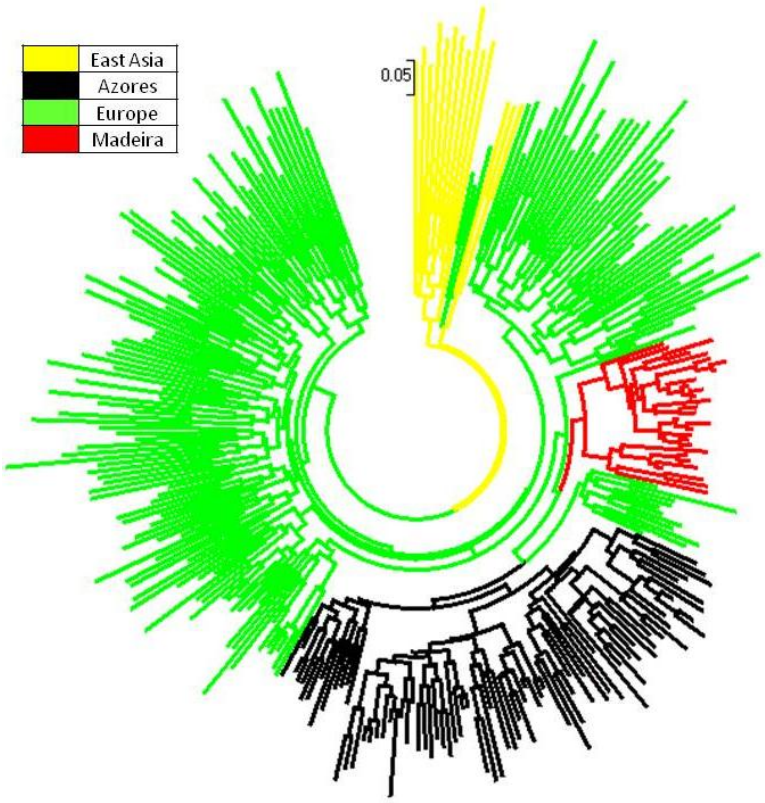

Figure 2.3 Allele sharing tree for Palearctic wasp spiders, based on 15 microsatellite loci. The tree is rooted using East Asian outgroup specimens. Azores and Madeira form monophyletic groups, but render European specimens paraphyletic with respect to them. Colors correspond to those on the sample map in Figure 2.1.

As I refrained from microsatellite genotyping for most East Asian samples, I use a dataset of five diagnostic nuclear SNP loci to identify gene flow between Asian and European populations (Chapter 1 - Krehenwinkel & Tautz, 2013). I genotyped 310 specimens from all over the Palearctic region for these loci. The SNP data largely confirms the

microsatellite based phylogeny, with a genetic cluster in East Asia and one in the Western Palearctic (Figure 2.4). Azorean and Madeiran populations carry the same alleles as European mainland spiders. The Azores however, are distinguished by a unique mitochondrial haplotype. Asian nuclear alleles enter the European gene pool via Central Asia and Russia. The difference of mitochondrial and nuclear introgression is quite pronounced. For example are Central Asian populations completely fixed for western mitochondria despite a high amount of Asian allelic background (33 %). And Russian spiders carry less than one percent of Asian mitochondria, but harbor many nuclear alleles from Asia (26 %). Asian introgression is largely limited to these two regions. It is much less evident for Southern Europe (12 %) as well as the Macaronesian islands (5 % on the Azores and 10 % on Madeira). Moreover, introgression appears to be unidirectional, with few European alleles entering East Asia (2% European background in China and 1% in Japan).

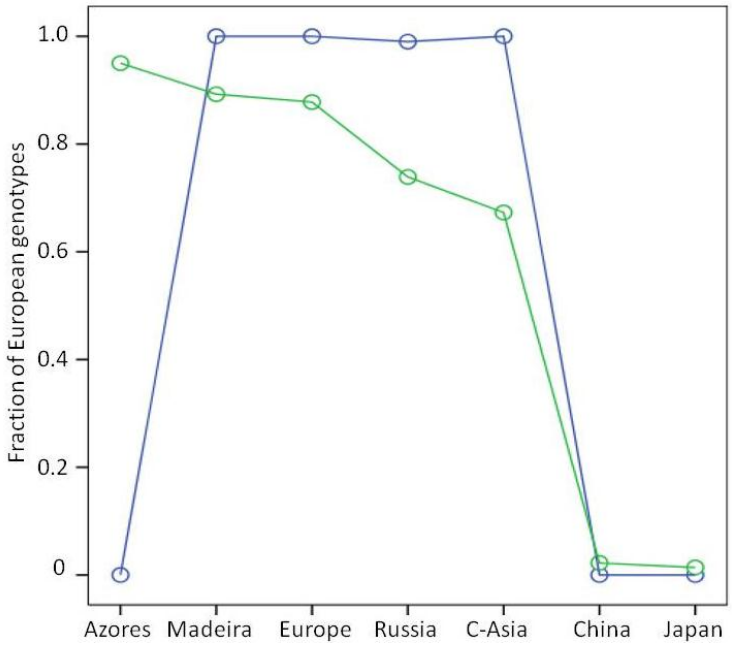

Figure 2.4 Fraction of European genotypes for wasp spider populations from different Palearctic regions. The *blue* dots depict the fraction of European mitochondrial haplotypes and the *green* ones are based on five nuclear SNP loci. I find a clear increase of Asian alleles, when moving towards the Eastern Palearctic, while mitochondria follow a step cline without any introgression. Azorean populations share the same nuclear alleles with European ones, but are fixed for a different mitochondrial haplotype.

Nuclear DNA sequences

In addition to the SNP genotyping assay, I sequenced two nuclear fragments of ~350 bp each for 150 samples from the Azores, Madeira, Western Europe and East Asia (Figure 2.5). The nuclear DNA networks generally confirm the microsatellite analysis. Europe, Madeira and the Azores largely share the same alleles. The network analysis shows these

alleles to be derived from an East Asian ancestral sequence by just two to three mutations. This result is again very different from the mitochondrial data. Populations from Europe, the Azores and Madeira are distinguished by genetic homogeneity. They carry very few, slightly derived alleles, originating from a widely distributed ancestral sequence. Spiders from East Asia harbor a huge variety of distantly related sequences for these nuclear markers. Interestingly, the two sequenced fragments are completely divergent between East Asia and Europe. Introgression is not evident for the sequenced samples. However, I did not sequence Russian specimens for these markers, which might show a certain amount of Asian alleles.

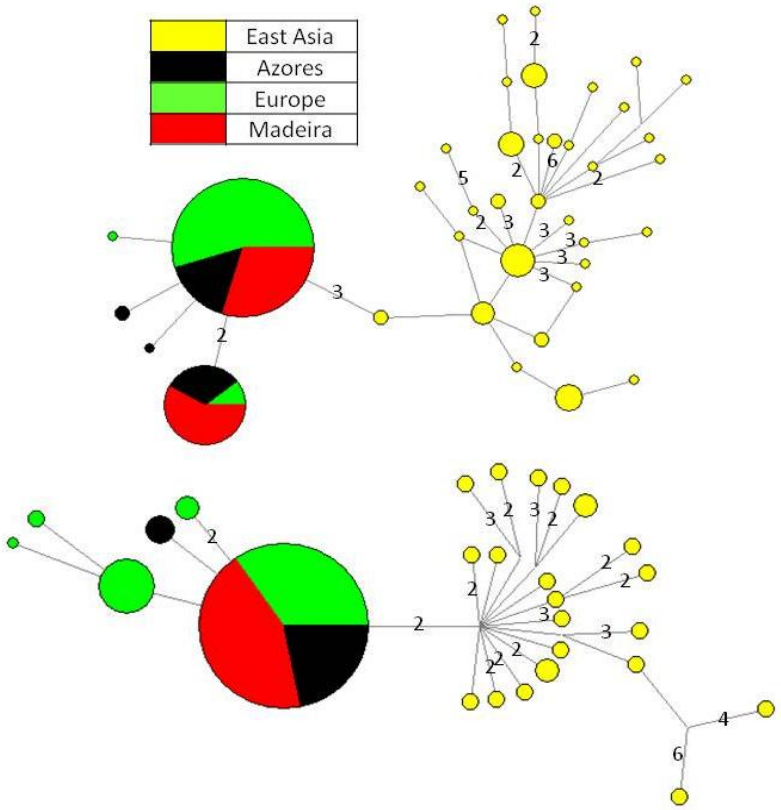

Figure 2.5 Median joining networks for Palearctic wasp spiders, based on two nuclear sequences. While East Asian spiders form a diverse and separate genetic cluster, European, Madeiran and Azorean spiders are genetically more homogeneous and share the same nuclear alleles. If not denoted by numbers, branches on the network signify a single substitution. Colors correspond to those on the sample map in Figure 2.1.

Genetic diversity

I find quite contrasting diversity measures between mitochondrial and nuclear markers as well as between different Palearctic populations of the wasp spider (Figure 2.6). Nucleotide diversity is highest in Eastern Palearctic populations (nuclear $\pi = 0.0116$ and mitochondrial $\pi = 0.0037$). This difference is particularly pronounced for nuclear DNA markers, whose diversity is several-fold higher compared to the Western Palearctic and to

the East Asian mitochondrial diversity. European, Madeiran and Azorean populations are not significantly different in their nuclear genetic diversity (π = 0.0012, for Europe, Madeira and Azores). On the other hand, mitochondrial variation is significantly lower in Madeiran (π = 0) and Azorean (π= 0.0005) populations than in Europe (π = 0.001). This is especially evident on Madeira, where I find only one single mitochondrial COI haplotype (significant differences were identified using an ANOVA, Tamhane posthoc test, $p < 0.05$).

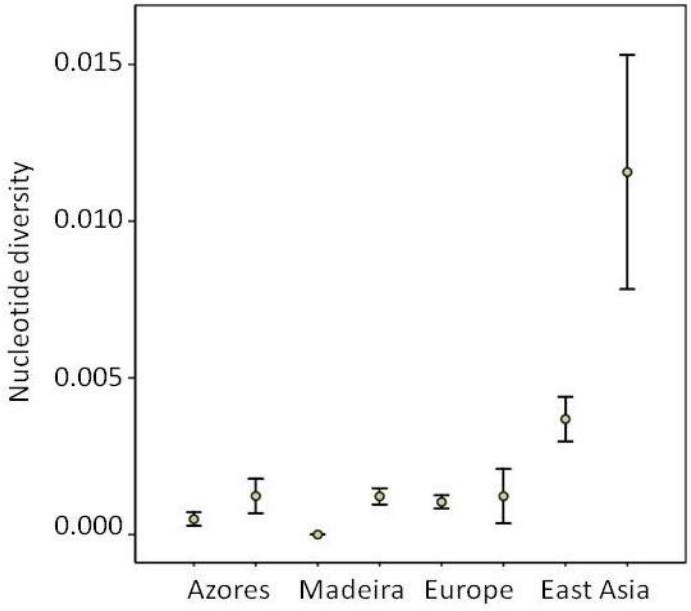

Figure 2.6 Mean nucleotide diversities for the mitochondrial COI gene and two nuclear DNA fragments for Palearctic wasp spiders. I plotted data for Azorean, Madeiran, European and East Asian populations. For each of these geographic regions, the left bar represents mitochondrial and the right one nuclear diversity. Bars depict the 95 % confidence intervals of the mean. I find significant differences between mitochondrial diversity of East Asian, European and Macaronesian populations. Nuclear diversity is only significantly different in East Asia. (ANOVA, Tamhane posthoc test, $p < 0.05$).

The nursery web spider *Pisaura mirabilis*

I sequenced 650 bp of the mitochondrial COI gene for 138 European *Pisaura mirabilis* specimens. The mitochondrial haplotype network reveals a much deeper genetic differentiation over shorter geographic distances than in *Argiope bruennichi* (see Figure 2.7 & 2.8). European nursery web spider populations are split into three geographically separated mitochondrial lineages, different by 4.3 - 5.1 % sequence divergence. It is possible to distinguish a Southwestern lineage from Madeira and Spain, one from the Balkans and one from Northern and Central Europe. I will refer to these lineages as the Southwestern, Balkan and Central one. The three clades are well separated from each other. Only the Central group carries 3.6 % of introgressed haplotypes from the Southwestern one (Figure 2.8). With a nucleotide diversity of 0.006-0.009, European

Pisaura mirabilis populations are rather diverse in comparison to wasp spiders (mitochondrial $\pi = 0.001$).

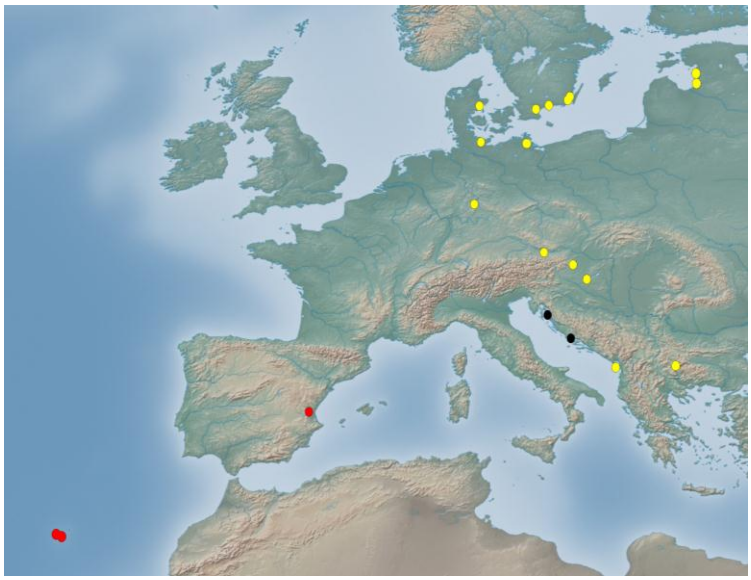

Figure 2.7 Sampling locations for the studied nursery web spider (*Pisaura mirabilis*) populations. The colors represent the three identified mitochondrial clades.

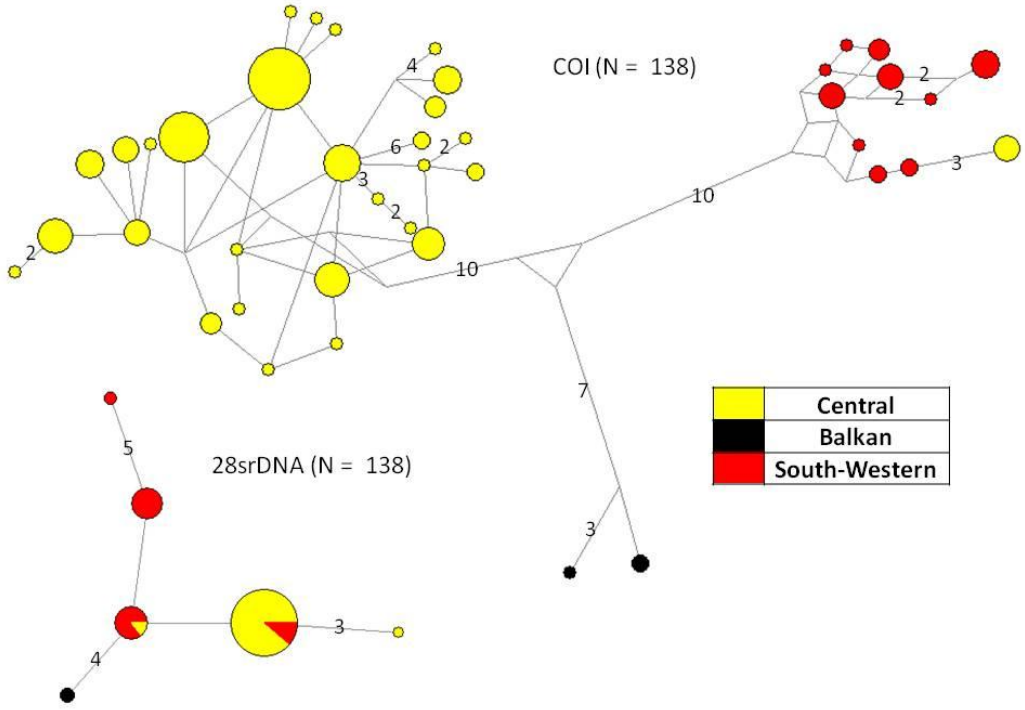

Figure 2.8 Median joining haplotype networks for ~1000 bp of the mitochondrial COI gene (upper network) and the nuclear 28SrDNA gene (lower network). Colors represent the collection sites from Southwestern Europe (red), the Balkans (black) or Central - and Northern Europe (yellow). Both COI and 28S networks indicate the existence of several divergent genetic lineages within Europe. Different populations share a considerable fraction of alleles. If not denoted by numbers, branches on the network signify a single substitution. Colors correspond to those on the sample map in Figure 2.7.

The nuclear genetic analysis for *Pisaura mirabilis* generally confirms the mitochondrial data. The microsatellite analysis uncovers the aforementioned three genetic groups in Europe. However, only the Balkan specimens seem to be clearly isolated from the other European populations (see Figure 2.9). They form a deeply split clade on the microsatellite allele sharing tree and carry private alleles in the 28SrDNA gene as well as the analyzed four nuclear DNA fragments (see Figure 2.8, 2.9 & 2.10). Southwestern and Central populations are also forming largely distinct genetic clusters, according to the microsatellite data (see Figure 2.9). However, their differentiation in the allele sharing tree is much shallower than that of the Balkan clade. Moreover, the 28SrDNA and nuclear fragments indicate a considerable fraction of shared alleles between the two groups (Figure 2.8 & 2.11).

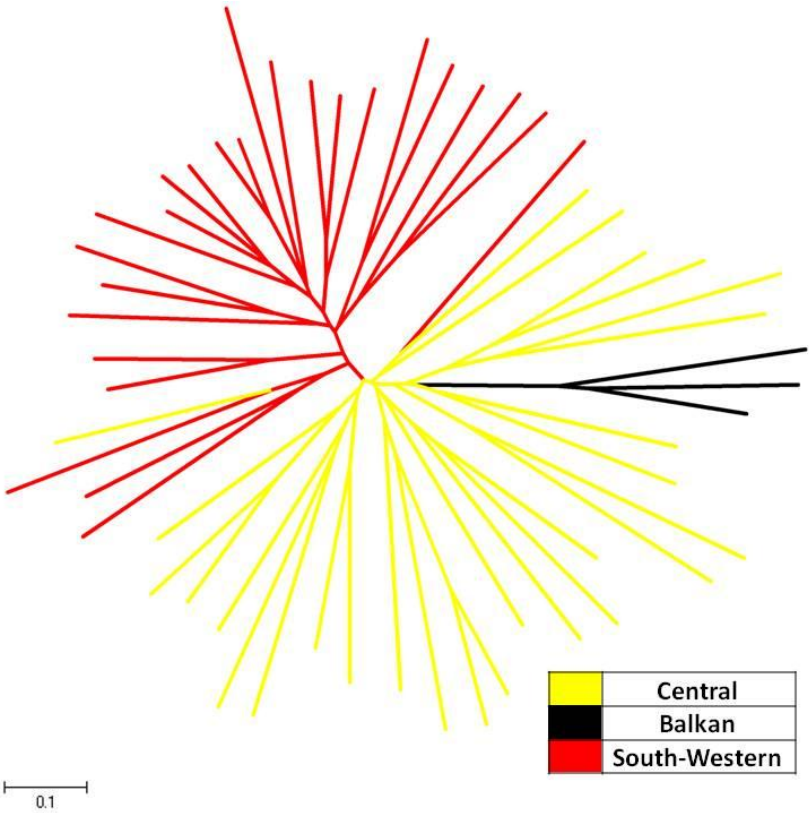

Figure 2.9 Allele sharing tree for European *Pisaura mirabilis* samples, based on 13 nuclear microsatellite loci. The colors correspond to sampling locations in Central - and Northern Europe (yellow) the Balkans (black) and South-Western Europe (red). Colors correspond to those on the sample map in Figure 2.7.

All four examined nuclear loci allow the identification of divergent alleles for the Central- and South-Western groups (Figure 2.11a), but only few are clearly fixed

between regions. On average, South-Western and Central European nursery web spiders are distinct by 1.7 % nuclear distance (4.4 % mitochondrial distance). Balkan and South-Western samples are distinct by 3.1 % at the nuclear level (5.1 % mitochondrial distance). Central and Balkan nuclear alleles are on average divergent by 3.6 % (4.3 % mitochondrial distance) (Figure 2.11a).

By scoring the frequencies of divergent nuclear alleles between South-Western and Central clades, I find an average introgression of 18 % South-Western alleles into the Central genepool, but only 1 % Central alleles in South-Western populations. As for the mitochondrial data, introgression is thus largely unidirectional (Figure 2.11b).

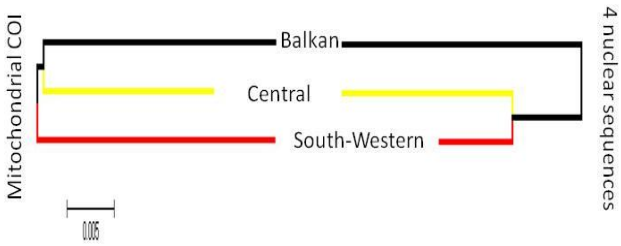

Figure 2.10 These neighbor joining trees depict the general phylogenetic structure for European *Pisaura mirabilis* populations as found for the mitochondrial COI gene (~650 bp, left tree) and four random nuclear DNA sequences (~800 bp, right tree). The trees were midpoint rooted. Colors represent the Balkan, Central and Southwestern clades. Although the tree topology is not identical, the three general geographic groups are clearly recovered using both marker types. Colors correspond to those on the sample map in Figure 2.7. The scale bar shows uncorrected, pairwise genetic distance.

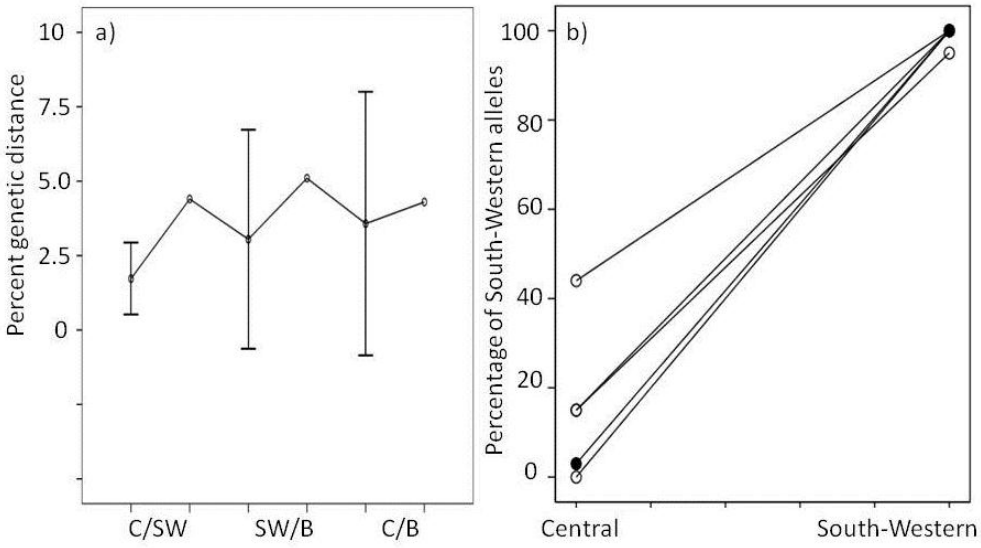

Figure 2.7 a) depicts the genetic distance between Central and South-Western, (C/SW), South-Western and Balkan (SW/B) and Central and Balkan (C/B) populations of *Pisaura mirabilis.* The bars to the right represent the average distance for four nuclear sequences of ~200 bp each, while the single dot shows mitochondrial distances. The bars represent the 95 % confidence interval of the mean. b) shows the percentage of presumably South-Western European alleles in South-Western and Central European populations, respectively. Mitochondrial data is shown in black, that for the four nuclear SNP loci in white.

2.4 Discussion

In the following, I first discuss the results for the wasp spider *Argiope bruennichi*. Then, I compare the findings to the pattern observed in the nursery web spider *Pisaura mirabilis*.

Pleistocene isolation and postglacial recolonization in *Argiope bruennichi*

The mitochondrial phylogeny indicates the existence of three distinct glacial refugia, one in East Asia, one in Europe and one on the Azores. The genetic distance between these regions corresponds to roughly 1 million years of divergence time (Knowlton & Weigt, 1998), agreeing with a Pleistocene isolation of lineages. Nearly trichotomous splits between the three regions and very similar genetic distances between them suggest a single isolating event (Hung et al 2012). A high genetic diversity indicates the existence of a large glacial refugium in the Eastern Palearctic. East Asia was not affected as much by the Pleistocene glaciations as the Western Palearctic (Adams, 2002). This could have enabled the persistence of large populations during glacial periods. The deep divergence between mitochondrial radiations in East Asia even hints at the presence of several refugia in this region, as has been suggested for e.g. hares (Nunome et al, 2010) or bats (Flanders et al, 2011). Large stretches of tropical grassland dominated the landscape of Southern China during the last glacial maximum (Ray & Adams, 2001). And a simulation of suitable habitat for Palearctic Rosefinches during the last glacial maximum shows much larger refugial areas in East Asia than in Europe (Hung et al, 2013). East Asia thus possibly served as important Pleistocene refugium for many plant and animal species.

Western Palearctic wasp spiders, probably survived the glaciations in small bottlenecked populations, as evident by their reduced diversity. Europe offered rather unsuitable conditions for the spiders during the last glacial maximum. It was covered by large ice sheets or cold forest steppe habitat. This habitat was connected to the Saharan desert in the South (Ray & Adams, 2001). European wasp spider populations were thus trapped between two hostile environments and possibly went extinct on most of the continent. A likely refugium for European spiders is found in the south of the Caucasus Mountains (Krehenwinkel & Tautz, 2013). This assumption is supported by previous work, showing

the Caucasus region as an important center of endemism and possible glacial refugium (Hung et al, 2012).

The island of Madeira was probably colonized in the course of the last postglacial expansion into Europe. The colonization was accompanied by a pronounced founder effect, leading to the high mitochondrial homogeneity on Madeira. While the species went extinct in most of Europe and Madeira during the Pleistocene glaciations, it probably survived on the Azores. *Argiope bruennichi* can be found virtually everywhere and in huge populations sizes on the Azorean archipelago. This finding is contrary to many studies, which emphasize the importance of Madeira and the Canaries as Macaronesian centers of endemism for other species (Carine & Schaefer, 2010; Amorim et al, 2012; Cameron et al, 2006). However, the comparatively low measures for mitochondrial nucleotide diversity indicate a recent, postglacial bottleneck of Azorean mitochondria (Avise, 2001), similar to Madeira.

To summarize, deep mitochondrial divergence between East Asia, Europe and Azores indicate the importance of Pleistocene glaciations for a long isolation of different wasp spider populations. However, this phylogeographic framework is only partly corroborated by an analysis of nuclear markers. In the following, I discuss possible causes of this incongruence.

Secondary contact, differential introgression and limits to gene flow in a highly dispersive species

Argiope bruennichi is a high gene flow species, which can cover large distance by passive, wind mediated dispersal (Follner & Klarenberg, 1995). Their dispersal ability allows the spiders to quickly expand their range in the limits of their ecological niche, as evident by a current range shift into Northern Europe (Kumschick et al, 2011). In this regard, secondary contact of formerly isolated spider populations during their postglacial range expansion is very likely. And indeed, I find signatures of extensive gene flow between glacial lineages. Little genetic structure over geographic distances of several hundred km and even over huge oceanic barriers underlines the efficient mode of ballooning dispersal.

East Asian and European populations meet and hybridize in Central Asia. Introgression is mostly unidirectional and limited to Asian nuclear alleles, entering the Western gene pool. From Central Asia, Asian alleles move into Russian populations north of the Caspian Sea. The area of admixture spans several thousand km, with no evidence for a clear contact zone, as they have been described for numerous other species (Hewitt, 1999; Taberlet et al, 2002). The ballooning mode of dispersal rather entails the establishment of broad areas of admixture. Interestingly, introgression is predominantly limited to nuclear genes. Mitochondrial introgression is mostly absent. While Central Asian and Russian populations carry a substantial amount of East Asian nuclear alleles, they are largely fixed for Western mitochondria. An even more extreme case of such differential gene flow involves the Azorean archipelago. Here, I find a unique and deeply divergent mitochondrial haplogroup, but largely shared nuclear alleles with the European mainland. In the following, I discuss possible reasons for such nuclear mitochondrial discordance.

1. Paternal gene flow, as e.g. found in bats is a common explanation (Mao et al, 2010; Turmelle et al, 2011). Ongoing nuclear gene flow would then contribute to genetic homogenization, despite mitochondrial differentiation. Published work on Southern German wasp spider population indicates a high dispersal rate of all offspring from a wasp spider eggsac, regardless of sex (Follner & Klarenberg, 1996). But dispersing females could suffer from a reduced reproductive success in established populations, leading to quick loss of their mitochondrial lineages. This explanation is not unlikely, considering the wasp spider's life cycle. Females of the species grow much larger and are longer-lived than males. Consequently, they require access to more resources in a habitat and are prone to longer episodes of selection during their life cycle. Established wasp spider populations could thus be resilient against introduction of new females by long range dispersal.

2. Interspecific incompatibilities between male and female reproductive organs constitute another possible reason for differential gene flow between mitochondrial and nuclear genes. Spider genital morphology can evolve quickly and is highly species specific, efficiently preventing interspecific mating (Kraus, 2000). It has been suggested, that these reproductive barriers could act unidirectional, allowing females of one spider species to

mate with males of the other, but not the other way round (Croucher et al, 2004). However, I would expect mitochondrial introgression happening in at least one direction, e.g. from Europe into Asia, if the isolation is unidirectional in this way, but this is not observed.

3. Selection acting on mitochondria constitutes a third possible explanation for the observed incongruence. Contrasting diversity and divergence patterns between mitochondrial and nuclear DNA indicate that mitochondria might be affected by natural selection. Admixture could be coupled with purifying selection on mitochondrial lineages or the evolution of mitochondrial lineages speeded up due to positive selection and recurrent sweeps. The incongruent pattern of nuclear and mitochondrial differentiation on the Azorean Archipelago, in Russia and Central Asia might be explained by gene flow and following loss of one mitochondrial lineage. The near complete loss of one mitochondrial haplotype after secondary contact is not unusual. Many studies have shown strong mitochondrial gene flow or even complete mitochondrial replacement (e.g. Martinsen et al, 2007; Chen et al, 2009; Nevado et al, 2009; Wilson & Bernatchez, 1998; Renoult et al, 2009; Melo-Ferreira et al, 2005; Gantenbein & Largiader, 2002). Selection on mitochondria can have several causes, which I discuss in the following.

3.1 Cytoplasmic incompatibility, induced by different strains of bacteria, e.g. *Wolbachia* spp. is one possible reason (Hurst & Jiggins, 2005; Toews & Brelsford, 2012). The observed pattern is not a case of actual selection on mitochondria, but a consequence of mitochondria, being inherited along with the bacterial lineages. Invasions by such reproductive parasites can induce recurrent sweeps of mitochondria and speed up evolution of a mitochondrial lineage, compared to the nuclear genome. The contrasting mitochondrial and nuclear pattern of divergence for Azorean populations could be induced by such an infection. By accumulating mutations at a faster rate, the Azorean mitochondrial genome would be divergent from mainland populations, while nuclear markers could be freely exchanged. But the nearly trichotomous pattern of mitochondrial divergence and similar genetic distances between Azorean, East Asian and European populations contradicts this assumption. It rather indicates a single incident during the Pleistocene as isolating agent, possibly glaciations (Hung et al, 2012).

In a PCR based assay, I did not find evidence for *Wolbachia* infection for the examined populations (unpublished data). Other studies confirm these results, showing no infection with the endosymbionts *Wolbachia* and *Cardinium* in *Argiope bruennichi* in Europe (Duron et al, 2008a) and *Wolbachia* in East Asia (Yun et al, 2011). A test for the presence of a wide range of reproductive parasites in the congeneric species *Argiope lobata* did not uncover any infections (Duron et al, 2008b). So far, there is no published evidence for an infection with reproductive parasites in the genus *Argiope*. Although I currently can not rule out an unknown reproductive parasite causing mitochondrial sweeps, other reasons seem more likely.

3.2 Incompatibilities between nuclear and mitochondrial genomes are assumed to contribute to reproductive isolation (Lee et al, 2008; Burton et al, 2006; Hutter & Rand, 1995). Such incompatibilities could have evolved between different glacial refugia and prevent the interaction of e.g. Asian mitochondria and a European nuclear background. Secondary contact of these genetic clusters would lead to limited introgression or sweeps of one mitochondrial lineage, as observed in e.g. Central Asian or Azorean populations. The probability of being a worse maternal parent in a hybrid cross is linked to increased mitochondrial substitution rate (Bolnick et al, 2008). Interestingly, I find that usually mitochondria from the less diverse populations are fixed during secondary contact in wasp spiders. A more detailed sequence analysis of, e.g., Azorean mitochondrial genes and their nuclear interaction partners will help elucidating this issue.

3.3 A contribution of mitochondria to adaptation to environmental conditions, e.g. climate (Balloux et al, 2009; Mishmar et al, 2003; Melo-Ferreira et al, 2005; Doiron et al, 2002; Bazin et al, 2006; Rand, 2001), is another possible explanation for mitochondrial sweeps. If mitochondria contribute directly to adaptation, divergence of haplotypes would occur despite ongoing nuclear gene flow.

The phylogeography of *Pisaura mirabilis* - differences and similarities with *Argiope bruennichi*

The phylogeographic analysis for the nursery web spider *Pisaura mirabilis* reveals clear differences, as well as surprising similarities with that of *Argiope bruennichi*.

The high genetic variation within all European *Pisaura mirabilis* populations indicates comparably large glacial refugia and no pronounced loss of diversity during the ice ages.

Moreover, the very similar mitochondrial divergence between all three European lineages suggests a single isolating event during the Pleistocene. Based on these data, one can speculate about the existence of a Western- and Eastern Mediterranean refugium for the Southwestern and the Balkan lineage, as they have been described for many plant and animal species (Hewitt, 1999; Taberlet et al, 2002). Central populations might have survived the glaciations in more eastern regions of Europe, possibly even in Asia. This phylogeographic difference to *Argiope bruennichi* is surprising. Both species occur in very similar habitats and are often found synanthropically. However, *Pisaura mirabilis* is distributed far more to the north (Platnick, 2013), suggesting that it is more tolerant towards cold temperatures and should consequently have better survived the European glaciations. Even if species share a similar distribution and ecology, one should consequently be careful to generalize phylogeographic patterns. Even subtle ecological differences can lead to rather different genetic structure.

Despite these major differences, I still find one important similarity between the wasp spider´s and nursery web spider´s phylogeographies. The incongruence between mitochondrial and nuclear markers in *Pisaura mirabilis* is comparable to that observed for *Argiope bruennichi* populations from the Azores, Europe and East Asia. The deep divergence in the COI gene suggests an isolation of several million years for refugial lineages in *Pisaura* (Knowlton & Weigt, 1998). In the course of their postglacial northward movement, these lineages probably established secondary contact. Reproductive isolation was only completed for the Balkan populations, which retain their genetic distinctness and are thus probably a different species. The current Central and South-Western European populations however, started to exchange genetic material. As for *Argiope bruennichi*, the ballooning mode of dispersal probably prevents the establishment of stable contact zones and leads to the emergence of broad geographic areas of admixture. The observed lack of mitochondrial introgression is most likely explained by a selection against mitochondria or resilience of populations against female colonizers.

I interpret the observed divergent nuclear genetic lineages as evidence for a glacial isolation. However, incomplete lineage sorting might be another explanation for this divergence in *Pisaura mirabilis*. But considering the deep mitochondrial splits (Figure

2.8 & 2.11) and the clear divergence of nuclear fragments (Figure 2.10 & 2.11), incomplete sorting of nuclear alleles is unlikely.

2.5 Summary

The present study highlights the importance of extra-European glacial refugia for a thermophilic Palearctic species. East Asia probably offered the largest area of suitable habitat during the Pleistocene and thus serves as the most important source of unique genetic variation in the wasp spider. Consequently, a range-wide sampling is inevitable for a proper phylogeographic reconstruction of widespread species. In contrast, the more cold tolerant *Pisaura mirabilis* did survive the glaciations in several refugia in Europe.

Due to their efficient mode of passive aerial dispersal, the studied spiders are capable of very high gene flow, leading to homogenizations of nuclear gene pools over huge distances. However, mitochondrial introgression is nearly absent during secondary contact of glacial lineages. One can assume selection on mitochondria or resilience of populations against introduction of females as most likely reasons for these incongruent patterns of gene flow.

Chapter 3: An assessment of genotyping success in museum specimens identifies unexpected predictors of DNA integrity and shows an historical increase in genetic diversity in an expansive spider species

3.1 Introduction

Natural history museums provide a rich source for historical DNA studies. DNA from museum specimens can be used for taxonomic assignments (Hajibabaei et al, 2006; Puillandre et al, 2012), phylogenetic reconstructions (Houde & Brown, 1988; Cooper et al, 2011), conservation biology (Paplinska et al, 2010) or to trace historical genetic changes in populations (Lister et al, 2011; Wandeler et al, 2007). However, molecular work on museum specimens is not unproblematic. When natural history collections were set up and until a few decades ago, the importance of molecular studies was still unforeseen. Storage conditions aimed for a long term preservation of the specimen's phenotype and not its DNA integrity (Zimmermann et al, 2008). For example, unsuitable preservatives or insecticides dramatically decrease the molecule's lifetime (Espeland et al, 2010). Consequently, DNA from museum specimens is often degraded, broken apart into small pieces and present in much lower concentrations than in fresh samples (Wandeler et al, 2007). Considering the degradation of historical DNA, mitochondrial sequences are usually the preferred genetic marker (Casas-Marce et al, 2010). Each cell has only one nuclear genome, but can contain more than thousand mitochondria (Robin & Wong, 2005). The probability of extracting stretches of well-preserved mitochondrial DNA is thus much higher, compared to unique nuclear sequences. However, the use of nuclear DNA is inevitable, when fast evolving markers are needed to study recent genetic processes, e.g. population subdivisions.

Here, I present a detailed study on the feasibility of PCR analysis of historical DNA from spiders. Natural history museums all over the world house huge arachnological collections, usually stored in Ethanol, a well suited DNA preservative. Consequently, spiders are promising targets for work on historical DNA. I present a comparative study of PCR -and genotyping performance in Ethanol preserved museum specimens of the wasp spider *Argiope bruennichi* (Platnick, 2013). I include several hundred specimens from two large German natural history collections.

I test the amplification success of mitochondrial- and nuclear DNA markers of different fragment length and in relation to collection date and body size of the preserved specimens. Moreover, I discuss the influence of immediate treatment of a sample after collection on its DNA quality.

The results show that molecular work on historical spiders is indeed a promising endeavor, although it is clearly hampered by DNA degradation. Not only the age of a specimen, but the immediate treatment and proper storage after its collection and the specimen´s body size explain most differences in amplification success. In addition, I provide an example for the practical use of historical DNA samples. Using mitochondrial sequences and microsatellite markers, I show that a range expansion of the studied species in Europe is associated with historical admixture and increasing genetic diversity at its range edge.

3.2 Material and methods

Sample preparation and tests for genotyping success

Samples were acquired from two large German natural history collections, the Senckenberg Museum in Frankfurt (182 specimens) and the Naturkundemuseum in Berlin (215 specimens). Selected specimens were examined under a Leica MZ95 binocular and their prosoma width was measured, using a Leica measuring eyepiece (Leica, Wetzlar, Germany). One leg of each specimen was removed with heat sterilized forceps and then kept in pure Ethanol. Laboratory work was carried out at the Max Planck Institute for Evolutionary Biology in Plön, Germany. For DNA extractions, the spider leg was removed from its preservative and allowed to air dry on a piece of tissue paper for a few minutes. Legs were then cut into several pieces with sterile scalpel blades. After being cut down, the tissue was transferred to lysis buffer and disrupted on a Tissuelyser by using 5 mm stain less steel beads (both Qiagen, Hilden, Germany) for 30s at 30 hz. The Archivpure Cell & Tissue Kit (5PRIME, Hamburg, Germany) was used for the DNA extractions according to the manufacturer’s protocol. Modifications of the protocol include the increase of all reaction volumes by ½ and the substitution of glycogen solution (20 mg/mL, 5PRIME, Hamburg, Germany) as a DNA carrier during

Isopropanol precipitation. Moreover, I used only between 20 µl of hydratation solution (10 mM Tris, 1 mM EDTA, pH 7–8) to eventually dissolve the DNA pellet. A negative control extraction was added. Work with museum specimens was carried out in a different room than that on contemporary specimens to avoid DNA carryover.

Primer design was done using the primer 3 software (Untergasser et al, 2007). Primers for the mitochondrial COI gene were designed based on an alignment of 1200 bp of contemporary sequences (see Krehenwinkel & Tautz, 2013) and targeting two distinct fragment sizes of 130 and 350 bp. The respective PCR fragments both contained informative SNPs to distinguish the major mitochondrial haplogroups within the wasp spider. In addition to the mitochondrial DNA, I targeted four nuclear microsatellite fragments, two of approximately 150 and two of 250 bp, respectively (Primers MA53, MA55, MA56 & MA60 from Krehenwinkel & Tautz, 2013).

PCRs were run with 1 µl of the DNA extract on an Applied Biosystems Veriti Thermal Cycler (Applied Biosystems, Foster City, US), using the Qiagen Multiplex PCR kit (Qiagen, Hilden, Germany) with 40 cycles, according to the manufacturer's protocol. A negative control reaction was added to each PCR. Positive PCRs served as a measure of DNA extraction success. Due to limited amount of DNA extract, I refrained from other quantification measures, e.g. gel electrophoresis or photometry. DNA sequencing and microsatellite genotyping followed the protocols described in chapter 1. DNA sequences were edited using the Codon Code Aligner software (Codon Code Corporation, Dedham, MA, USA) and aligned with MEGA (Tamura et al, 2007) under default alignment parameters. Sequence diversity estimates were generated with DnaSP (Librado & Rozas 2009). Microsatellites were called using Genemapper (Applied Biosystems, Foster City, USA) and allelic richness estimated with the Microsatellite Analyzer software (Dieringer & Schlötterer 2003).

I then tested for an association of genotyping success and several other factors. First, the dependence of marker type and size and genotyping success was analyzed. Moreover, the influence of the age of the samples was estimated. I then tested for an effect of the specimen´s body size. As I used one leg per specimen, the body size relates directly to the amount of tissue used for DNA extraction. Last, I analyzed for the influence of museum collection and proximity of the specimen´s sampling site to the collection on DNA

integrity. The according museum collections are located in the federal states of Berlin and Hessen. I defined a spider as being collected in close proximity, if it was sampled in these states or in one of the bordering states. Using these criteria, I included samples from Berlin or Brandenburg as being close to the Naturkundemuseum. Samples from Hessen, Rhineland-Palatinate and Baden-Württemberg were considered close to the Senckenberg Museum.

Changes of genetic diversity during a range expansion

The success of range expansions and biological invasions is associated with genetic diversity of expanding populations (Kolbe et al, 2004). I analyzed changes of genetic diversity from populations at the wasp spider's former northern range edge in Europe. The historically northernmost populations of *Argiope bruennichi* have been found in the area around Berlin. Due to its proximity to the natural history museum, a large collection of wasp spiders from this range edge population has been compiled. From around 1930 onwards, *Argiope bruennichi* started expanding its range into more Northern latitudes. The range expansion than increased its pace in the past decades and by today the species can be found as far north as Finland (Guttmann 1979; Terhivuo et al, 2011). I split the museum samples into two time periods. The first corresponds to the time before the range expansion and includes specimens sampled until 1930. The second group accounts for the early phase of the range shift and comprises spiders collected until 1960. Finally, I analyzed specimens from after 1960, with a focus on those collected in the past few years. The contemporary specimens were genotyped for the same set of markers like the museum samples and as described above. The museum specimens comprise spiders collected from many different locations and at different years around Berlin. However, they do not include a large series from a single collection site. I thus can not estimate genetic diversity for single sites, but have to work on pooled data.

3.3 Results

Factors, which affect DNA integrity in wet preserved spiders

Overall, I extracted DNA from 397 specimens and tested it for PCR amplification and genotyping success. Mitochondrial DNA amplification was generally more successful compared to nuclear DNA (Fisher's exact test $p < 0.05$). Moreover, PCRs on shorter DNA fragments yielded higher success rates for the mitochondrial DNA (Fisher's exact test $p < 0.001$), but not the nuclear microsatellite markers (Figure 3.1).

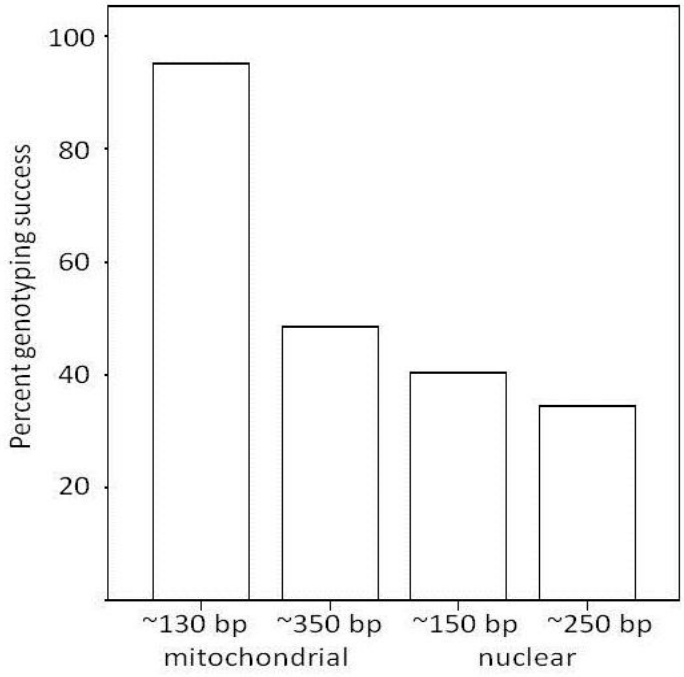

Figure 3.1 Percent of museum samples (N = 397) yielding genotyping data for a ~150 bp and a ~350 bp mitochondrial fragment as well as nuclear microsatellite loci of ~150 bp and ~250 bp. Mitochondrial markers show significantly more genotpyping results. This is especially evident for the short mitochondrial fragment (Fisher's exact test $p < 0.05$).

I was able to acquire exact collection dates for 340 samples. As nearly all samples yielded sequences for the short mitochondrial fragment, I did not analyze the influence of collection date for this marker. For the remaining markers, the sample age is significantly associated with PCR success, with younger samples yielding more positive PCRs than older ones (ANOVA, Bonferroni corrected p-value $p < 0.05$). On average, samples with positive genotyping results have been collected in 1926 for the mitochondrial marker, in 1929 for the short microsatellites and in 1930 for the longer microsatellite markers. The average collection date for non-amplifying samples was 1918 for the mitochondrial sequence and 1919 for both microsatellite markers (Figure 3.2).

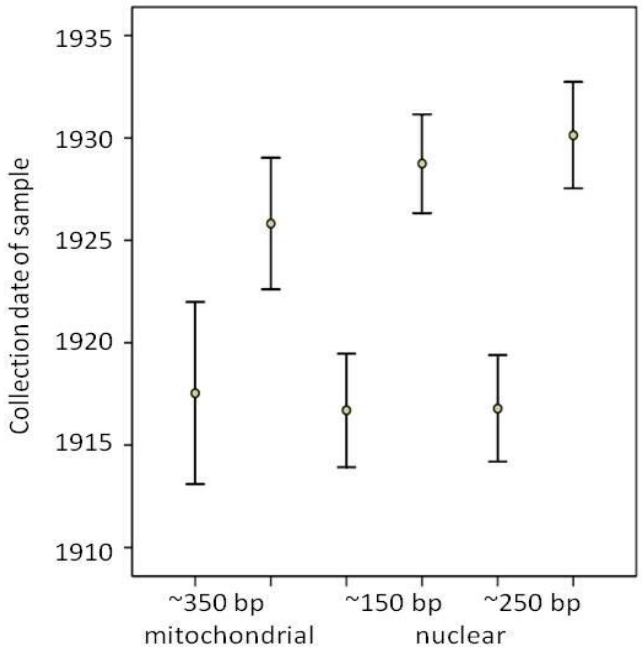

Figure 3.2 Average ages of museum specimens (N = 340), which did or did not yield genotypes for a ~350 bp mitochondrial marker as well as ~150 bp and ~250 bp nuclear microsatellite markers. For each marker type, the right bars represent positive genotyping results and the left bars negative ones. Bars represent the 95 % confidence interval of the mean. The mean age difference between amplifying and non-amplifying samples is significant for all markers types (ANOVA, Bonferroni corrected p-value $p < 0.05$).

I find a highly significant association between the museum collection of origin and the usefulness of tissue samples for genotyping in all marker types. Except of the short mitochondrial fragments, all markers amplify significantly better in samples from Berlin (Fisher's exact test $p < 0.001$). While only between 26 % and 29 % of samples from the Senckenberg Museum yielded positive genotyping results, I could genotype 42 to 66 % of the specimens from the Naturkundemuseum in Berlin (Figure 3.3).

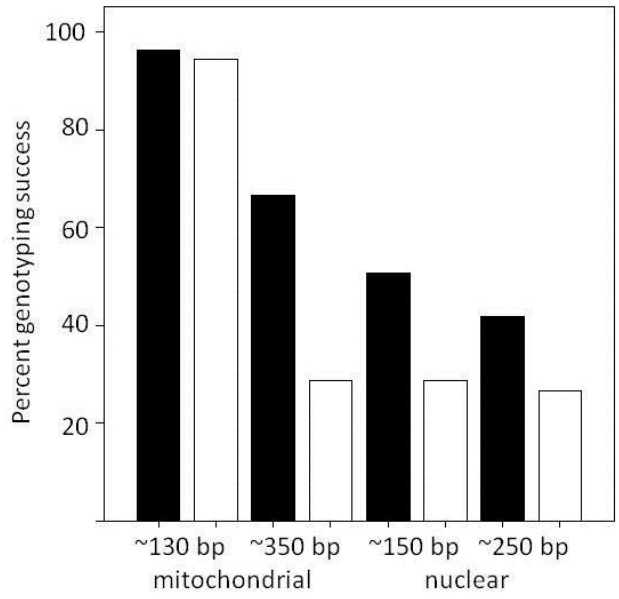

Figure 3.3 Fraction of genotyping data for samples from the Naturkundemuseum Berlin (black bars, N = 215) and the Senckenberg Museum Frankfurt (white bars, N = 182). I tested for genotyping success of a ~150 bp and a ~350 bp mitochondrial fragment as well as nuclear microsatellite loci of ~150 bp and ~250 bp. Samples from the Naturkundemuseum Berlin perform significantly better for all markers, except of the short mitochondrial fragment (Fisher's exact test $p < 0.001$).

The data uncover a clear association between sampling location and genotyping success for samples from the Naturkundemuseum in Berlin (Figure 3.4). Specimens collected from regions in close proximity to the museum result in significantly higher genotyping success, than those collected in more distant localities. This association can be found for all marker types, except for the short mitochondrial fragment (Fisher's exact test $p < 0.05$). The difference is particularly pronounced for the nuclear microsatellites. They can be reliably genotyped for more than 70 % of the samples from the area around Berlin, but only about 30 % of the samples from outside of Berlin (Figure 3.4). In contrast, I do not find any significant association of genotyping success and collection origin for samples from Frankfurt.

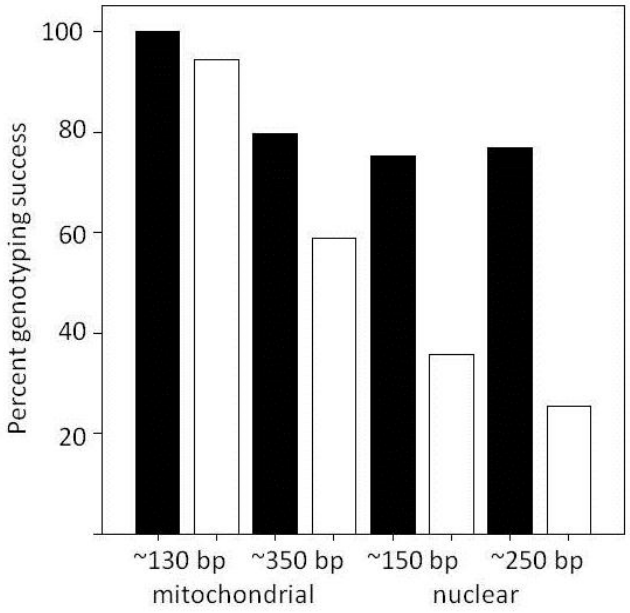

Figure 3.4 Percentage positive genotyping data for samples from the Naturkundemuseum Berlin, which have been collected in close proximity to the museum (black bars, N= 71), or from distant localities (white bars, N = 144). I tested for genotyping success of a ~150 bp and a ~350 bp mitochondrial fragment as well as nuclear microsatellite loci of ~150 bp and ~250 bp. Except of the short mitochondrial fragment, all markers perform significantly better for samples collected in close proximity ot the museum (Fisher's exact test $p < 0.05$).

Another significant association is found for the body size of the analyzed specimen and it´s utility for genotyping. The smaller a specimen is, the more likely it was suited for genotyping (Figure 3.5). However, this association was again only valid for the Naturkundemuseum in Berlin (ANOVA, Bonferroni corrected p-value $p < 0.05$). Generally, specimens from the Naturkundemuseum in Berlin are distinguished by a smaller body size (4.1 mm on average) than those from the Senckenberg Museum (4.5 mm on average) (t-test, $p < 0,05$, Figure 3.6).

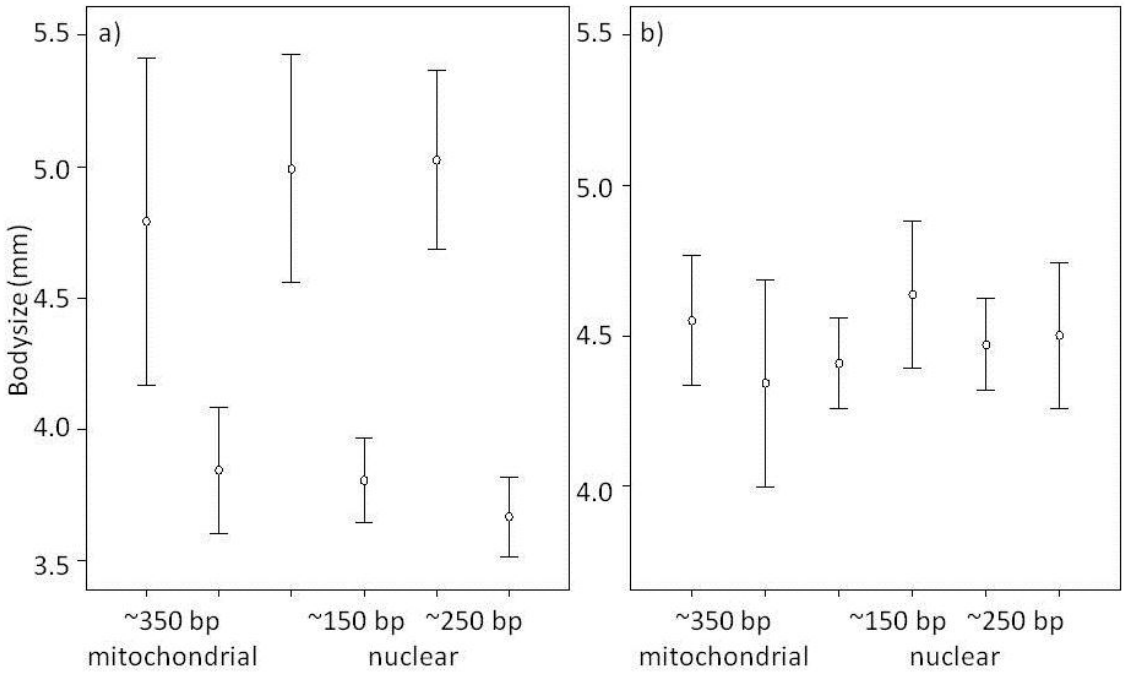

Figure 3.5 Association of genotyping success and average body size of the analyzed specimen for a 350 bp mitochondrial fragment and nuclear microsatellites of ~150 bp and ~250 bp for samples from a) the Naturkundemuseum Berlin (N = 74) and b) the Senckenberg Museum Frankfurt (N = 105). For each marker type, the right bars represent positive genotyping results and the left bars negative ones. Bars represent the 95 % confidence interval of the mean. The association of smaller body size and higher genotyping success is only valied for samples from the Naturkundemuseum Berlin (ANOVA, Bonferroni corrected p-value, $p < 0.05$).

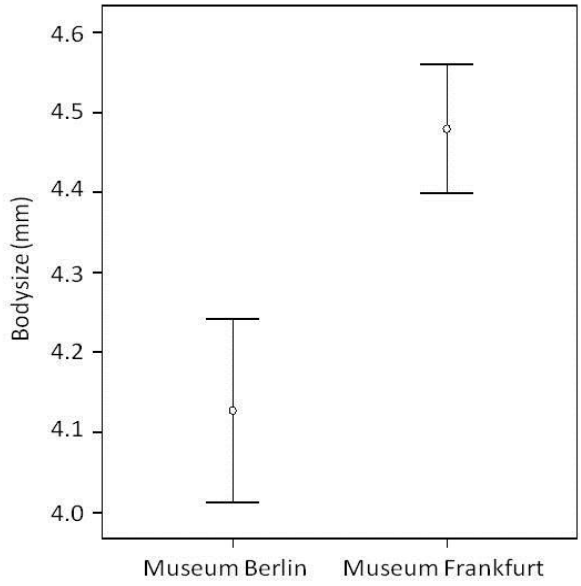

Figure 3.6 Average body sizes of the analyzed specimens from the Naturkundemuseum Berlin (N = 74) and the Senckenberg Museum Frankfurt (N = 105). Bars represent the 95 % confidence interval of the mean. The analyzed samples from the Naturkundemuseum Berlin are significantly smaller than those from the Senckenberg (t-test, $p < 0.05$).

Changes of genetic diversity during a range expansion

The mitochondrial and microsatellite data show a progressive increase of genetic diversity in the Berlin area, associated with the wasp spider's range expansion (Figure 3.7). I find the lowest estimates of mitochondrial (nucleotide diversity $\pi = 0.0009$) as well as microsatellite (allelic richness = 6.99) diversity for historical populations from before 1930. Until 1960, diversity measures increase sharply, corresponding to the beginning range expansion ($\pi = 0.0024$; allelic richness = 9.25). Until today, both measures have additionally increased, although not as steep as between 1930 and 1960 ($\pi = 0.0026$; allelic richness = 11.16).

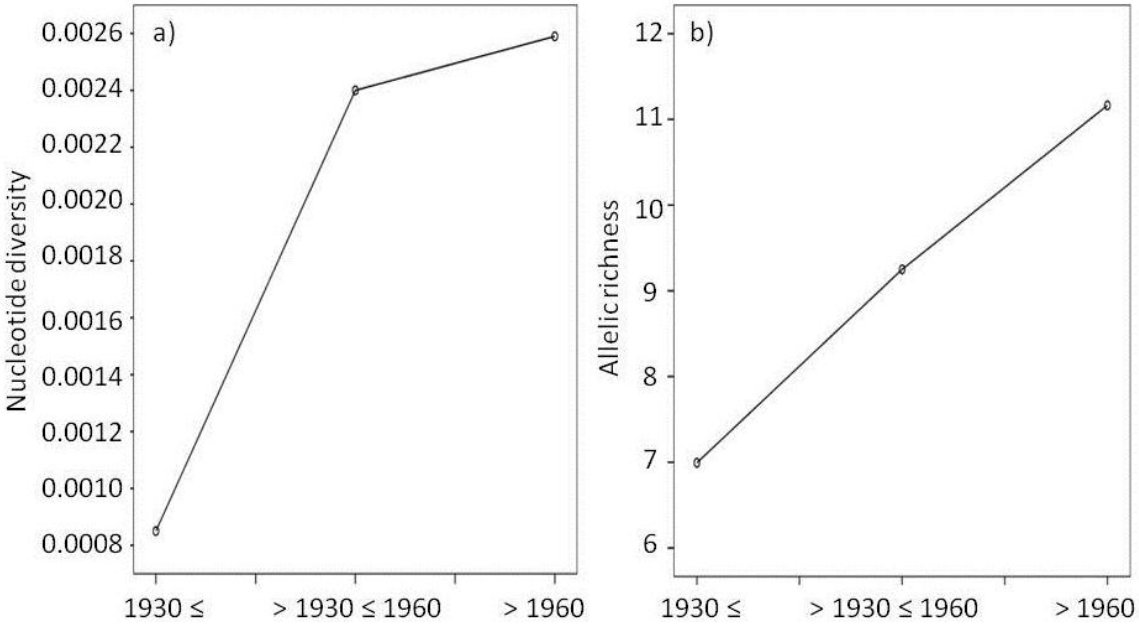

Figure 3.7 Changes of genetic diversity in wasp spider populations around Berlin from before the spider's range expansion (≤ 1930), at the early phase of the range shift (> 1930 ≤1960) and today (> 1960). The plots represent changes in a) mitochondrial nucleotide diversity $N_{\leq 1930} = 41$; $N_{>1930 \leq 1960} = 37$; $N_{>1960} = 130$ and b) microsatellite allelic richness ($N_{\leq 1930} = 37$; $N_{>1930 \leq 1960} = 37$; $N_{>1960} = 51$);

3.4 Discussion

Factors, which affect DNA integrity in wet preserved spiders

Historical DNA can be routinely extracted and sequenced from preserved spiders. However, spider DNA seems to suffer from pronounced degradation. Only short mitochondrial PCR fragments can be amplified in nearly 100% of samples and irrespective of age. Molecular studies on historical spiders should thus rely on short PCR

fragments, only a few 100 bp long. Such short fragments can be sufficient for taxonomic or phylogeographic studies (Hajibabaei et al, 2006). However, the marker type is an important predictor of genotyping success. Due to their high copy number, mitochondrial markers will perform better than nuclear ones. A specimen's utility for a molecular analysis does depend on its age. But I did not observe a clear cutoff after which a sample will not be useful anymore, as it has been described for insects (Watts et al, 2007). Even an analysis of very old specimens, collected more than 100 years ago, can yield positive results in many cases. Interestingly, not a specimen's age, but collection schemes may determine their utility for PCR and genotyping. Samples from Berlin performed much better in my analysis than those from Frankfurt. Spiders are usually stored in 70% Ethanol and kept at room temperature. Moreover, the arachnological collections in Berlin and Frankfurt both use and have historically used similar Ethanol denaturants (Jaeger & Dunlop pers. comment). Consequently, no huge differences in storage conditions between collections should arise. Moreover, I find a clear association of genotyping success and the proximity of sampling sites to the museum collection in Berlin. This allows to imply that the immediate treatment of a specimen right after its collection may have a major influence on long term DNA integrity. Specimens from the area around Berlin have probably been transferred to the museum shortly after their collection. From work on contemporary wasp spider samples, I know that DNA of these animals degrades very quickly, possibly due to very effective DNAses in their digestive system. A dead spider, which is not immediately transferred into Ethanol, will show extreme DNA degradation after only 24 hours (pers. observation). Many historical samples have been collected during long expeditions, sometimes lasting several years. Unsuitable storage of these specimens could have destroyed their utility for molecular work shortly after their collection and before they have actually been stored in their respective museum. Many of the historical specimens from the Berlin area have been collected by the museum's curators and are thus well preserved. On the other hand, the former curators of the Senckenberg Museum did not sample many spider specimens around the Frankfurt area (Jäger pers. comment). This could explain the lack of association between proximity of sampling sites to the Senckenberg Museum and genotyping success. Museum collections should consequently aim for an immediate and proper storage of samples. Another

explanation for the superior quality of samples from the Berlin museum is possibly found in the body size of spiders. Since I discover a very strong association of small body size and long term DNA preservation, the tissue of a larger specimen might simply be less accessible for the Ethanol preservative, leading to more DNA degradation.

Changes of genetic diversity during a range expansion

The analysis of historical and contemporary populations from Berlin allows insight into genetic changes, associated with range expansions. The historical Berlin populations show a lower genetic diversity than the more recent ones, which may be due to the fact that it was at a range edge during historical times (Eckert et al, 2008). Possibly they have just been a demographic sink, maintained by immigration from southern populations (Kawecki, 2008). From around 1930 onwards, the genetic diversity around Berlin substantially increased, probably due to immigration from Eastern European lineages (Krehenwinkel & Tautz, 2013). This admixture possibly enabled a range shift into more northern latitudes. The association of increasing genetic diversity and range expansions has been found in many other taxa (Kolbe et al, 2004; Keller & Taylor 2010; Lucek et al, 2010). Bottlenecked range edge populations possibly have a reduced adaptive potential (Pujol & Pannell 2008), which can be restored by admixture of new lineages.

3.5 Conclusion

The age of spider specimen seems to be of minor importance for their utility for DNA work. Instead, the immediate and proper treatment after collection and possibly the size may be more important. My data suggest that it is advisable to immediately place spiders in alcohol, or to at least remove a leg for immediate storage in alcohol for optimal future DNA extraction. The removal of a single leg will not alter the utility of a spider specimen for morphological analysis.

Chapter 4: Whole genome sequencing reveals genetic signatures of ecological differentiation during a contemporary range expansion of a spider species

4.1 Introduction

"Starting with Darwin and continuing until very recently, speciation has been considered to be usually a very slow process - perhaps requiring millions of years" (Hendry, 2009). Populations were assumed to gradually diverge in allopatry and accumulate incompatibilities, which would finally lead to reproductive isolation and speciation (Mayr, 1942). But as the above statement implies, this view has been challenged by recent research. The emergence of new species can occur within short timeframes (Filchak et al, 2000) and even despite ongoing gene flow (Savolainen et al, 2006). Divergent selection for different ecological opportunity has been suggested to drive such differentiation (Via, 2009). Gene flow between ecologically diverging populations would be only reduced for genomic regions of adaptive significance. Small islands of elevated divergence in the vicinity of divergently selected mutations would constitute the genomic footprint of such recent ecological differentiation (Via, 2009). Theory suggests that these islands could quickly grow in size, contributing to increasing genetic differentiation (Via, 2012). An association of traits, involved in, e.g. mate choice to the vicinity of growing islands, could then culminate in reproductive isolation and speciation (Feder et al, 2012; Via, 2009 & 2012).

A major obstacle to ecological divergence is the prevention of gene flow and recombination in genomic regions of adaptive significance. Recombination coupled with gene flow could effectively prevent the built up of co-adapted gene complexes, for example those, which associate with ecologically relevant loci (Via, 2012). On the other hand, gene flow does not necessarily impede adaptation. Genetic exchange has been identified as an important driver of adaptation in many plant- and animal species (Arnold, 2006). Instead of waiting for new adaptive mutations to arise, selectively advantageous variants could be introduced into populations by secondary contact (Nolte & Tautz, 2010). Especially the success of biological invasions has been associated with admixture of formerly isolated lineages (Keller & Taylor, 2010; Kolbe et al, 2004). In the face of

climate change and human impact on ecosystems, such secondary contact is becoming increasingly likely for many species (Garroway et al, 2010; Crispo et al, 2011). This in turn could lead to large numbers of new invasive species and pose a problem of major economic importance.

In the past years, next generation sequencing has enabled a refined view into the actual genomic basis of ecological differentiation with gene flow (e.g. Renaut et al, 2012; Nadeau et al, 2012; Roesti et al, 2012; Turner et al, 2005; Hohenlohe et al, 2012). Today, even screens of whole genome sequences are becoming an affordable option for speciation researchers (Feder et al, 2012). So far no genomic study has particularly considered the importance of admixture during range expansions. Here, I provide a first insight into these processes.

I apply a whole-genome sequencing approach using populations of the European wasp spider *Argiope bruennichi*. Originally predominantly inhabiting the Mediterranean region, this species has recently expanded its range into continental Northern Europe and can now be found as far North as Finland (Guttmann, 1979; Kumschick et al, 2011). The spider's range expansion has probably been enabled by a recent ecological niche shift and is associated with historical genetic admixture (Chapter 1 - Krehenwinkel & Tautz, 2013). Here I describe the results from whole genome and transcriptome sequencing of population samples from the historical native range in Portugal and Italy and the range expansion front in Sweden and the Baltic states. In addition, I include sequences from outgroup samples from five locations along the Japanese Islands.

Currently, no spider genome has been fully sequenced. I thus first present an initial draft genome and transcriptome of the species and some general genomic features. Then, I discuss genomic differences between native and invasive populations. I initially focus on an association of admixture and increasing genetic diversity in Northern Europe with the species' range expansion success. I examine which traces of divergence the range expansion left in the genomes of invasive wasp spider populations. I then discuss this genomic differentiation in regard to recent adaptation and demographic influences. Finally, I confirm a selection of candidate loci for reciprocal fixation between Northern and Southern Europe by PCR and sequencing.

In summary, I find a significantly increased genome wide genetic variation in expanding Northern European wasp spider populations, possibly as the result of recent admixture. At the same time, I identify a very low genetic divergence between Northern and Southern Europe. Despite this limited divergence, I discover genomic islands of high differentiation between expanding and native populations. The PCR screen shows that the examined candidate loci might indeed have been subject of recent selection.

4.2 Material and methods

Generation of a reference transcriptome from an ontogenetic series

In order to cover as many genes as possible from the wasp spider's genome, I generated a reference transcriptome based on an ontogenetic series of RNA samples. I prepared four different total RNA extractions using the Qiagen RNeasy Kit according to the manufacturer's protocol (Qiagen, Hilden, Germany). In order to avoid DNA contamination on columns, a DNAse digestion step was carried out using Qiagen RNase-Free DNase Set, according to the manufacturer's protocol. Extractions were prepared from a whole adult male and female specimen. In addition, one extraction was carried out with 20 eggs and one using 20 spiders in the first nymphal instar. Female spiders are usually well nourished and their opisthosoma is filled with eggs and digested food remains. I consequently extracted the female in two separate reactions, one containing the prosoma including the legs and one containing the opisthosoma. All extractions were done with specimens from a population from Plön, Schleswig Holstein, Germany. The extraction series thus covered early and late developmental stages, as well as sex specific differences in gene expression. The RNA extracts were sent to the Center for Genomics at the University of Cologne, where tagged cDNA libraries with an average insert size of 250 bp were prepared. These libraries were then sequenced on one lane of an Illumina HiSeq 2000 (Illumina, San Diego, USA) according to the manufacturer's protocol.

The raw data was quality trimmed using PoPoolation (Kofler et al, 2011) by using a minimum quality of 20. The quality was checked using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The paired reads were then assembled using SOAP de Novo (Li et al, 2009b) with a maximum read length of 100 bp.

I included a mapping and a scaffolding step. I tried assemblies of all four separate libraries and at distinct k-mer sizes (19-32). The best assembly was generated using a pooled dataset of all four libraries and a k-mer size of 25.

Subsequently, I used CD-HIT-EST (Li & Godzik, 2006; Fu et al, 2012) with a similarity cutoff of 90% to remove redundant contigs from the de novo assembly. Last, the Galaxy server (Goecks et al, 2010) was used to remove contigs and scaffolds of less than 250 bp from the final assembly. I included this filtering step to remove a large part of contigs, which corresponded to very few assembled reads. The final reference dataset of transcripts was blasted (using BLASTX with an E-value cutoff of 10^{-3}) using Blast2Go (Conesa et al, 2005). I then separately aligned the reads for every ontogenetic stage onto the reference transcriptome using CLC genomics workbench (CLC Bio, Cambridge, USA), with a mismatch cost of 1, insertion cost of 3 and a deletion cost of 3. The RPKM values of the resulting four alignments files were normalized using quantile normalization (Bolstad et al, 2003) and each dataset filtered for RPKM values of above 0.5. This value served as a cutoff for reliably counting a transcript as expressed (as e.g. in Strout et al, 2011). I then calculated the number of uniquely expressed transcripts per ontogenetic stage.

Generation of a wasp spider draft genome

At present, no spider genome has been sequenced. In order to provide a suitable reference for my study, I consequently had to determine the genome sequence of the target species *Argiope bruennichi*. The quality of a de novo assembly can be greatly improved by reducing the amount of heterozygous loci, e.g. by sequencing DNA of an inbred specimen (Vinson et al, 2005). However, as I did not have inbred lines, I relied on a wild caught specimen from a very homozygous population of the wasp spider on the island of Madeira. The spider was collected in August 2012 (near Camacha, 32.7°N, -16.8°E) and brought to the laboratory alive. I tried to maximize the DNA yield and integrity and used the whole spider for extraction. In previous DNA extractions, I experienced very fast post mortem DNA degradation in spiders. Consequently, I carried out the DNA extraction using the living specimen. DNA was extracted using the Archivpure blood and tissue kit

(5 PRIME, Hamburg, Germany). An RNA digestion step was included using RNase A solution (7000U/ml) (5 PRIME, Hamburg, Germany). The extractions were carried out according to the manufacturer's protocols. The spider was first anesthetized using carbon dioxide. Then it´s prosoma was removed using a sterile scalpel blade, cut in two pieces and each transferred into a 2 ml Eppendorf tube. The tissue was disrupted at 30 hz for 30 s on a Qiagen tissuelyzer (Qiagen, Hilden, Germany). The spider´s opisthosoma was not used for extractions, as it contains the hindgut and large parts of the digestive system, leading to impure DNA.

DNA integrity and purity was checked on a 1% agarose gel and using a Nanodrop (PEQLAB, Erlangen, Germany). A paired end DNA library with an average insert size of 250 bp was prepared by the Center of Genomics at the University of Cologne. The library was sequenced on one lane of an Illumina HiSeq 2000 to an approximately 20-fold coverage and according to the manufacturer's protocols (Illumina, San Diego, USA). I did not know the exact genome size of *Argiope bruennichi*, and relied on available data for its sister species *Argiope aurantia* and other members of the genus *Argiope*. All of them have similar genome sizes of about 1.5 Gb (Gregory, 2006). The haploid number of chromosomes for *Argiope bruennichi* is known to amount to 13 (Araujo et al, 2011).

The data was quality trimmed as described above for the transcriptomes. The trimmed data was then assembled using CLC genomics workbench, with a word size of 45, a bubble size of 98, a minimum contig length of 1000 and including a scaffolding step. The resulting DNA assembly was aligned to the previously generated reference transcriptome using BLAT (Kent, 2002) under default settings and at a similarity threshold of 98%. I subsequently filtered all hits of genomic DNA into transcripts of more than 100 bp length. I then assigned the according transcript names to each genomic hit, leading to combined names of transcript and genomic contigs. As a transcript usually consists of several exons separated by introns, the same RNA sequence can map into different stretches of DNA. Assuming that all exons of a gene are located in close distance to each other, DNA fragments can be combined to linkage groups. This way, the complexity of an assembly can be reduced.

Estimation of genomic divergence during a range expansion

I analyzed specimens from several European and Asian populations, to investigate genomic divergence during the wasp spider´s range expansion. European spiders were sampled between 2010 and 2012 from each of two regions of Northern (Sweden and Baltic states) and Southern Europe (Portugal and Italy). While the latter sampling sites represent the historical native distribution of the spider, the first correspond to the current range edge in Northern Europe with populations established since less than ten years. I sampled five specimens from three populations in each of those four regions, resulting in 15 specimens per region and 60 spiders in total. DNA was extracted from single animals as described above for the Madeiran reference specimen. The DNA quantity was then measured using a ND-3300 Fluorospectrometer (PEQLAB, Erlangen, Germany) according to the manufacturer's protocol and equal DNA amounts of each specimen pooled for the 15 specimens per region. Each DNA pool was sequenced on one lane of an Illumina HiSeq 2000, as described above. Moreover, genomic DNA of five Japanese specimens from distinct populations from Northern Honshu to the Southern Pacific Amami Oshima Islands was sequenced on one HiSeq 2000 lane (see Figure 4.1 for the collection sites).

The paired end reads were trimmed using PoPoolation as described above and then aligned to the Madeiran reference genome using Bowtie2 under default alignment settings (Langmead & Salzberg, 2012). The SAMtools (Li et al, 2009a) package was used to convert the SAM alignment files into sorted BAM files with a minimum alignment quality of 2. The mpileup command in SAMtools was applied for SNP calling from the generated alignments, using a maximum per BAM file depth of 200 and with disabled indel calling. PoPoolation and PoPoolation2 (Kofler et al, 2011) were then used to infer genome-wide nucleotide diversity and genetic differentiation between Northern and Southern European as well as Japanese populations. Allele frequency differences were calculated for each variable position in the genome alignment, while a pairwise FST and nucleotide diversity were estimated using sliding windows of 5000 bp. The analyses were run with a minimum quality per base of 20, at least 0.5 fold coverage per window and a minimum and maximum coverage of 10 and 50, respectively. I generated a distance matrix, based on the genome-wide averaged FST. This matrix served to construct a

neighbor joining tree for the studied populations using PHYLIP (Felsenstein, 1989). In addition, I generated mean values of nucleotide diversity for each population.

Theory predicts that divergent selection will initially manifest itself in genomic islands of differentiation between ecologically diverging populations (Via, 2012). These islands correspond to ecologically relevant alleles and their linked, neutral variation. To gather a first insight into genomic divergence, I chose to filter the top percentile of divergent loci, based on the sliding windows of FST. I filtered genomic contigs with regions of at least 5000 bp of elevated divergence from the genome assembly. I applied this filter for a pairwise comparison of Northern and Southern European populations (based on the means of Baltic and Swedish as well as Italian and Portuguese populations), which correspond to two ecologically divergent geographic regions. A recent fixation of adaptive variants by selection will likely reduce genetic variation around the selected allele (Biswas & Akey, 2006). Consequently, I crosschecked the filtered genomic regions for a reduction in nucleotide diversity.

An admixture of Asian and European populations has previously been shown to be involved in the range expansion of *Argiope bruennichi* (Chapter 1 - Krehenwinkel & Tautz, 2013). This Asian introgression seems to be primarily limited to invasive Northern European spiders. I consequently used the genomic data to estimate the amount of introgression from Japanese alleles into the European genepool. For that reason, I used the allele frequency data, to filter contigs of complete differentiation between European and Japanese populations.

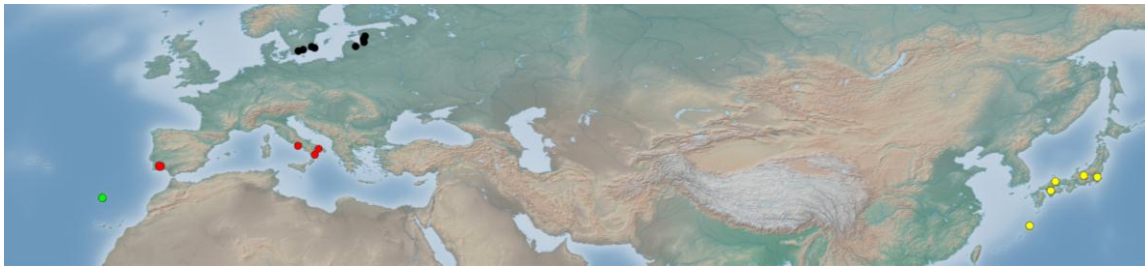

Figure 4.1 Sampling sites of the specimens, which were used for generating whole genome sequences of Palearctic wasp spiders. The green dot corresponds to the Madeiran reference specimen, the yellow ones to the five Japanese samples. Sampling sites for Northern and Southern European DNA pools are highlighted in black and red, respectively. The map was generated using GenGis (Parks et al, 2009).

A PCR based screen for range-wide genetic differentiation in genomic candidate loci

I assume that an introgression of genetic material from East Asia could have conferred adaptive variation to Northern European wasp spider populations (Krehenwinkel & Tautz, 2013). Selection and migration should interact during this introgression, leading to a clinal pattern of Asian allele frequency across Europe. *Argiope bruennichi* is a high gene flow species, which is expected to flatten this genetic gradient. Very strong selection on the other hand, could override the effect of gene flow and steepen a cline (Futuyma, 2005; Sotka & Palumbi, 2006). I tested a subset of genomic candidate loci for clinal allele frequency patterns across Europe. These loci were sequenced for a selection of individuals from European populations. Candidate regions were chosen according to the following criteria: 1. They showed a pronounced genetic differentiation between Southern and Northern European populations over at least 5 kb. 2. A reduction of nucleotide diversity could be observed for the candidate region in Northern European populations, or both Northern and Southern European ones. 3. The candidate SNP was largely fixed between Southern European and East Asian spiders; it thus probably introgressed into Northern European populations from East Asia. The respective genomic regions were amplified in a multiplex PCR and genotyped as described in chapter 1. Primers were designed on an alignment of Japanese and European consensus sequences for the respective genomic contigs and using the Primer3Plus software (Untergasser et al, 2007). The consensus sequences were generated from BAM alignment files and using the 50% consensus option in Geneious (Biomatters, Auckland, New Zealand). In addition, I used published data from seven SNP loci, which were largely fixed between East Asian and European populations (see Krehenwinkel & Tautz, 2013 and chapter 1). This data served as a neutral background control for the potentially selected genomic candidate loci. I analyzed samples from several geographic regions over the European range of *Argiope bruennichi* and from two Japanese populations (see Figure 4.11 for collection sites). Allele frequencies for each population were plotted against geographic location and inspected for clinal patterns. As *Argiope bruennichi* is invading Continental climate zones in North-Eastern Europe, longitude alone will not suffice to show a clear clinal genetic pattern. Populations from e.g. the Balkans are expected to share alleles with those from Spain, although they originate from very distinct longitudes. I accounted for this

Northeastern ecological transition, by generating average measures for longitude and latitude.

4.3 Results

Reference transcriptome and - genome

After quality trimming, the ontogenetic RNA sequencing yielded a total of 28,247,869 paired reads for the eggs, 48,319,049 for the spiderlings, 42,878,220 for the male and 38,369,736 for the female dataset. 622,782 reads of the eggs, 802,297 for the female, 852,624 for the male and 883,171 for the spiderlings were removed during quality trimming. Even before quality trimming, the sequence quality was generally very high, with the majority of bases having a Phred score of above 30 (Figure 4.2).

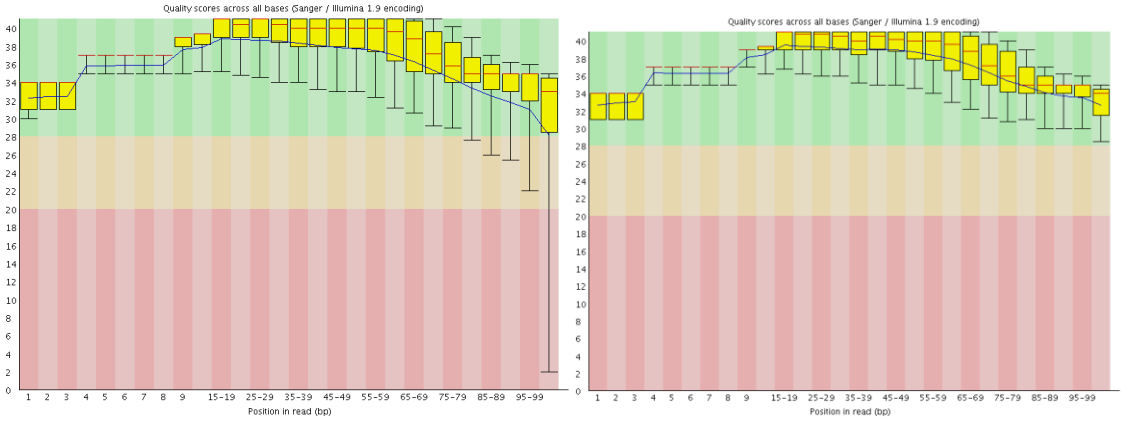

Figure 4.2 Sequence quality (Phred scores) from the FastQC analysis for an RNA-seq library, before and after quality trimming using PoPoolation.

The Madeiran reference specimen yielded 180,119,468 paired DNA reads of ~100 bp to each side. The sequencing of pooled DNA yielded 177,066,940 paired reads for the Swedish, 160,091,602 for the Baltic, 170,432,904 for the Portuguese and 142,163,319 for the Italian populations. The Japanese samples yielded between 33,480,135 and 45,529,677 paired reads per library. The sequence quality was generally very high, comparable to the RNA-seq data (Figure 4.3).

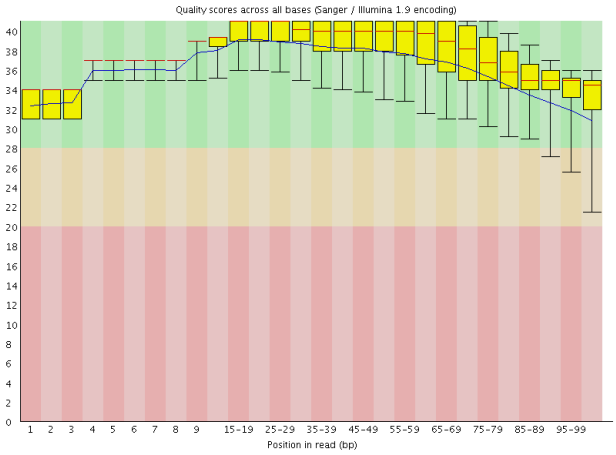

Figure 4.3 Phred quality scores from the FastQC analysis for the untrimmed genomic DNA library of the Madeiran reference specimen.

The initial transcriptome assembly yielded more than 150,000 contigs in approximately 130 million bases. After filtering redundant contigs with CD-HIT-EST and application of a 250 bp size cutoff, the final assembly contained 87,262 transcripts. Only 20,764 of these yielded a significant BLAST hit with genes from other organisms.

Using a minimum RPKM value of 0.5 per sample, 58,627 of these transcripts were sufficiently covered in at least one ontogenetic stage. 33,973 transcripts were expressed in all ontogenetic stages, while 24,654 were unique to a certain developmental stage. The smallest number of unique transcripts was identified in first instar nymphs (4.654), while the largest number was observed for eggs (7,490). The number of uniquely expressed male (5,154) and female (7,356) genes is intermediate (Figure 4.4). The average GC-content of the transcriptome assembly is 32 % GC.

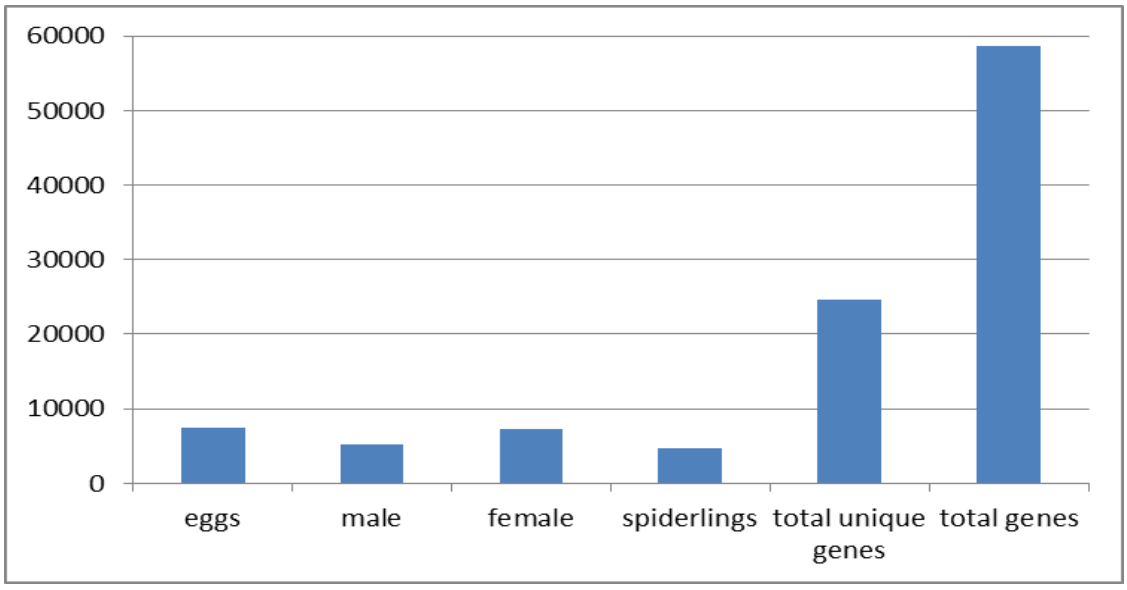

Figure 4.4 Numbers of uniquely expressed transcripts for different ontogenetic stages of the wasp spider. A considerable amount of genes is only expressed at a certain ontogenetic stage. 7,490 transcripts are exclusively found in developing eggs, 4,654 genes are only expressed in first instar nymphs, 7,356 in the female and 5,154 only in the male.

The reference genome assembly amounted to roughly 1.4 gigabases in 240,178 contigs with an average contig length of 6,055 bp, an N50 value of 11,151 bp and a maximum contig size of 201,766 bp (Table 4.1). In comparison to the RNA data, I find a considerably lower GC-content in the genomic DNA (29 % GC). Assuming, that the total assembly size is comparable to the actual genome size, the Madeiran DNA sequencing reads amount to an approximately 20-fold coverage. Using BLAT, about 77,000 transcripts could be mapped into nearly 130,000 of these contigs. This allowed to combine previously separated contigs and reduced the total amount of contigs to approximately 200,000.

Table 4.1 General sequence statistics for the genomic DNA and RNA assemblies

	RNA seq	DNA
Assembly software	SOAP de Novo	CLC genomics workbench
Total size of assembly	130,769,830	1,454,196,420
Number of contigs	165,709	240,178
Mean contig size	798	6,055
Longest contig	35,942	201,766
N50	2,055	11,151
GC-content	32	29

Estimation of genomic divergence during a range expansion

After mapping all genomic population pools to the Madeiran reference assembly, I measured genome-wide nucleotide diversity using PoPoolation. With an average π of 0.016 Japanese spiders carry the highest diversity, while the lowest diversity is found in Mediterranean populations (π = 0.005 in Portugal and π = 0.006 Italy respectively). The invasive Northern European populations are distinguished by a significantly higher diversity than their native Southern European relatives (π = 0.008 on average for Swedish and Baltic populations) (Figure 4.5, ANOVA, Bonferroni posthoc test, $p < 0.05$). This result is especially noteworthy, as these populations are located at the species´ northern range edge and have been found only a few years ago.

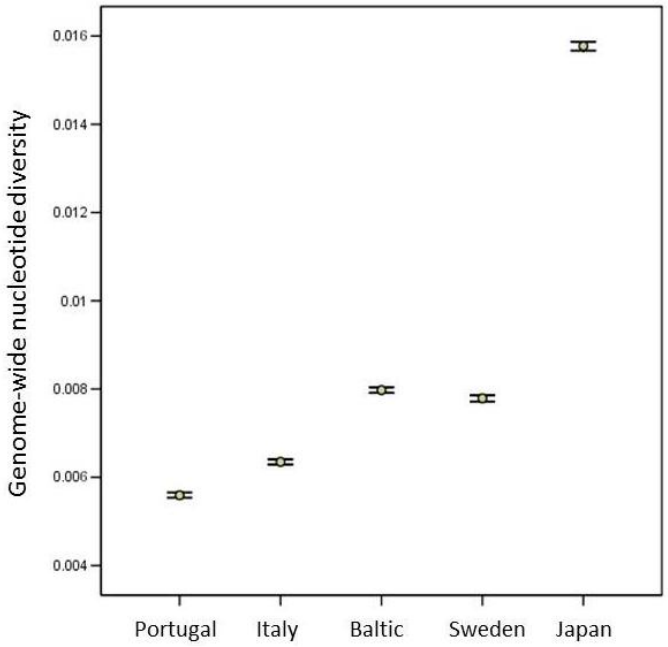

Figure 4.5 Averaged genome-wide nucleotide diversity for Palearctic wasp spider populations. Each plot shows the mean of ~150,000 separate sliding windows of 5,000 bases over the whole genome. A clear increase of diversity from Southern to Northern Europe and then towards Japan is evident. The difference between Northern and Southern Europe, as well as Europe and Japan is significant (ANOVA, Bonferroni posthoc test, $p < 0.05$). Bars around the mean represent the 95 % confidence interval.

A genome-wide comparison for allele frequency differences yielded 32,000,000 polymorphic nucleotides (SNPs) for all studied populations. Only about 1 % out of these are completely fixed between all European and Japanese populations. The divergence between Japan and Europe is biased towards southern European populations. Southern European populations are more divergent from Japan by roughly 4 % fixed differences on average. This corresponds to 89,306 genomic contigs. Northern populations are different from Japanese in only about 2 % of variable SNPs and 49,812 contigs. I find a large overlap of differentiated regions between Northern and Southern Europe and Japan (Figure 4.6). Northern European populations are distinguished by very few contigs, which exclusively distinguish them from Japanese ones (6,075). Southern European populations, on the other hand, carry a large amount of uniquely differentiated contigs (45,569). These numbers correspond to roughly one completely fixed difference between all European and Japanese populations per kilobase (supplementary figure 4.1). To summarize, Northern European spiders are distinguished from Southern ones by many more shared alleles with East Asian spiders.

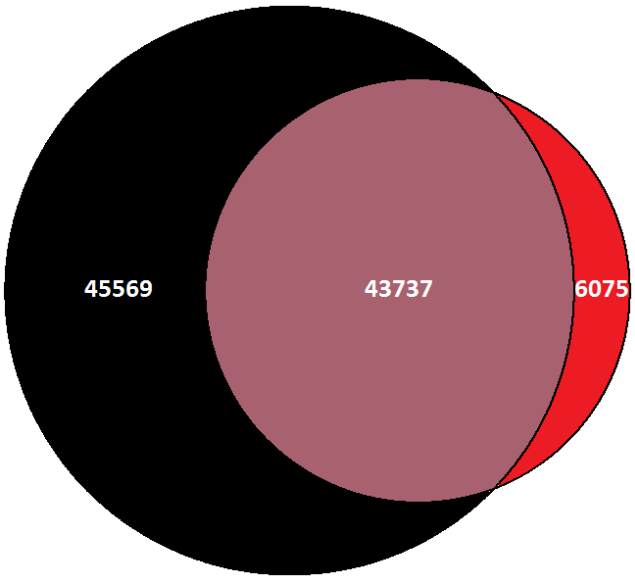

Figure 4.6 Number of contigs, which contain at least one completely fixed SNP between European and Japanese populations. The left Venn shows divergent contigs between Southern European and Japanese (45,569) and the right one between Northern European and Japanese populations (6,075). I also find a considerable overlap, of divergent contigs between all European and Japanese populations (43,737).

A comparison of genome-wide pairwise FST confirms the observed pattern of European and Japanese divergence (Figure 4.7). Northern European spiders are less divergent from Japanese (FST = 0.29) ones than their Portuguese and Italian relatives (FST =0.32). As these FST values indicate, Europe and Japan are not completely divergent, but still share a considerable fraction of alleles (supplementary figure 4.1). With an FST of just 0.1 on average, European populations appear very closely related to each other. Native European (Portuguese and Italian) populations form one monophyletic group (FST = 0.09), with the two Northern European populations basal to this clade. Northern European populations are most closely related to each other (FST = 0.07). Northern and Southern European populations are distinct by an FST of 0.1 on average.

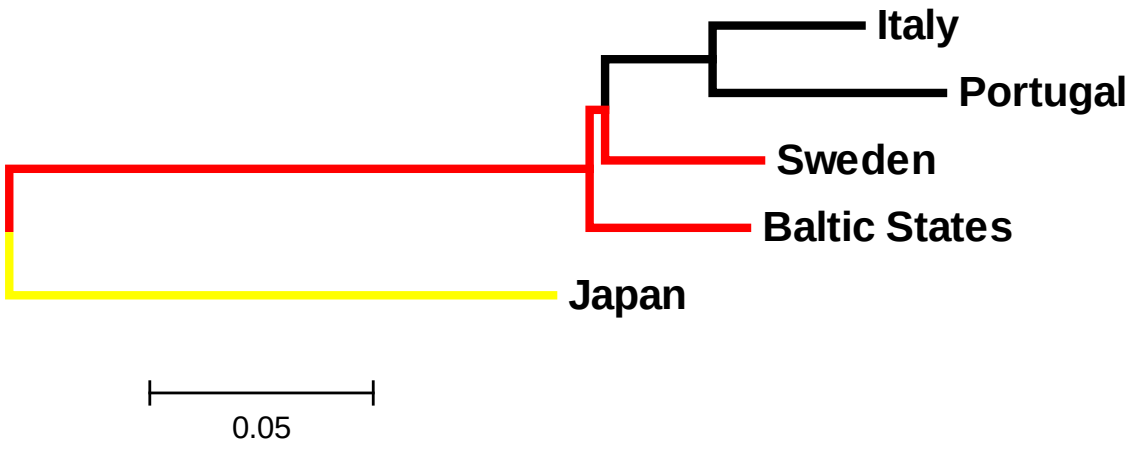

Figure 4.7 FST-based neighbor joining phylogeny for European and Japanese wasp spider populations. Colors correspond to those in the sampling map (Figure 4.1).

Despite a low average genome-wide FST, Northern and Southern European populations are distinguished by several genomic regions of very high differentiation. The top percentile of divergent regions between Northern and Southern Europe contained 1712 contigs and spanned an FST range from 0.24 to 0.60. However, a part of these regions showed an overlap with contigs, which belonged to the most divergent ones between Portuguese and Italian populations. After filtering these, 1102 contigs remained, which showed a high differentiation between Northern and Southern Europe. In parallel to the increased FST, I find a reduction of nucleotide diversity in those candidate contigs in Southern Europe (average π = 0.004 for the candidate regions vs. π = 0.006 for the whole genome) and Northern Europe (average π = 0.006 for the candidate loci vs. π = 0.008 for the whole genome). The parallel increase of genetic differentiation and drop of nucleotide diversity is exemplarily shown for one candidate contig in Figure 4.8. However, it should be noted that we did not always observe a simultaneous decrease of nucleotide diversity in Northern and Southern European populations. For some contigs, a nucleotide diversity decrease was observed only Northern or Southern Europe.

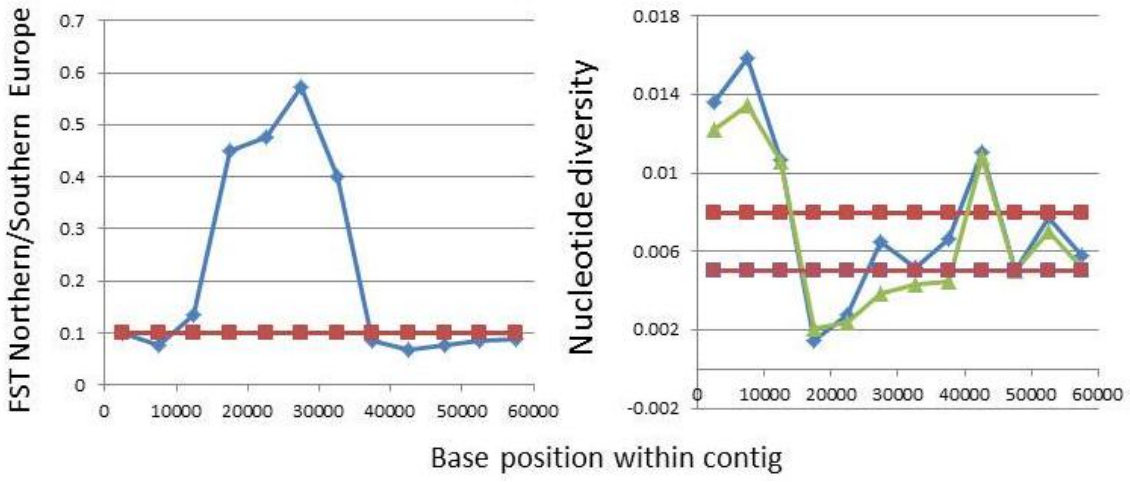

Figure 4.8 Patterns of genomic divergence and diversity between Southern and Northern European populations for a candidate contig. The left plot shows a steep increase of FST values over several kilobases for the candidate region (blue line) and in comparison to the average FST (red line). The right graph presents the same genomic region, but nucleotide diversity is plotted. A decrease of nucleotide diversity (blue line for Northern Europe & green line for Southern Europe) is evident in comparison to the genome-wide average (red lines). The windows of increased FST and decreased nucleotide diversity do clearly overlap in this example. Such an overlap of reduced diversity is, however not found for all diverged contigs.

A visual inspection of divergence patterns revealed two types of candidate contigs. The first shows a sudden and steep increase of divergence after an initial sequence stretch of low differentiation. The latter is distinguished by a high rate of divergence over the whole contig sequence. These two patterns are approximately equally prevalent among my candidate contigs and show a continuous transition into each other (Figure 4.9).

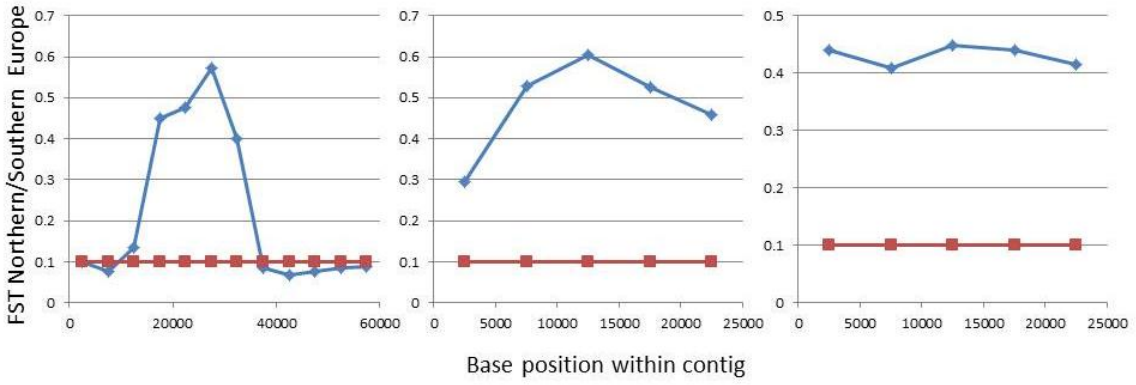

Figure 4.9 Different patterns of divergence, which I observe for candidate contigs between Northern and Southern Europe. The left plot shows a steep increase and then drop of FST (blue line) in comparison to the genome-wide average (red line), giving the appearance of an island. The right plot shows an elevated FST over the whole coting sequence. And the graph in the middle represents a transition of those two states.

A PCR based screen for range-wide genetic differentiation in genomic candidate loci

An inspection of candidate contigs for selective sweeps of introgressed haplotypes in the integrative genome viewer IGV (Robinson et al, 2011) revealed many shared SNPs between Japanese and Northern European populations (Figure 4.10, supplementary figure 4.2). The following PCR screen is based on these alleles in four such candidate contigs.

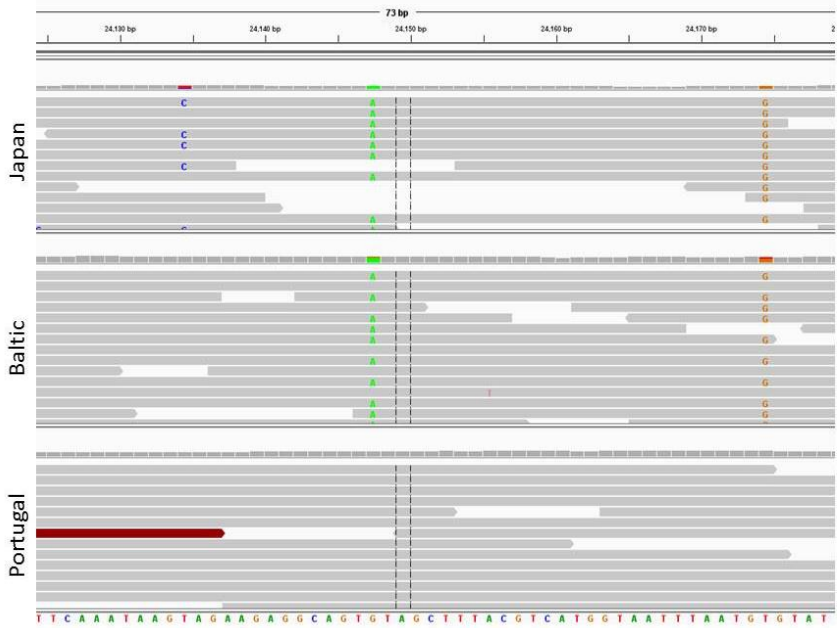

Figure 4.10 Integrative genome viewer tracks of one candidate contig for divergence between Northern and Southern Europe. The divergence between Baltic and Portuguese populations is based on shared SNPs with Japanese spiders.

I screened four genomic candidate SNPs in 200 specimens from 20 Palearctic populations (Figure 4.11). The respective contigs shared a pronounced FST between Northern and Southern Europe, and reduced nucleotide diversity compared to the genome wide average. Three of them map into a transcript with a significant BLAST hit (see Table 4.2).

Table 4.2 Genetic divergence, diversity and BLAST hits for the four genomic candidate contigs.

Name	Outlier contig	Average FST North.-South.	Average nucleotide diversity π Northern Europe	Southern Europe	BLAST hit	GI No.
G1	C609506_contig_52661	0.32	0.0013	0.0004	Hypothetical protein	328714313
G3	scaffold30462_contig_60388	0.39	0.0019	0.0004	Dentin matrix protein	241608386
G4	C441899_contig_75705	0.43	0.0017	0.0004	NA	NA
G7	C601208_contig_38174	0.36	0.0031	0.0014	Cysteine proteinase	301103674

The SNP genotyping for the candidate loci revealed Japanese specimens to be mostly fixed for their respective diagnostic allele. The frequency of the four genomic candidates then decreases from Northeastern to Southern European populations in a steep clinal pattern (from 82 to 0 %). Longitude and latitude both seem to be involved in building up this frequency gradient. Using information for longitude and latitude, I find a less noisy clinal pattern (Figure 4.12). The emerging gradient approximates a transition from Southern and Western European Mediterranean and Oceanic climates towards Northeastern European Continental climate zones (Peel et al, 2007). While the frequency of Asian alleles is very low in Mediterranean and Oceanic populations, it quickly rises in Continental Europe. Populations from Berlin and Brno, which approximate the transition between both climate zones, are distinguished by an intermediate allele frequency (39 % and 34 %).

Using the background dataset of seven SNP loci, I find a similar pattern of decreasing Asian allele frequencies towards Southern Europe. But the cline is much shallower, slowly increasing from around 5 % in Western Mediterranean to 21 % in Northern Continental Populations and up to 23 % in Central Asia. The allele frequency then peaks in Japanese populations with 98-99 %. A comparison of the slopes for the four candidate loci and background SNP shows a less steep clinal pattern within Europe (slope 0.02 vs. 0.04). The background SNPs follow a two-step cline. A first, shallow cline is evident

from Southern - towards Northeastern Europe. The steep cline, which is observed for the four genomic candidates, is then shifted towards Central Asia.

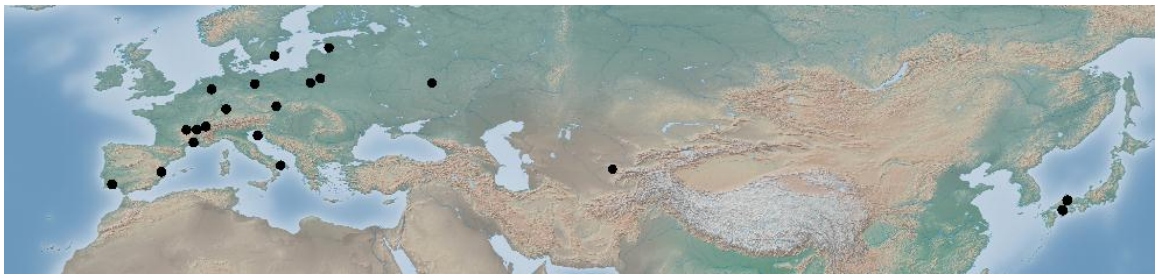

Figure 4.11 Sampling locations of Palearctic populations, which were PCR-genotyped for four genomic candidate loci for selective sweeps. The map was generated using GenGis (Parks et al, 2009).

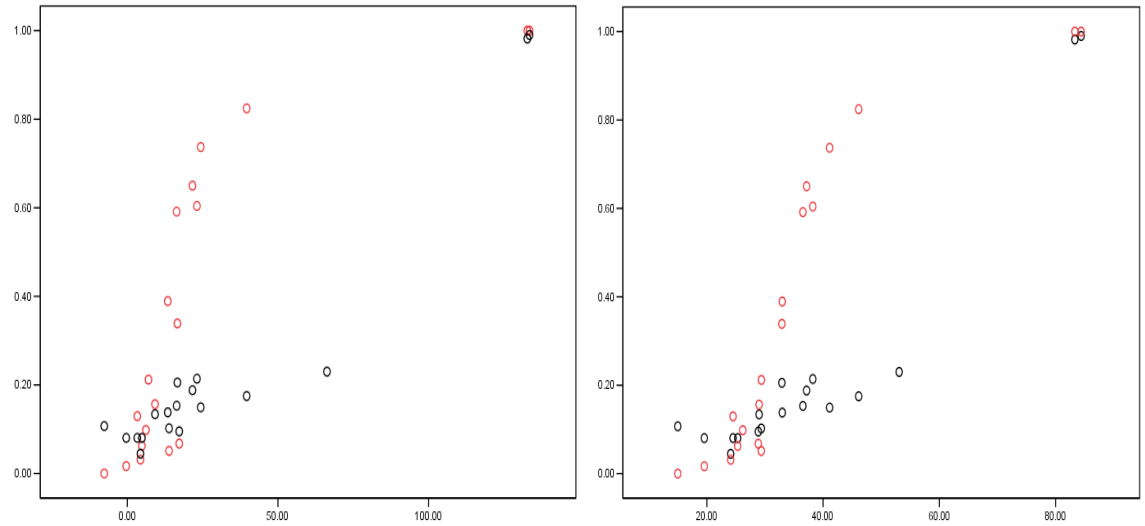

Figure 4.12 Average fraction of Asian alleles for Palearctic wasp spider populations, genotyped for a set of four genomic candidate loci (red) or seven random background SNPs (black). Each dot corresponds to the mean allele frequency of 10 genotyped specimens. The left plot shows allele frequency plotted against geographic longitude. The right graph accounts for a genetic change in northeastern direction, by plotting allele frequencies vs. the mean of latitude and longitude.

4.4 Discussion

Reference genome and transcriptome

Considering the total number of only 13 chromosomes (Araujo et al, 2011) for *Argiope bruennichi*, my genome assembly of ~200.000 contigs is far from being complete. Using further mate pair information, which bridges long stretches of DNA, might however result in a significant improvement (Schatz et al, 2010). An additional linkage mapping will eventually be necessary, to group all major scaffolds onto chromosomes. With

around 1.4 gigabases, the species has a comparably large genome. Spider genomes seem generally to be quite large, usually above one billion basepairs (Gregory & Shorthouse, 2003). The observed higher GC-content for the transcriptome is not surprising and is found in most animal and plant genomes. Moreover, my data clearly shows the importance of an ontogenetic series for transcriptome reconstructions. Using only one ontogenetic stage or sex, a large fraction of the species' actual genes would not have been recovered.

Genetic structure, - diversity and introgression

The genomic data is well explained by the Palearctic-wide phylogeography of the wasp spider (see chapter 1 & 2). The deeply divergent Asian and European clades have probably been isolated since the last glaciations, leading to a considerable amount of nucleotide differences (chapter 2). The highly increased nucleotide diversity, which I observe for the Japanese population, could be explained by a much larger glacial refugium in the Far East. The importance of East Asia as major glacial refugium has been already shown in several species (e.g. Hung et al, 2012).

East Asian spiders did apparently not undergo pronounced reductions in population size during the last glaciations, while European ones have possibly experienced bottlenecks (chapter 2). At least in part, however, the elevated diversity for Japanese samples might also be explained by a different sampling scheme, which I applied for theses spiders. My sampling of Japanese specimens aimed to cover a large part of the islands, includes samples from five distant populations, and thus probably artificially increases diversity.

After their glacial bottleneck, European wasp spiders recolonized the Mediterranean region from their Caucasian refugial area (chapter 1). This probably led to an additional reduction in genetic variation and explains the low diversity in Southern Europe. Such genetic impoverishment is well known to reduce the adaptive potential of a population (Pujol & Pannell, 2008). Southern European populations might have consequently lacked the evolutionary potential to expand their range into continental Northern Europe.

In this regard it is quite interesting that the currently ongoing range expansion is associated with a distinctive introgression of Asian alleles into Northern Europe. This

introgression of Asian genetic material is probably causing the pronounced increase of genetic diversity in Northern European populations. Moreover, an influx of Asian alleles could have supplied new genetic variation to adapt to new environmental conditions at the former range edge and enable a niche shift.

Interestingly, the hybridization with Asian spiders seems to be largely limited to the invasive Northern European range of the species. This observation corresponds well to the assumption that hybridization and admixture will unfold their adaptive value only in a newly colonized ecosystem. For a well-adapted population from the range center, hybridization might lead to swamping, push the population form its adaptive peak and would thus be probably selected against (Verhoeven et al, 2011).

But even in invasive Northern European populations, the amount of Asian alleles, entering the European gene pool, is very limited. With an averaged FST of 0.1 the invasive European spiders are still very closely related to their Southern European relatives, while they share much less genetic variation with East Asia. This highlights a mixed blessing of hybridization even in a newly colonized habitat. The divergent Asian and European populations might have accumulated many genetic incompatibilities during their long isolation (Presgraves, 2010). Loci involved in reproductive isolation, will not cross between different populations and introgression will be limited to selectively neutral or adaptive genomic regions (Arnold & Martin, 2009). This possibly allows Northern European spiders to keep most of their European genomic ancestry. On the other hand, the data could just represent a snapshot in time of an ongoing process of introgression and genomic replacement. Hybridization of distinct species might eventually culminate in assimilation of one lineage by the other (Rhymer & Simberloff, 1996). Previous analyses have shown that the fraction of Asian alleles in Northern Europe has been increasing since about 100 years (Krehenwinkel & Tautz, 2013). This process could still be ongoing, leading to a step-wise gain of Asian ancestry in Northern Europe.

Genomic divergence during a recent range expansion

Northern and Southern European lineages of the wasp spider have genetically diverged since only about 100 years. Nevertheless, the expanding spiders have probably undergone

an ecological niche shift, which enabled their spread into colder continental climate zones (Krehenwinkel & Tautz, 2013). Considering the short time period of separation, the expected genomic signature of such recent divergence would be islands of elevated differentiation in an otherwise undifferentiated genomic background (Via, 2012). Such a pattern of differentiation has already been shown to be directly involved in cold adaptation of eukaryotic microorganisms (Ellison et al, 2011). Genomic islands are expected to start from rather small blocks of DNA around adaptive nucleotides, creating a genome-wide mosaic of divergence (Smadja et al, 2008). The association of further loci of only modest adaptive importance to such divergent regions can then lead to rapid growth of genomic islands to blocks of several million bases. This process was termed divergence hitchhiking (Via, 2012; Feder et al, 2012). Considering the very young divergence of wasp spider lineages, I would still expect rather small regions of divergence. On the other hand, I find recent Asian introgression to be involved in the species´ range shift. If adaptive variation is introduced by hybridization, it will initially be present in very large, introgressed blocks, before recombination can break it down. A recent study in mice showed introgressing blocks to be of several hundred kilobases on average (Staubach et al, 2012). The comparatively small size of my genomic contigs is still a limiting factor for the analysis. A more detailed view on the surrounding regions of each contig would be desirable to gather a significant understanding of genetic variation around possibly selected loci. In fact, the observed divergent contigs might actually be part of recently hitchhiked blocks of many megabases (Michel et al, 2010). However, I find several small divergent regions of only few kilobases. A possible explanation for these comparatively small candidate genomic islands in my data is selection from standing genetic variation (Barrett & Schluter, 2007). Adaptive nucleotides from standing variation have usually been subjected to many generations of recombination and might even have independently arisen several times. A sweep around these variants would consequently leave a smaller and less steep window then a hard sweep on a newly arisen mutation (Barrett & Schluter, 2007). The introgressing Asian genetic variants might have historically recombined with European genomes, before entering the Northern European gene pool. East Asian and European lineages meet in Central Asia, from where Asian genetic material has been entering the Russian steppe since at least 100 years

(Krehenwinkel & Tautz, 2013). The continental steppe regions of Southern Russia offer similar climatic conditions, like the currently colonized Northern European wasp spider habitats (Peel et al, 2007). Potentially adaptive Asian variants could have gone through a filtering process in Central Asia and Russia, before entering Europe. Many generations of recombination might have broken down initially large blocks of Asian DNA and possibly pre-packed it into adaptive complexes. Moreover, the observed regions of divergence could be located in genomic hotspots of recombination (Myers et al, 2005). A vastly increased recombination frequency around these regions could have quickly led to the observed genomic blocks. However, a high recombination rate might prevent the establishment of co-adapted gene complexes and foil adaptation in the first place (Via, 2012). Signatures of genomic divergence are most often observed in regions of low recombination (Nachman & Payseur, 2012).

Apart from a focus on single loci contributing to evolutionary change, one has to consider the possibility of multilocus adaptation. Many subtle allele frequency changes between Northern and Southern European populations could enable adaptation (Pritchard & di Rienzo, 2010) and successful range expansion. Based on my dataset of very closely related populations, I currently cannot rule out this pattern, nor can I confirm it. A parallel screen for allele frequency changes in wasp spider population along similar environmental gradients might help identifying such adaptive alleles. East Asian populations, which colonize a climatic gradient from the tropical South of China up to Southern Siberia (Guttmann, 1979), will probably be well suited candidates for that purpose.

And last, the observed genomic changes between Northern and Southern Europe do not need to have any adaptive explanation. Demographic processes do not necessarily act evenly on each part of the genome and could consequently mimic islands of adaptation (Noor & Bennet, 2009). A parallel test, with different populations under the same selective pressure will help disentangling ecological from demographic responses (Freeland, 2005). By using each two populations from Northern and Southern Europe for my analysis, I am comparing potentially independent evolutionary events. However, these populations are still very closely related. A common bottleneck in the ancestors of

current Baltic and Swedish spiders could have easily affected the current genomic make up of both.

Clinal allele frequency variation over an environmental gradient

While moving from the Mediterranean region towards Northeastern Continental Europe, climatic conditions do not abruptly change. Instead, they transform gradually from e.g. warm to very cold winters. The genomic footprint of adaptation to such an environmental gradient could constitute a genetic cline of increasing allele frequencies. A mutation conferring, e.g. cold tolerance, could be fixed in Northern- and absent from Southern Europe. At the same time, intermediate allele frequencies or heterozygote genotypes might be advantageous in climatic transition zones, where the severity of winter cold is not predictable (Futuyma, 2005). In the absence of gene flow and if one homozygote genotype is favored by selection, a genetic cline is expected to occur in the form of a step. This even holds true for a gradually changing environment (Futuyma, 2005). However, no effective geographic barriers seem to disrupt gene flow in the wasp spider's European range, leading to high genetic homogeneity (Krehenwinkel & Tautz, 2013). Selection for adaptive variants thus needs to counter the effects of migration. Such interplay of gene flow and selection will result in a less steep clinal pattern of allele frequencies. This is especially true for a highly dispersive species like *Argiope bruennichi*. The higher the homogenizing effect of gene flow and the less selection is acting on an allele, the less pronounced a genetic cline is (Sotka & Palumbi, 2006). All the four screened candidate loci show a pronounced clinal pattern of allele frequencies over Europe. This is especially true in comparison to the seven analyzed background loci, which increase in a comparably shallow manner. Interestingly, the observed clines are located in the climatic transition zone between Northern Continental to Oceanic and Mediterranean climate zones (Peel et al, 2007). Selection for adaptation to these climates might consequently shape the clines. *Argiope bruennichi* occurs in huge populations sizes, with a near complete colonization of Europe (Kumschick et al, 2011). A role of genetic drift in affecting the shape of the genetic clines is thus unlikely.

I cannot assign the direction of selection in this European cline. Asian alleles could be adaptive in Northern Europe, but might also be associated with genetic incompatibilities and selected against in Southern Europe. Theoretically both these assumptions could hold true (Sotka & Palumbi, 2006). In this regard, the observed shifted clinal pattern for the seven background loci is particularly noteworthy. The shift might actually indicate an adaptive importance for the four candidate loci in Northern Europe. Introgression of the background loci, on the other hand, could be deleterious even in Continental Northern Europe. Interestingly, one of the screened candidates maps into a cysteine proteinase gene. These proteins are known to be involved in cold tolerance in plants (Grudkowska & Zagdańska, 2004). Moreover, we find a cysteine proteinase gene to be associated with differential gene expression between Northern and Southern European wasp spiders (see chapter 5).

4.5 Outlook

First of all, the connectedness of my current genome assembly will have to be improved. The inclusion of long distance mate pairs is currently under way and will probably help to join contigs, separated by moderately repetitive sequences (Schatz et al, 2010). In addition, I have bred more than 1000 F_2 spiders for the generation of a linkage map. Using these animals' DNA for high throughput genotyping, I might be able to sort the scaffolded contigs into linkage groups. Ideally, these would correspond to the species' 13 chromosomes. Linkage mapping might additionally help to clarify the position of candidate regions, which could be tightly linked or scattered throughout the genome.

Moreover, I will have to develop an appropriate population genetic model to quantify the actual amount of outlier loci and the relevance of introgression in the dataset. The filtering of the upper percentile of divergence only served to gather an initial overview of differentiation.

Studying the role of inversion polymorphisms might be worthwhile for *Argiope bruennichi*. Inversions are well known to be associated with reduced gene flow and adaptive divergence between populations (Feder et al, 2012; Feder & Nosil, 2009). They are, for example, directly associated with climate tolerance in *Drosophila* flies (Sax et al, 2005).

A screen for clinal variation in candidate allele frequency over the spider´s range might be advisable to study the interplay of gene flow and selection shaping genetic structure (Freeland, 2005). An analysis of historical DNA might reveal the pace of the genomic adaptation in *Argiope bruennichi* and its underlying structural changes. By quantifying patterns of linkage in historical DNA, I might be able to estimate the importance of recent recombination during the introgression of adaptive Asian alleles.

The candidate regions, identified in my genome screen, could be cross checked using a QTL or association mapping approach. Genomic candidate regions for certain phenotypic traits should ideally fall into previously identified genomic islands of divergence (Via, 2012).

Eventually, functional test will be necessary to identify the actual contribution of candidate genes to adaptation. Genes could be knocked out or overexpressed in vivo, in order to uncover their role in e.g. cold tolerance (Noor & Feder, 2006).

In the long run, genomic studies on closely related or even distinct species will help to uncover parallel genomic responses in recent adaptation. *Argiope bruennichi* populations in East Asia are already distributed along a comparable climatic gradient, like those in Europe are currently colonizing (Guttmann, 1979). The underlying genomic basis might be similar for European and Asian wasp spiders. The same might hold true for the wasp spider´s sister species *Argiope aurantia*, which is found along an environmental gradient from tropical Central America up to Southern Canada (Platnick, 2013). Several other European species currently undergo similar range expansions into continental climate zones, for example the toxic yellow sac spider (Muster et al, 2008), several butterflies (Hill et al, 2008), dragon flies (Hickling et al, 2005), praying mantids (Krawczynski & Wagner, 2013), and slugs (Engelke et al, 2011). Fundamental evolutionary change during range expansions might be shared between all those species.

Chapter 5: Genome-wide patterns of gene expression divergence and evidence for a thermal niche shift during a contemporary range expansion of a spider species

5.1 Introduction

Gene regulatory changes are believed to play an important role in evolution (Davidson & Erwin, 2006; Oleksiak et al, 2002; Tirosh et al, 2009; Ranz & Machado, 2006). Expression phenotypes are highly flexible and can be fine-tuned on many different levels (Chen & Rajewsky, 2007). Gene expression variation can be based on mutations in regulatory DNA (Wittkopp et al, 2004), posttranscriptional regulation of mRNA levels (Chen & Rajewsky, 2007) and posttranslational modifications of proteins (Gallego & Virshup, 2007). The flexibility of gene expression is additionally increased by an integration of genes into large regulatory networks with conserved hubs and interchangeable external elements (Davidson & Erwin, 2006; Chen & Rajewsky, 2007; Erwin & Davidson, 2009). These numerous levels of gene regulation provide ample raw material for natural selection to act on (Whitehead & Crawford, 2006a; Whitehead & Crawford, 2006b; Oleksiak et al, 2002). And indeed researchers have found large amounts of gene expression divergence between different taxa (Whitehead & Crawford, 2006b) and even closely related populations of the same species (Bryk et al, 2013; Whitehead & Crawford, 2006b). Gene expression responses to environmental stress are known to evolve quickly under laboratory conditions (Bettencourt et al, 1999; Sorensen et al, 2007; & Cooper et al, 2003; Dekel & Alon, 2005; Ferea et al, 1999). And regulatory changes have been directly associated with adaptation to ecological conditions in nature (Mandel et al, 2009; Carleton & Kocher, 2001).

Variation of gene regulation could consequently be an important driver of contemporary evolution (Larsen et al, 2007; Lopez-Maury et al, 2008). In the context of ongoing global change, populations are exposed to rapid alterations of their environment. Temperature is a particularly limiting ecological stressor for many species. (Preisser et al, 2008; Chinnusamy et al, 2007). Evolution of gene expression could constitute an early phenotypic response to such environmental stress (Crawford & Powers, 1992; Lopez-Maury et al, 2008). On the other hand, a large extent of gene expression variation seems

to have evolved by the acquisition of selectively neutral changes in regulatory sequences (Staubach et al, 2009). And despite its potential flexibility, the regulation of many genes is stabilized by selection and expression changes can be highly maladaptive (Whitehead & Crawford, 2006b). Recent adaptation by positive selection might thus be the exception rather than the rule (Gilad et al, 2006). Phenotypic plasticity of gene expression could allow circumventing this constraint. A gene's expression level would only be altered during certain environmental stress, while it remains unchanged during normal conditions. Even genes, whose transcription rate is highly stabilized by selection, could be sub-functionalized this way.

In this chapter I use the European wasp spider *Argiope bruennichi* for studying transcriptome changes, associated with thermal adaptation during a contemporary range expansion. This originally Mediterranean species has expanded its range into increasingly colder regions of Northern Europe within the past decades (Kumschick et al, 2011). A previous reciprocal transplant experiment has shown that this range expansion is associated with rapid local adaptation to the novel climatic conditions in Northern Europe (Chapter 1 - Krehenwinkel & Tautz, 2013). Here, I introduce additional evidence for a climatic niche shift from a heat and cold tolerance experiment with first instar wasp spiders from Northern and Southern Europe. Based on these experiments, I analyze genome-wide gene expression variation between native Southern and expansive Northern European spiders in a reciprocal transplant design. I exposed first instar offspring to heat or cold before measuring gene expression. This pretreatment was supposed to mimic the actual ecological stress in respective habitats. I discuss the results in relation to the probability of ecological divergence in gene regulation. I then ask, whether Northern European wasp spiders have acquired novel plastic expression responses or if expression changes largely rely on modifications of already existing expression patterns. Last, I search for an enrichment of known stress related genes in the data to uncover possible evolutionary divergence due to thermal stress.

To summarize, the data indicates a clear divergence of gene expression during a climatic niche shift. A large number of genes are only differentially expressed during thermal stress conditions. This pattern of plasticity did not newly evolve in Northern Europe. Instead, invasive wasp spiders seem to rely on fine tuning of already existing plastic

expression responses. A majority of expression changes is based on down-regulation in Northern European populations. I speculate that this down-regulation might be a signature of an obligate diapausing state in Northern European wasp spiders as initial adaptation to colder winters.

5.2 Methods

Analysis of gene expression divergence

Reciprocal transplant experiment and transcriptome sequencing

Argiope bruennichi is an annual species, which overwinters as first instar nymphs in the protective silk case of the eggsac, before they hatch in late spring (Köhler & Schäller, 1987). The spiders construct their eggsac on grass stalks, leaving them directly exposed to environmental influences. Temperature probably constitutes a major environmental difference for invasive and native spider populations (Krehenwinkel & Tautz, 2013). Southern European spiders can be exposed to pronounced heat in early autumn, while Northern European ones will have to cope with subzero temperatures. The first instar is thus ideally suited to study thermal adaptation in *Argiope bruennichi*.

The subsequently described reciprocal transplant experiment served to study gene expression responses to opposing temperature stresses in Northern and Southern Europe, respectively. The experiments were carried out in the early overwintering phase and thus simulated Northern and Southern European locations during autumn. The normal conditions in the experiment were supposed to simulate a common autumn temperature for Northern and Southern Europe. The heat treatment simulated a hot autumn day in the Mediterranean region. And the exposure to subzero temperatures mimicked a cold autumn day in Northern Europe.

Mated wasp spider females were collected in August 2012 in Southern Portugal, Latvia and Estonia. Portuguese spiders represent the historical native Mediterranean range of the species. The Baltic populations have been found only a few years ago and thus constitute the invasion's wave front (see Figure 5.1 for collection sites).

Figure 5.1 Sampling sites for wasp spiders, which were used for the gene expression experiment. The black dots correspond to samples used for the chill tolerance experiment. Red dots signify sampling locations of specimens, which were additionally used for the gene expression experiment. The map was generated using GenGis (Parks et al, 2009).

The spiders were kept in the laboratory at room temperature (20°C) in 200 ml plastic cups, sprayed with water every second day and supplied with house flies, till they constructed an eggsac. I initially set up a day and night cycle of 14 hours light followed by 10 hours of darkness.

After eggsac construction, the mother was removed from the cup, and stored in 70% Ethanol for further analysis. All eggsacs were then kept at room temperature (20°C) for four weeks and sprayed with water every day. Subsequently, the temperature was lowered to 15°C, the light cycle reverted to 10 hours of light and the eggsacs kept for additional two weeks. After six weeks, six eggsacs each from the Baltic and Portuguese populations were chosen for the reciprocal transplant experiment. I chose eggsacs, which had been built only a few days apart, to reduce age effects in the experiment. Each eggsac was opened and the spiderlings forced to emerge. 20 spiders per eggsac were then transferred to three Petri dishes, equipped with a slightly wetted paper tissue. One dish per eggsac was transferred into a Memmert IPP800 thermal chamber (Memmert, Schwabach, Germany) at 15°C. Three distinct thermal profiles were then set for the incubators. A control treatment was permanently set to 15°C, a cold treatment started at

15°C and lowered by 5°C every 2 hours. A heat treatment started at 15°C and increased by 5°C every two hours. The spiders were kept under these conditions for 10 hours, allowing the cold treatment to reach -10°C and the heat treatment to peak at 40°C. The Petri dishes were then taken out of the thermal chambers. The spiderlings were immediately snap frozen in liquid nitrogen and then stored on dry ice.

Total RNA was extracted from the spiders using the Qiagen RNeasy Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The samples were sent to the Center for Genomics in Cologne for library preparation and sequencing (as described in chapter 4). The 36 samples were sequenced on two lanes of an Illumina HiSeq 2000 according to the manufacturer's protocol (Illumina, San Diego, USA).

Gene expression analysis

The reciprocal transplant data was analyzed using CLC genomics workbench (CLC Bio, Cambridge, USA). Each library was quality trimmed with a minimum quality score of 0.05 and a maximum number of two ambiguous nucleotides. The trimmed reads were then aligned to the previously generated reference transcriptome (Chapter 4), with a mismatch cost of 1, insertion cost of 3, deletion cost of 3. Gene expression was analyzed based on RPKM values (reads per kilobase per million). A quantile correction (Bolstad et al, 2003) was applied for data normalization and a normalized RPKM of 0.5 per group used as threshold for a transcript to be considered in the analysis (as e.g. in Strout et al, 2011). Significant differences in gene expression were evaluated using Baggerley's test (Baggerly et al, 2003) at an FDR of 0.05. I first tested for expression changes between Northern and Southern European populations. In addition, I tested for differentially expressed genes between cold treatment and normal conditions, as well as heat treatment and normal conditions within Northern and Southern European populations. Using these conditions, lists of differentially expressed genes were prepared. The resulting sets of differentially expressed genes between Northern and Southern populations were then subjected to further analysis. As Northern European populations have been recently established in the course of the spider's expansion, I interpreted the expression data of these samples as derived state. Expression of Portuguese specimens, on the other hand, served as ancestral trait. I compared the direction of expression changes between

Northern and Southern European populations by counting the numbers of up- or down-regulated transcripts per region and experimental condition. I additionally analyzed fold-change in expression for all differentially expressed genes. I further quantified and compared the amount and variation of differential gene expression for the six tested RNA pools. I blasted all transcripts using the BLASTX implementation in Blast2Go (Conesa et al, 2005) with an E-value cutoff of 10^{-3}. BLAST hits were then annotated (with an E-value cutoff of 10^{-6}, an annotation cutoff of 55 and a GO-weight of 5) and datasets of differentially expressed genes tested for GO-term enrichment (using Fisher's exact test with an FDR of 0.05).

Testing for plasticity of gene expression

I evaluated the contribution of phenotypic plasticity to the observed differential expression values between Baltic and Portuguese populations. For that reason, I quantified condition dependent changes in transcript abundance. A gene could respond to environmental stress in several ways. Its expression might be similar under all experimental conditions or it could only change during certain stress conditions. This, in turn, would lead to different reaction norms of expression at changing temperatures. I thus estimated the amount of changes in expression patterns for differentially expressed transcripts between Portuguese and Baltic spiders across the experimental temperature gradient. First, I tested for significant departures from even expression levels within populations, but between experimental conditions by using an ANOVA (using a Bonferroni posthoc test and a p-value cutoff of 0.05). A significant difference between e.g. normal and cold conditions signifies environment dependent plasticity. All genes with a significant plastic response were then tested for population differences in that response.

Heat and cold tolerance experiments

I tested cold tolerance and chill coma recovery in spiderlings from 16 Portuguese and 36 Baltic eggsacs. They originated from the same stock of mated females as described above for the gene expression analysis. In addition, I collected mated females from Southern

Sweden and included 15 Swedish eggsacs (Figure 5.1). Ten spiders per eggsac were put into a petridish. As soon as five petridishes were prepared, they were placed in a freezer for six hours at -20°C. This freezing time was chosen based on a test series from 10 minutes to 12 hours, with six hours showing the most distinctive differences. It is well known that cold adapted arthropods can easily withstand long periods of subzero temperatures (Preisser et al, 2008). Spider mites for example did easily survive many hours of freezing at -24°C (So & Takafuji, 1992). After the cold treatment, the spiders were allowed to recover in a tempered room at exactly 22°C for 120 minutes. During that time, the number of recovered spiders was counted every 30 minutes. 24 hours after the cold treatment, the fraction of spiders per eggsac was counted, which did not survive the freezing or were permanently damaged (showed problems walking properly). Moreover, I used data from a previous heat tolerance experiment (Chapter 1 - Krehenwinkel & Tautz, 2013). To make the data comparable to the cold stress experiment, I used only Baltic and Swedish data from the thermal stress test. Briefly, the little spiders were gradually heated up until they fell into rigor. The temperature of heat knockdown was then noted (see chapter 1).

5.3 Results

Heat and cold tolerance experiments

Compared to their Southern relatives, Northern European wasp spiders have shifted their thermal tolerance. They show a significantly reduced heat resistance (average heat knock down temperatures are 48°C vs. 44°C; t-test, equal variances assumed, $p < 0.001$), but are more tolerant to cold. 24 hours after a six hours cold shock at -20°C, I find 79 % of the Southern European spiderlings to be dead or severely damaged (could not walk properly), but only 37 % of the Northern European ones. The difference is highly significant (t-test, equal variances assumed, $p < 0.001$) (Figure 5.2). Northern European spiders do also recover significantly faster from cold knockdown than Southern ones (ANOVA, Bonferroni post hoc test, $p < 0.05$). 30 minutes after freezing, on average 12 % of the Northern European spiders have already recovered. At the same time only 1% of the Southern European ones have recovered. After 60 minutes this difference shifts to 41 %

vs. 15 %, after 90 minutes to 58 % vs. 31 % and after 120 minutes to 71 % vs. 50 % (Figure 5.3).

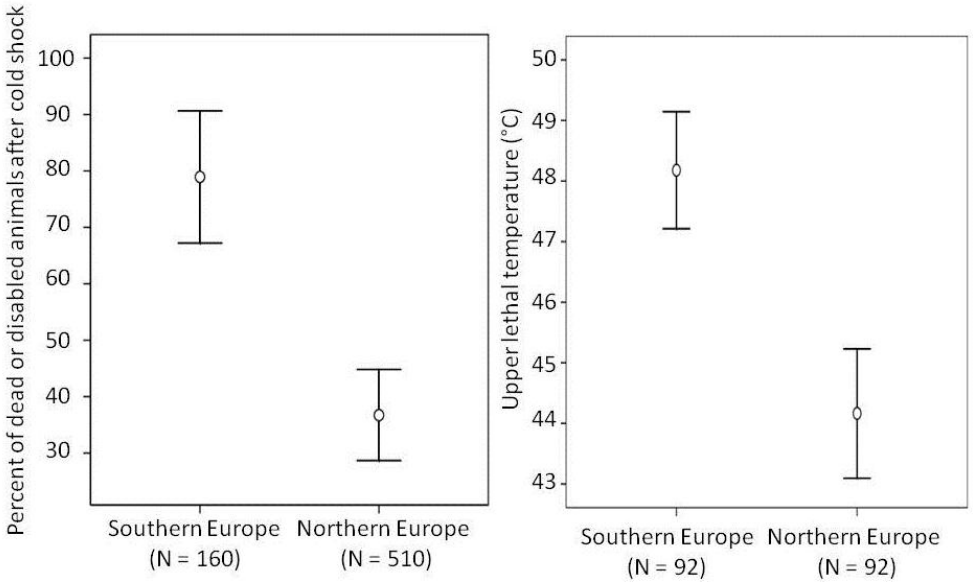

Figure 5.2 Heat and cold tolerance of Southern and Northern European wasp spider nymphs. The left plot depicts the mean percentage of dead or severely damaged spiders 24 hours after a cold shock of -20°C. The right plot shows the mean upper lethal temperature. The bars show the 95 % confidence intervals. The differences between Northern and Southern European spiders are signifcant for both experimental treatments (t-test, p < 0.001).

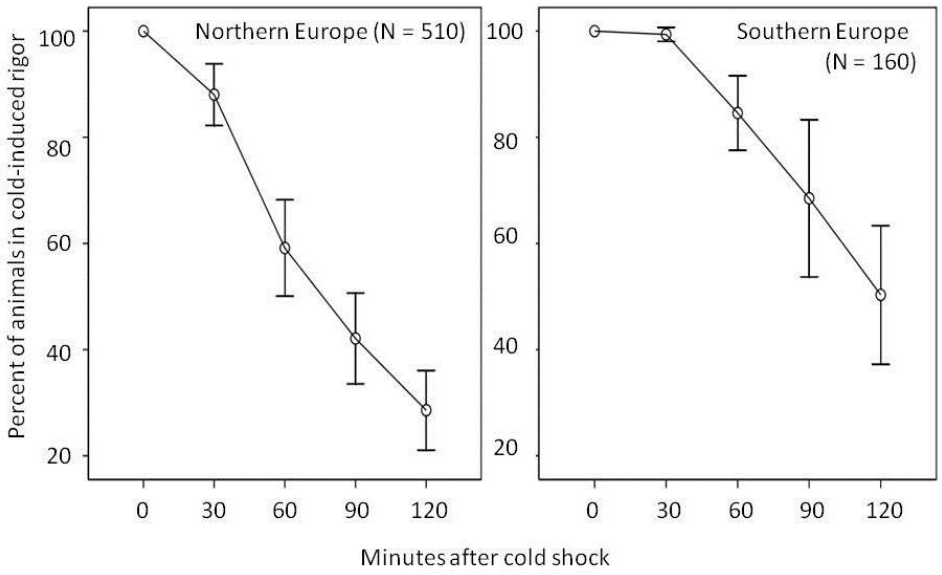

Figure 5.3 Cold shock recovery for Northern and Southern European wasp spider nymphs. The plots depict the mean percentage of spiders in cold rigor directly after a -20°C cold shock, and after 30, 60, 90 and 120 minutes of recovery time. Bars show the 95 % confidence interval of the mean. The differences between groups are significant (ANOVA, Bonferroni post hoc test, p < 0.05).

Gene expression analysis

Condition dependent gene expression within populations

First, I present an analysis of gene expression differentiation within populations, i.e. the innately regulated differences. I highlight significant expression differences between the heat and control treatment and between the cold and control treatment for Baltic and Portuguese spiders separately.

The analysis revealed generally a very pronounced response to heat stress (Figure 5.4). Out of more than 50,000 analyzed transcripts, nearly 4,000 genes were differentially expressed during heat stress compared to normal conditions in Baltic and Portuguese populations. This response is very similar for Portuguese and Baltic populations. This holds true for the number of differentially regulated genes, as well as the direction of expression. 1,717 out of 3,575 genes in Portuguese and 1,700 out of 3,593 in Baltic spiders were up-regulated during heat stress (Figure 5.4). This difference between up- and down-regulated transcript numbers is significant for the Baltic populations (Fisher's exact test, $p < 0.05$), but not for the Portuguese. GO-term enrichment for heat responsive transcripts indicates an association with protein synthesis. Genes connected with the nucleolus, the endoplasmatic reticulum and the ribosomes were significantly overrepresented.

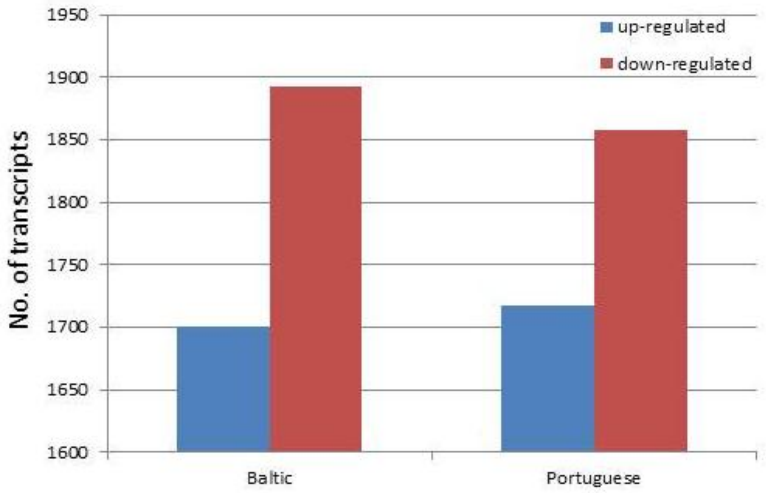

Figure 5.4 Number of differentially expressed transcripts during heat stress compared to control conditions for Baltic and Portuguese populations. Slightly more transcripts are down-regulated in the Baltic population. The bias towards down-regulated transcripts is significantly different from even expectations in Baltic populations (Fisher's exact test, $p < 0.05$)

In contrast to the large number of heat responsive transcripts, only two genes were found to be differentially expressed between cold and normal conditions for Portuguese spiders. One additional transcript was exclusively cold responsive in Baltic spiders. The expression of all these genes follows a very similar trend in Baltic and Portuguese populations (Figure 5.5). The two shared differentially expressed transcripts have significant BLAST hits for glucose-6-phosphatase and alanine-aminotransferase. Both genes are associated with Gluconeogenesis. Ubiquitin-C is significantly up-regulated only in Baltic populations during cold stress. However, the expression level observed for Ubiquitin is very similar in Baltic and Portuguese spiders.

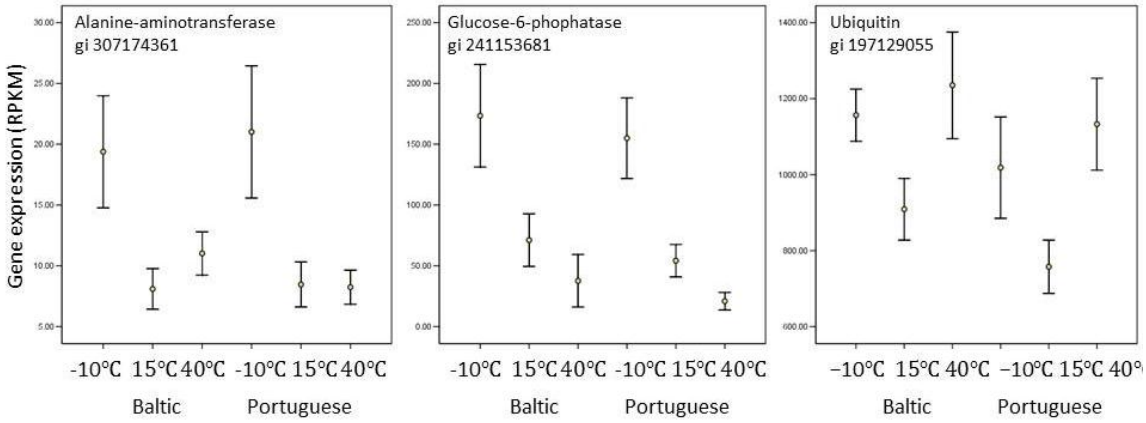

Figure 5.5 Expression levels of differentially expressed genes during cold stress, compared to control conditions. Alanine-aminotransferase (left plot) and glucose-6-phophatase (middle plot) are up-regulated during cold stress both in Baltic and Portuguese spiders. Ubiquitin (right plot) is significantly up-regulated only in Baltic spiders, although a similar trend of expression is evident in Portuguese ones. Plots depict mean expression and 95% confidence intervals of the mean.

Differential gene expression between Northern and Southern European animals

Now I will present differential gene expression between Northern and Southern European spider populations, i.e. the evolved expression differences. Using an RPKM cutoff of 0.5, I analyzed the expression profiles of 52,717 genes between Portuguese and Baltic spider populations. Only a small fraction of genes was differentially expressed between the two groups across all experimental treatments. Using an FDR of 0.05, I identified a total of 270 differentially expressed genes in the different treatments. Differential gene expression is generally biased towards heat responsive transcription. 162 genes were

differentially expressed during heat stress (40°C), 103 at control conditions (15°C) and 115 during cold treatment (-10°C). Many transcripts are differentially expressed in two or more experimental conditions. (Figure 5.6).

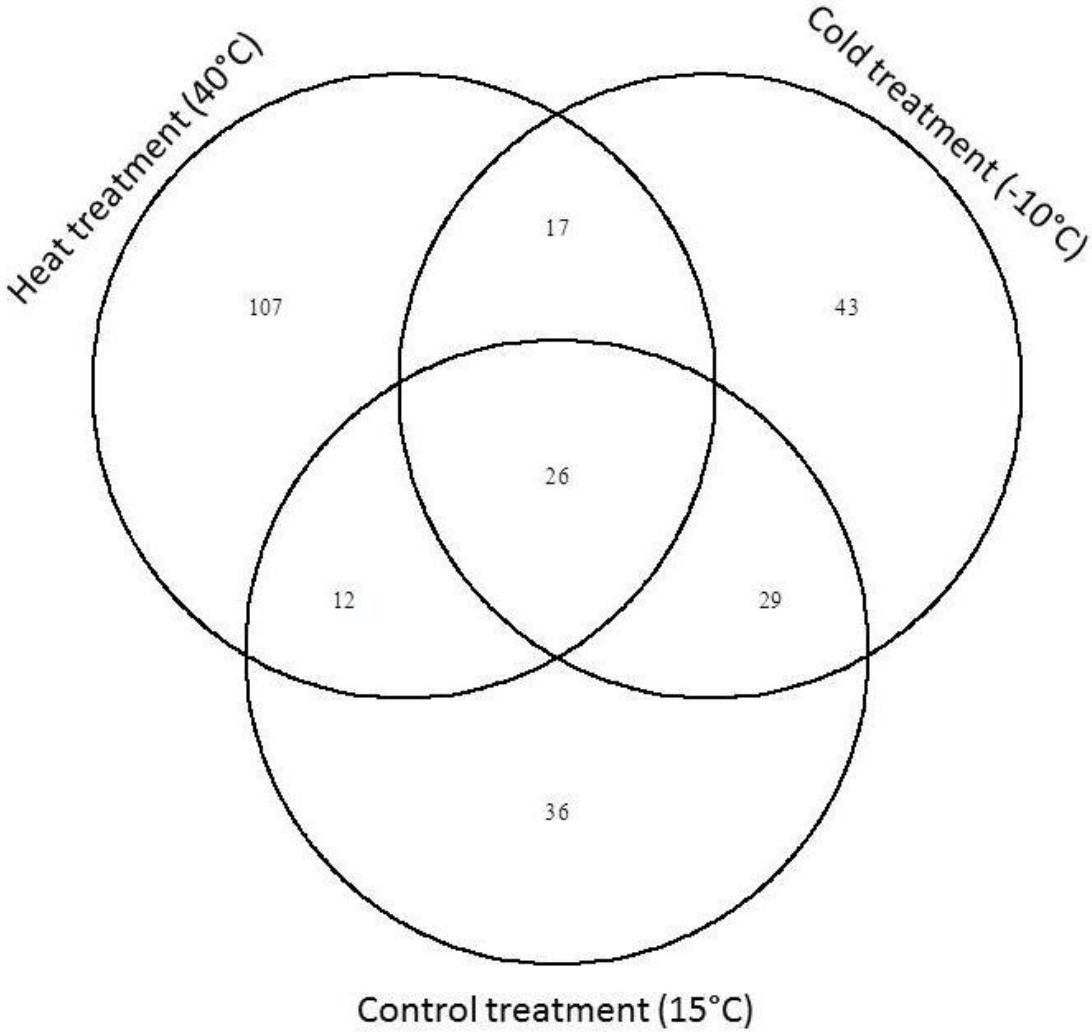

Figure 5.6 Number of genes differentially expressed between Northern and Southern European wasp spider populations during different experimental conditions.

I find a higher number of down-regulated genes for Baltic spiders during all experimental treatments compared to Portuguese ones (Figure 5.7). This difference departs significantly from an even expectation during cold stress and control conditions (Fisher's exact test, two tailed $p < 0.05$).

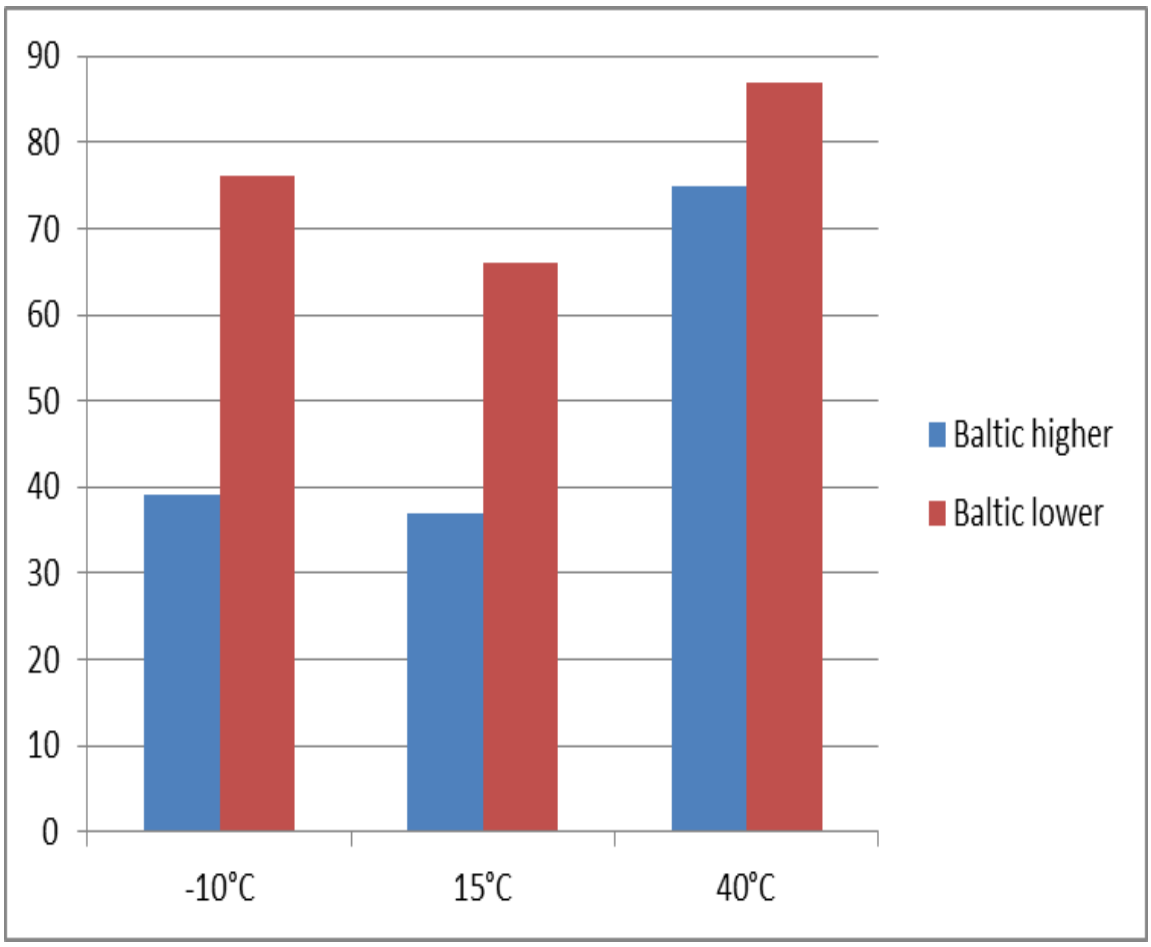

Figure 5.7 Number of differentially expressed genes, which are up-regulated (blue bars) or down-regulated in Baltic spiders compared to Portuguese ones. The fraction of down-regulated transcripts in Baltic spiders departs significantly from an even expectation during cold stress and control conditions (Fisher's exact test, two tailed $p < 0.05$).

I do not identify significant differences in fold change of gene expression between Baltic and Portuguese populations. However, I discover a reduced variation of fold-change for down-regulated genes compared to up-regulated ones (Levene's test $p < 0.05$). This increased variation is due to few extreme values of up-regulated transcripts with very high expression fold change (Figure 5.8).

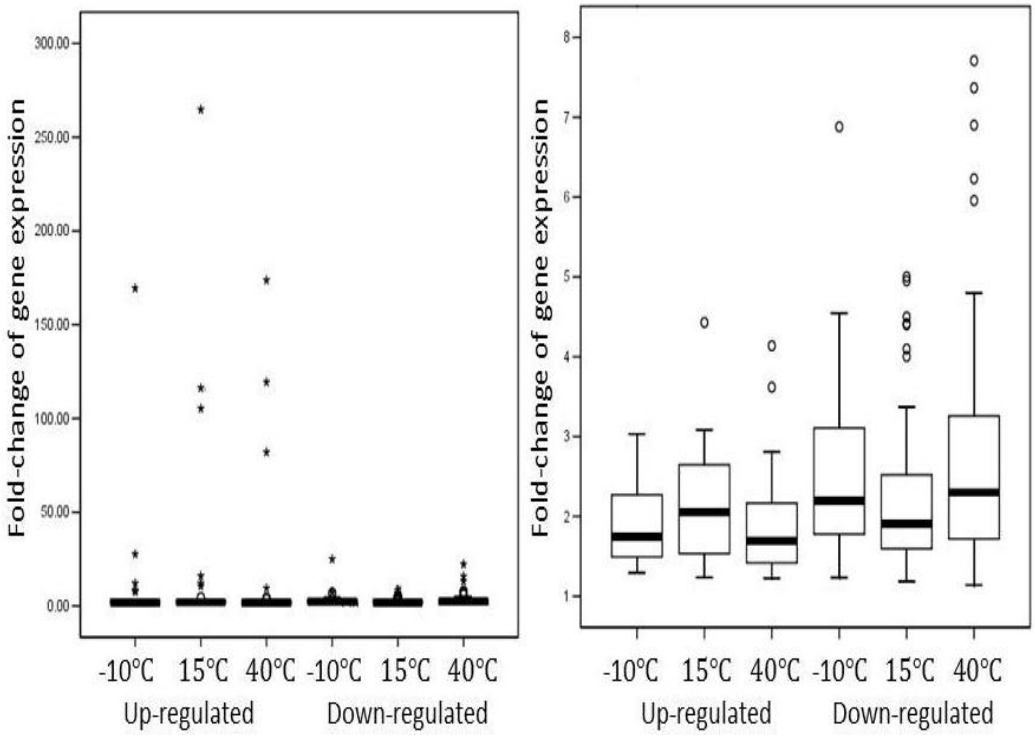

Figure 5.8 Fold-change of gene expression for differentially expressed transcripts between Baltic and Portuguese populations. The plots show up-regulated and down-regulated transcripts and different experimental conditions separately. The boxes show the median (black bars) and upper and lower quartile. Outliers are shown as circles, extreme values as stars. The left plot includes extreme values, while they have been removed in the right one. The significant difference of variation for fold-change between up- and down-regulated transcripts is due to a few highly differentially expressed extreme values.

I identified only slight differences in the mean expression and expression variation between Northern and Southern European populations. Generally, the higher expression levels are observed during heat stress and the lower during cold treatment or control conditions. The observed gene expression differences are, however, not significant (tested with an ANOVA). The same holds true for the variation of gene expression, which is not significantly different during all experimental conditions and between both populations (Figure 5.9).

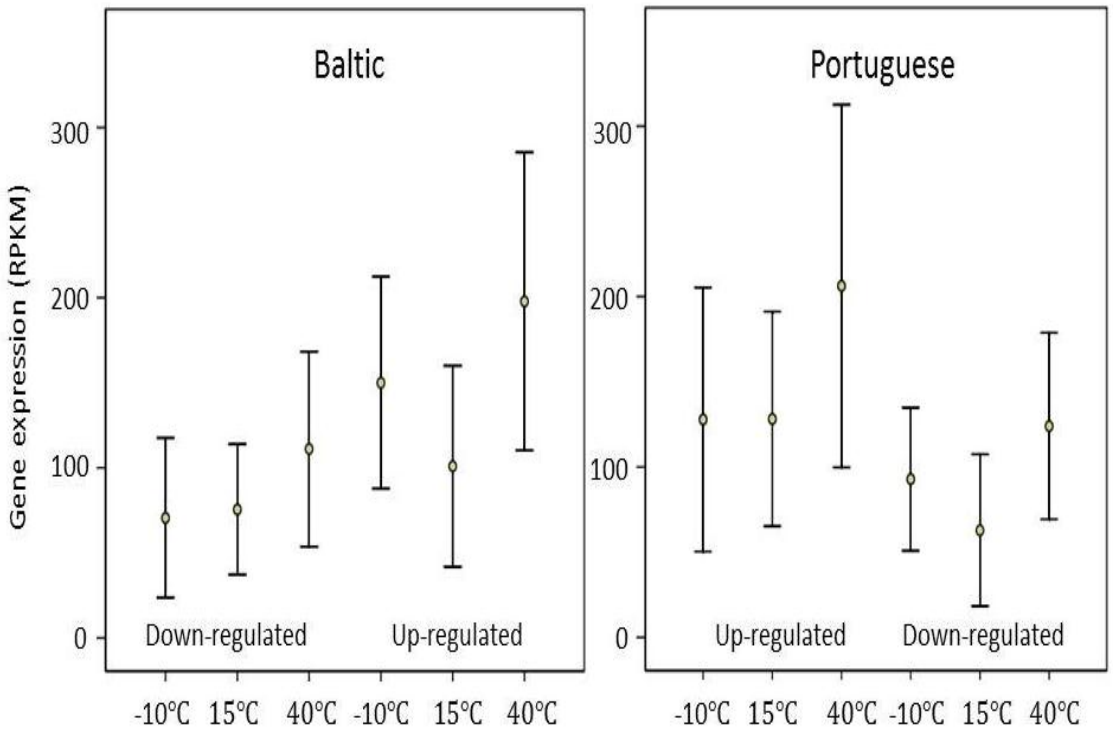

Figure 5.9 Mean gene expression values (RPKM) for down-regulated and up-regulated transcripts. The left plot shows mean expression values for differentially expressed genes in Baltic populations, which are down-regulated, or up-regulated in comparison to Portuguese ones. The right plot presents the same pattern for Portuguese populations. Bars depict the 95 % confidence interval of the mean.

Possible functions of differentially expressed genes between Northern and Southern Europe

A test for GO-term enrichment for the differentially expressed transcripts in comparison to the whole set of annotated genes, did not reveal any significantly enriched GO category. In order to gather insights into the functional importance of differentially expressed genes, I inspected the BLAST results for gene functions, with a focus on thermal stress. A selection of candidate genes and their respective BLAST hits is shown in Table 5.1. In the following, I will present some particularly interesting candidate genes, whose expression pattern distinguishes Northern and Southern European populations. Northern European spiders are more tolerant to cold, Southern European can endure higher temperatures. Gene expression patterns of the presented transcripts could thus directly relate to these tolerance phenotypes.

Table 5.1 Selection of BLAST hits for potentially ecologically important differentially expressed transcripts between Northern and Southern Europe. Rows printed in bold letters indicate transcripts, whose expression pattern is plotted in Figures 5. 8 - 5.11. Column two and three indicate if the transcript was up-regulated (B>) or down-regulated (B<) in Baltic populations. In addition the experimental conditions, at which differential expression was observed, are shown in column two and three (-10° C, Control, 40° C).

RNA contig no.	B>	B<	Gi number	Gene	Organism	Function
scaffold8541	**10**		**391348629**	**Cuticle protein**	***M. occidentalis***	**Cuticular structure**
C567890		**40**	**1345866**	**Cuticle protein**	***A. diadematus***	**Cuticular structure**
C576678		**10 40**	**1345866**	**Cuticle protein**	***A. diadematus***	**Cuticular structure**
scaffold19587	10 C 40		118094727	Cytochrome P450	*G. gallus*	Detoxification
C605328		10 C 40	30840237	Cytochrome P450	*H. sapiens*	Detoxification
scaffold9213		**10 C**	**405961234**	**Maltase**	***C. gigas***	**Digestion**
scaffold8606		**10 C 40**	**390340227**	**Deoxyribonuclease-1**	***S. purpuratus***	**Digestion**
scaffold14915		**10 40**	**318087032**	**Astacin-like metalloprotease**	***L. hesperus***	**Ecdysis**
C585262	**10 C**		**240953838**	**Phosphatidylinositol transfer protein**	***Ixodes scapularis***	**Lipid transport**
scaffold27303		**C**	**209165353**	**Aggregate spider glue**	***N. clavipes***	**Prey capture**
scaffold8666		40	475392972	Toxin GTx-VA1	*G. rosea*	Prey capture
scaffold31115		**10 C 40**	**209165611**	**Toxin-like structure LSTX-B7**	***L. singoriensis***	**Prey capture**
C491425		10 C	55274120	Dragline silk spidroin	*Macrothele holsti*	Prey capture
C530662		**10 C**	**193506891**	**Major ampullate spidroin**	***N. clavipes***	**Prey capture**
C587118		10 40	359552636	Cystatin (cysteinprotease inhibitor)	*A. ventricosus*	Stress
C585880		**10 C 40**	**344953542**	**Cathepsin**	***E. coioides***	**Stress**
scaffold24855	**10 C 40**		**307175778**	**Cysteine proteinase**	***C. floridanus***	**Stress**
scaffold28307	**10**		**391326081**	**Na-K ATPase**	***M. occidentalis***	**Thermal tolerance**

I find an up-regulation of cysteine proteinase in Northern European populations and an up-regulation of a cysteine proteinase inhibitor in Portuguese spiders (Table 5.1). Other up-regulated genes in Northern European populations are Sodium Potassium ATPase, a cuticular protein and a phospholipid transporter protein. All these genes have a potential association with cold tolerance (see discussion). Nevertheless, their expression pattern is not biased towards cold conditions. Instead, I find a general increase of condition

independent expression or an up-regulation during cold stress and control conditions (Figure 5.10).

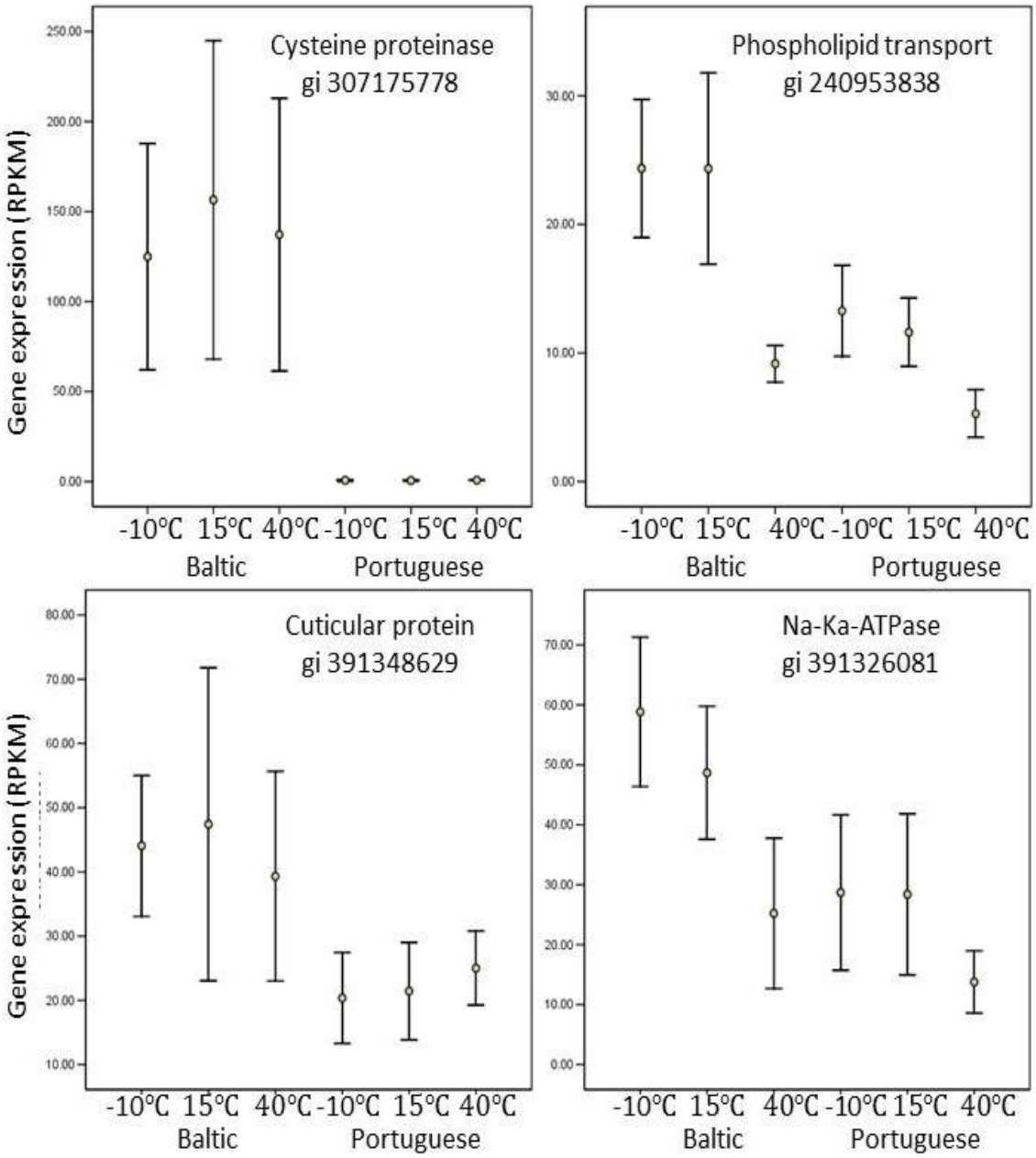

Figure 5.10 The plots depict mean expression values (RPKM) and 95% confidence intervals of the mean for selected differentially expressed genes between Baltic and Portuguese populations. Several up-regulated genes in Northern European populations can be associated with thermal stress tolerance (see discussion).

I observe an overexpression of cold stress-associated transcripts in Northern European spiders. At the same time the expression of several transcripts can be associated with heat conditions (see discussion). For example, I find several cuticular proteins and cathepsin to be down-regulated in Northern European spiders. Several presumptive heat responsive genes are exclusively up-regulated during high temperatures (Figure 5.11). Cold responsive ones, on the other hand, seem to be expressed less condition dependent (Figure 5.10).

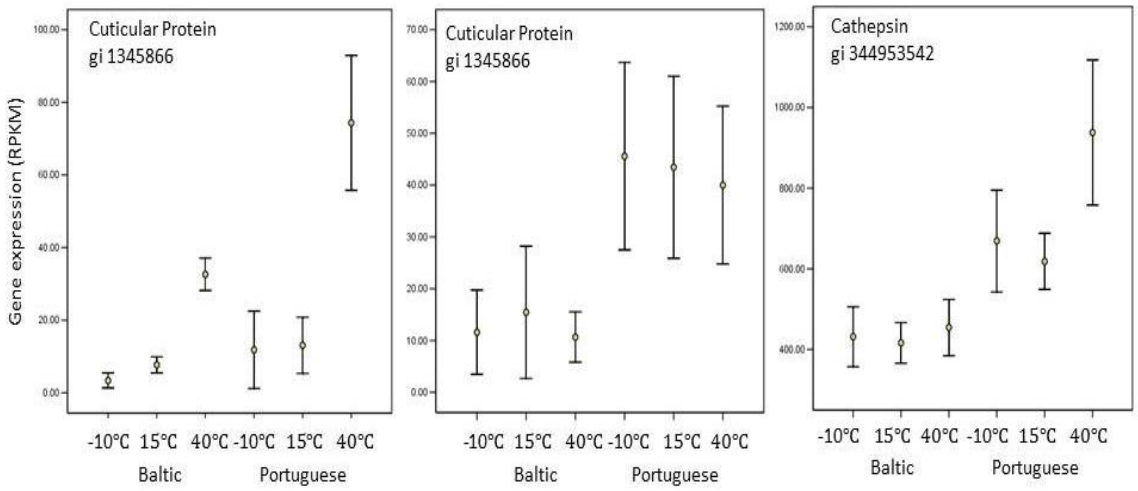

Figure 5.11 The plots depict mean expression values (RPKM) and 95 % confidence interval of the mean for selected differentially expressed genes between Baltic and Portuguese populations. Southern European populations show higher expression values for cathepsin and certain cuticular proteins during heat stress.

Apart from heat stress related gene expression, Southern European spiders show an up-regulation of several transcripts related to metabolic activity. Some of these are directly associated with prey capture, e.g., silk, toxin and capture glue genes (Table 5.1 & Figure 5.12). Moreover, genes related to digestion are overexpressed in Portuguese populations, e.g., a maltase and DNAse gene. In addition, I find increased expression of an astacin-like-metalloprotease in Southern spiders. This gene is known to be associated with ecdysis (Table 5.1, discussion).

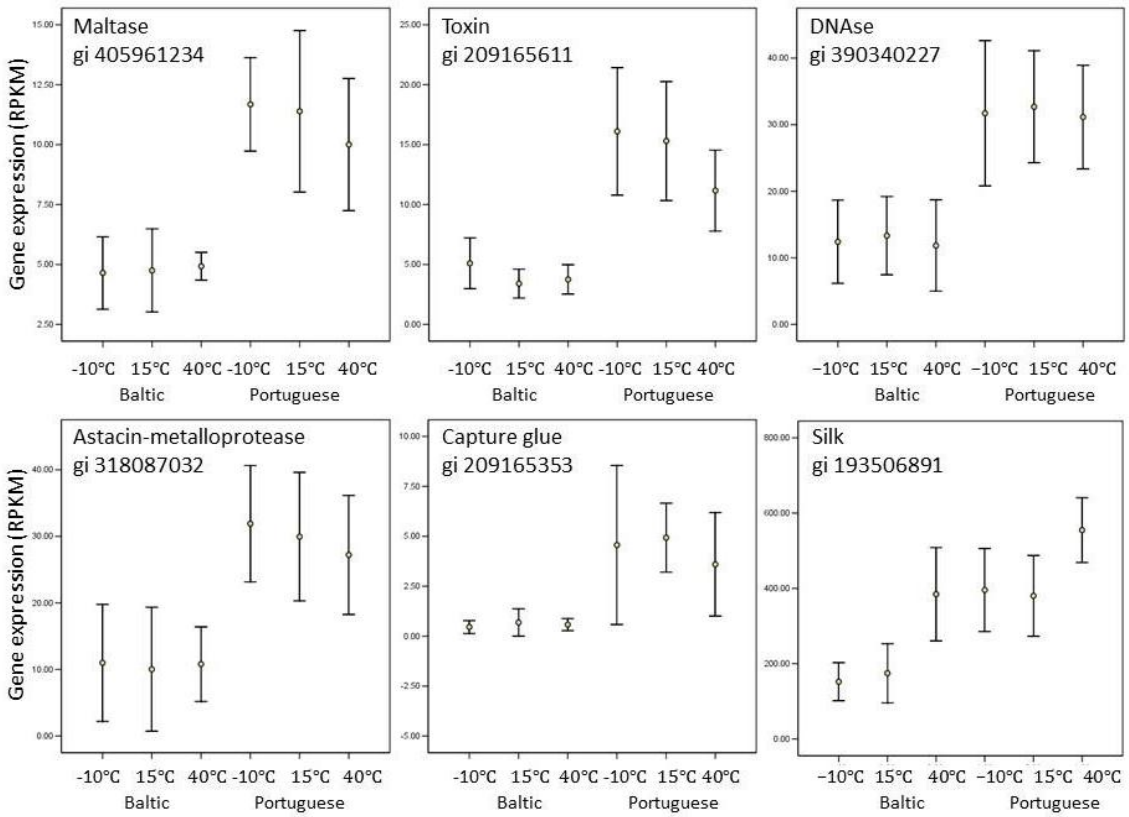

Figure 5.12 The plots depict mean expression values (RPKM) and 95% confidence intervals of the mean for selected differentially expressed genes between Baltic and Portuguese populations. Several genes related to prey capture (e.g. capture glue, silk, toxin), digestion (maltase, DNAse) and growth (e.g. astacin-metalloprotease) are down-regulated in Northern European spiders.

Testing for plasticity of gene expression

The screen for plastic responses of gene expression for differentially expressed transcripts suggested the following six general response forms of transcription at different thermal regimes (Figure 5.13). **1.** The gene did not show any significant plasticity of expression. **2.** An up-regulation during heat stress was observed. **3.** The transcript was down-regulated during heat stress. **4.** Cold stress induced elevated expression. **5.** The highest expression was observed during control conditions. **6.** The lowest expression was found during control conditions. I did not find any transcript that was significantly down-regulated only at -10°C. I also did not find significant stepwise patterns of expression with a gradual increase or decrease with temperature (ANOVA, $p < 0.05$).

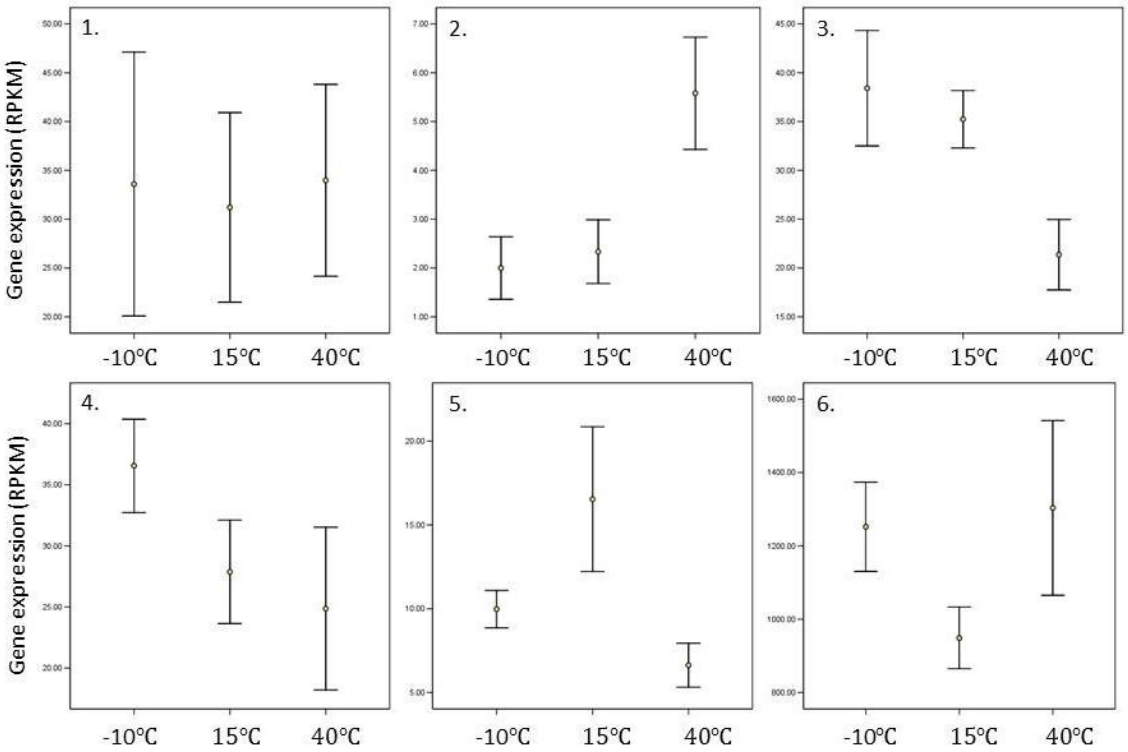

Figure 5.13 Exemplary reaction norms of gene expression during different experimental conditions. The plots depict mean expression values and 95% confidence intervals of the mean. 1. Shows an even pattern of expression, independent of thermal condition. 2. Gene expression is up-regulated during heat stress. 3. Gene expression is down-regulated during heat stress. 4. Cold induced up-regulation of a transcript. 6. Highest expression during control conditions and 6. lowest expression during control conditions.

The expression level of 154 out of 270 differentially expressed genes did not significantly depart from evenness (form **1.**) between experimental conditions. For these 154 transcripts, differential expression between Baltic and Portuguese populations is achieved by a general up- or down-regulation during all experimental conditions (an example is seen e.g. in the DNAse in Figure 5.12).

The 116 remaining differentially expressed transcripts followed forms **2. - 6.** in Baltic and/or Portuguese populations and thus show a plastic expression pattern. I found a change in the direction of the norms of reaction between Portuguese and Baltic populations for only 61 of these 116 transcripts. The reaction norm modifications are highly biased towards changes in the heat response. 39 expression patterns transform from heat responsive (form **2.** and **3.**) in Portuguese to an even pattern (form **1.**) in Baltic

populations (an example is seen in Cathepsin in Figure 5.11). An additional 18 transcripts respond in the reverse order from even to heat responsive in Baltic spiders.

I found only one transcript changing from highest expression during control conditions (form **5.**) in Portuguese samples to a decrease during heat stress in Baltic ones (form **3.**). One additional transcript changes from a pattern of up-regulation during heat stress to decreased expression at the control (form **6.**). And last, the expression patterns of only two transcripts of Baltic spiders transform towards up-regulation during cold stress. One changes from an even pattern and one from an up-regulated one at 40°C.

Most changes in reaction norm are the result of decreased transcription rates in Baltic specimens compared to Portuguese ones (Fisher's exact test, two tailed $p < 0.005$). I can confirm this pattern for 45 out of 61 genes. A significant down-regulation is evident for 29 transcripts during cold stress, 31 at control conditions and 19 during heat stress. At the same time, only 10 transcripts are contributing to reaction norm changes by significant up-regulation in Baltic populations during cold conditions, 8 in the control and 9 at the heat treatment.

To summarize, the majority of differentially expressed transcripts (154/270) is not expressed environmentally plastic. Moreover, plastic expression is largely biased towards heat responsive gene expression. A change in the actual reaction norm of expression between Northern and Southern European populations is rarely observed and mainly induced by down-regulation of transcripts in Baltic populations. Instead of *de novo* emergence of new reaction norms, the data indicate that expression differentiation relies on alterations of already existing plastic responses or a condition independent general up- or down-regulation.

5.4 Discussion

Range expansion, thermal niche shift and ecological divergence of gene expression

Climatic stress is known as major limitation to species distributions, and overcoming this limitation might enable rapid distributional shifts (Preisser et al, 2008; Bettencourt et al, 1999). At the same time, thermal tolerance is a trait known to evolve quickly (Barrett et al, 2011; Huey, 1991; Bettencourt et al, 1999; Gibert et al, 2001). My thermal tolerance

experiments support the idea of a recent ecological niche shift between Southern and Northern European wasp spiders. Corresponding to the actual climatic environment these animals are exposed to, they developed increased heat or cold resistance. Such clinal patterns of temperature resistance are well known from e.g. *Drosophila* species (Kimura, 2004). Environmental stress could consequently act as major driving force of evolutionary innovation (Lopez-Maury et al, 2008). As *Argiope bruennichi* has colonized Northern Europe less than 100 years ago, the underlying adaptation is probably of recent origin. My gene expression experiment exposed Northern and Southern European spider offspring to their native and non-native temperature conditions. The footprints of reciprocal adaptation should then be visible in stress related differential gene expression. Such adaptive gene expression divergence due to thermal stress is e.g. known from studies in fishes (Crawford & Powers, 1992).

I find a considerable number of genes, which are exclusively differentially expressed during temperature stress (167/270). This finding supports the idea of an ecological divergence between Northern and Southern Europe. Assuming selection has driven gene expression differentiation, I would find it to especially affect genes, which are involved in coping with actual environmental stressors. The data thus highlight a clear importance to quantify evolutionary young divergence in gene expression during ecologically relevant conditions. If I would have limited the analysis to control conditions, I would miss nearly two thirds of differentially expressed genes.

The observed condition dependence of differential expression might be interpreted as a proof for the importance of the evolution of plasticity in early adaptation. However, the analysis shows that a large fraction of differential expression does not entail a plastic change within populations. Many reaction norm changes in Northern European populations even involve assimilation, the loss of plasticity. Most plastic expression responses in Baltic populations rely on a modulation of already existing patterns of plasticity. Considering my data, a *de novo* evolution of plastic expression patterns thus seems to be the exception rather than the rule in *Argiope bruennichi*. Instead, adaptive plasticity of gene expression might be enabled by only a slight increase of the reaction norm´s slope.

In order to successfully colonize a new climatic zone in Northern Europe, *Argiope bruennichi* populations must have been supplied with novel genetic variation. And indeed, I find increased genetic diversity in Northern European spider populations (see chapter 4). However, this is not mirrored in elevated variation of gene expression. It will therefore be necessary to further study the question whether Northern climate adaptations are driven by regulatory or coding changes. Alternatively, recent selection in the invasive Northern European wasp spider lineage might have already eradicated variation for gene expression. Selection is expected to quickly favor few novel adaptive variants, which might lead to a rapid loss of variation for gene expression divergence (Czypionka et al, 2012).

Differentially expressed genes between Northern and Southern Europe and their possible association with thermal tolerance

The ecologically most relevant stress response for expanding wasp spiders might be genetic adaptation to cold exposure. And indeed, I identify several differentially regulated candidate genes, which might be directly related to cold tolerance. I find a pronounced up-regulation of cysteine proteinase in Northern European populations. At the same time, a cysteine proteinase inhibitor is up-regulated in Portuguese spiders. Interestingly, cysteine proteinase is known to be involved in cold tolerance in plants (Grudkowska & Zagdańska, 2004). One of the genomic candidate regions for reciprocal adaptation between Northern and Southern Europe maps into a cysteine proteinase gene (see chapter 4). And the expression level of Sodium Potassium ATPase has been assumed to be directly related to thermal tolerance in *Drosophila* (Feng et al, 1997). An alteration of cell membrane fluidity, as it is achieved by altering the phospholipid content, has also been described as important mediator of cold tolerance (Lee et al, 2006). An up-regulated phospholipid transporter could be directly involved in this process in Baltic spiders.

The up-regulation of these genes during cold stress might be an important adaptation of Northern European spiders. In contrast, differential gene expression during heat stress might be directly related to the increased heat resistance of Southern European *Argiope bruennichi*. Cuticular proteins constitute one interesting group of differentially expressed transcripts. They constitute an important part of the animal's exoskeleton and the

cuticle's protein composition may strongly influence its physical properties (Anderson et al, 1995). By integrating different proteins into its exoskeleton, a spider could cope with different thermal stress, e.g. evaporation or cuticular stability. Different cuticular proteins could consequently be involved in heat and cold tolerance. A cathepsin gene shows a clear heat responsive overexpression in Portuguese spiders. This gene is involved in Protein degradation and has been associated with heat stress response (Buckley et al, 2006).

Condition dependent gene expression within populations

I find a particularly strong general gene expression response during heat stress, with several thousand genes up- or down-regulated in populations in comparison to control conditions. This might be the result of extensive environmentally induced decanalization. Heat stress might release cryptic genetic variation leading to large numbers of up- or down-regulated genes (Gibson & Dworkin, 2004). It is unlikely that the expression of all these genes will have a pronounced biological function. Many genes are expressed in a highly unspecific manner during stress (Sorensen et al, 2007). Out of about 16,000 temperature responsive genes in *A. thaliana*, only 16 have an actual functional role during thermal stress (Lopez-Maury et al, 2008).

In comparison to the several thousand heat responsive transcripts, I found only three genes, differentially expressed between cold and control conditions. Two of these genes are associated with gluconeogenesis and highly up-regulated at -10°C in Portuguese and Baltic spiders. A stimulation of the glucose metabolism might be an initial cold stress response for wasp spiders. The use of saccharides as antifreeze is widely distributed in nature (Ohtsu et al, 1998). A significantly increased ubiquitin transcription is the only difference found between Baltic and Portuguese populations, when comparing cold and control conditions. However, both Baltic and Portuguese spiders express Ubiquitin C in a very similar manner and quantity. Its expression is up-regulated during heat and cold stress, an expression pattern known from other organisms (Müller-Taubenberger et al, 1988). Ubiquitin is involved in manifold cellular processes, from protein degradation and folding, DNA repair to signaling and many more (Pickart & Eddins, 2004). Its

association with thermal stress is thus not surprising. The experiment was conducted over a comparatively short timeframe of only 10 hours. Perhaps a longer exposure to cold stress will be necessary, in order to trigger the expression of more cold responsive genes. Acclimatization of plants to cold does require a certain time, but will then involve the differential expression of a large number of genes (Lee & Lee, 2003; Zhu et al, 2004). And flesh fly larvae will be much more cold tolerant after a period of several weeks of diapausing (Lee & Denlinger, 2008).

Diapausing as a simple and rapidly evolving adaptation during a range expansion

Transcriptional responses to thermal stress are a highly complex trait, involving many epistatic interactions (Somorjai et al, 2003). Fine tuning of a single element of such a network might affect the expression of many associated genes. An interesting example for such a change is provided by arthropod winter-diapause behavior. Diapausing is a complex physiological process. At the same time it seems to underlie a simple genetic basis. A single, dominant gene locus is sufficient to induce diapausing behavior in spider mites of the genus *Tetranychus* (Takafuji et al, 1999). And in fruit flies, diapausing has evolved repeatedly due to a single amino acid change in the *couch potato* gene (Schmidt et al, 2008). For a very young ecological split, such a simple evolutionary change is probably more likely than single regulatory changes in many independently acting genes. In fact, diapausing behavior was one of the first traits to be identified to evolve in response to global warming (Bradshaw & Holzapfel, 2001). Diapausing is known to evolve along latitudinal clines and can even be directly associated with cold tolerance (Emerson et al, 2009; Schmidt et al, 2005; Dingle et al, 1977). Generally, the genetic and transcriptional basis of diapausing is quite well understood (Srere et al, 1992; Zhang & Denlinger, 2012; Morris et al, 1996; Kimura et al, 1997; Schmidt et al, 2008; Dingle et al, 1977). It is a state of developmental arrest, involving a down-regulation of many metabolic genes (Yang et al, 2010; Kimura et al, 1997). In this regard, the observed bias towards down-regulated genes in Baltic populations is noteworthy. At the same time, Southern European spiders show up-regulation of genes like silk, capture glue and toxins and digestive enzymes, which are involved in prey capture and feeding. Moreover, I find

a repression of an astacin-like-metalloprotease in Northern European spiders. This gene is known to be associated with ecdysis (Stepek et al, 2011). Thus, Northern European spiders possibly have to go through an obligate dormancy phase, in which their whole metabolism is down-regulated. Southern European ones, on the other hand, could only facultatively choose to diapause. It is well known from laboratory experiments, that a pronounced dormancy is crucial for survival of wasp spiders from Northern Europe. This phase lasts several months and has to include subzero temperatures, for the spiders to actually hatch from their eggsacs and reach maturity (Zimmer pers. comment). Southern European wasp spiders will readily leave their eggsacs during warm winters and start developing without problems. In the course of the experiments, I could observe this for wasp spider eggsacs, which were kept at room temperature for several months. While most Portuguese spiders hatched, started building webs and caught prey, Northern European ones stayed inside of their eggs sacs and finally died. Overwintering behavior can be induced by different environmental triggers, of which photoperiod and temperature seem to play important roles in spiders and insects (Bradshaw & Holzapfel, 2001, Dingle et al, 1977; So & Takafuji, 1992). The factors responsible for the induction of diapause in *Argiope bruennichi* are however not yet known. It could be a predetermined character, independent of environmental cues, or e.g. induced by lowering temperatures or shorter day length.

A more simple explanation for the observed down-regulation of gene expression in Baltic spiders might be found in the genomic architecture of gene regulation. The repression of gene expression is achieved much easier than its activation (Chen & Rajewsky, 2007; Wray et al, 2003). Consequently, adaptation by transcriptional repression might be a more simple way to cope with rapid environmental changes.

The influence of non-genetic factors on gene expression divergence

Last, a non-genetic environmental influence could be responsible for the observed divergence in gene expression. As the analyzed spiders were kept under constant conditions, I can rule out environmentally induced plastic responses. But the mothers of eggsacs were collected in their natural environment. Consequently, I cannot exclude

maternal effects influencing gene expression between Northern and Southern Europe. Dependent on the environment it was exposed to, the mother could influence the phenotypic expression of its offspring. However, for unpredictable conditions, a dependence on the mother´s experience might lead to a less favorable response for her offspring. A very warm winter, followed by a very cold one, would then lead to a mass die off in Northern European wasp spiders. Such changing environmental conditions are very common in the wasp spider's habitat. This in turn, makes a strong dependence on maternal effects less likely.

5.5 Outlook

Gene expression has many more levels to study, apart from the simple transcript abundance of whole specimens. For example, expression changes are well known to be highly tissue specific (Chinnusamy et al, 2007; Staubach et al, 2009; Bryk et al, 2013). An analysis of distinct body parts might consequently yield a refined picture of regulatory divergence during the range expansion of *Argiope bruennichi*. Apart from regulatory DNA elements, gene expression divergence could also be based on copy number variation of genes (Bettencourt et al, 1999; Gu et al, 2004). Gene duplication is enabling a rapid change of expression levels and might thus be particularly important during contemporary evolution (Gu et al, 2004). Posttranscriptional alteration of transcript levels by small RNA is another important part of the gene regulatory cascade and well known to be involved in stress responses (Chinnusamy et al, 2007; Chen & Rajewsky, 2007). The same holds true for posttranslational modifications of proteins (Chinnusamy et al, 2007).

And last, an analysis of the genomic background of differentially expressed transcripts might yield interesting insights into the evolutionary divergence of gene regulation. An evolutionary divergence of gene expression could be based on external trans-acting factors or cis-regulatory mutations in close vicinity to the actual gene (Wittkopp et al, 2004). Selection on the latter might lead to detectable signatures of selective sweeps in the genomic vicinity of the according transcript. A correlative analysis of introgressed genomic regions (chapter 4) with the gene expression changes will provide further insights into the question of trans- versus cis-regulation of changes.

Concluding remarks

Poleward range expansions and biological invasions constitute a biological phenomenon of global ecological and economic importance. A main result of my PhD thesis is that these movements are not purely defined by environmental change. Instead, an interaction of environmental effects and rapid evolutionary adaptation appears to pave the way for many expansive species. Even in the face of very high gene flow, populations are capable to adapt to different ecological regimes. My study identifies a climatic niche shift as the potential driver of the wasp spider´s ongoing expansion into Northern Europe.

This finding is of twofold general importance. First, many species might be able to cope with global warming by evolutionary adaptation. Evolution could consequently counteract predicted mass extinctions in the coming decades. And second, evolutionary change has to be taken into account as a powerful force to promote biological invasions.

Interestingly, global change itself might contribute to evolution, by enabling secondary contact of formerly isolated populations. Human made alterations of ecosystems could thus indirectly mediate adaptation and trigger invasions.

The association of genetic admixture and the success of the wasp spider's range expansion is another essential result of my thesis. In recent years, increasing evidence links secondary contact and contemporary evolution. Considering this background, a focus on hybridization solely in the context of genetic incompatibilities and their destructive impact is not justified anymore.

And last, the emerging technologies of next generation sequencing do now enable an unprecedented view into the genomic basis of rapid adaptation. Whole genome sequencing allows obtaining insights into evolutionary processes in real time and will contribute to a revolution in evolutionary biology. A detailed analysis of my genomic data might eventually enable the identification of the underlying genomic changes of the wasp spider's climatic niche shift.

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My contributions to different chapters

Chapter one

Chapter one has been published in the Journal Molecular Ecology.

Krehenwinkel H, Tautz D (2013) Northern range expansion of European populations of the wasp spider *Argiope bruennichi* is associated with global warming–correlated genetic admixture and population-specific temperature adaptations. *Molecular Ecology*, **22**, 2232–2248.

I have acquired the samples, performed the laboratory work, ecological experiments and the data analysis. The manuscript was written together with Diethard Tautz. Sarah Lemcke assisted in the laboratory work.

Chapter two

Chapter two will be submitted to the Journal of Biogeography. I have acquired samples, performed laboratory work and data analysis and wrote the chapter. Susanne Meese collected the German *Pisaura mirabilis* samples and has established the microsatellite markers for *Pisaura mirabilis* under my supervision as part of her diploma thesis. Vanessa Gorsler and Nicole Thomsen assisted in lab work.

Chapter three

Chapter will be submitted to the Journal Molecular Ecology Resources. I have acquired samples, performed laboratory work and data analysis and wrote the chapter. Sarah Lemcke assisted in the laboratory work.

Chapter four & chapter five

I have acquired samples, performed laboratory work, ecological experiments, data analysis and wrote the chapters. Nicole Thomsen, Thoomke Brüning and Sarah Frehse assisted in the lab work, ecological experiments and spider caretaking. The Illumina sequencing runs were performed by the staff of the Cologne Center for Genomics at the University of Cologne.

Appendix

Chapter 1

Chapter 1 has been published in the journal Molecular Ecology. All data, on which the study is based, has been uploaded to the Dryad Digital Repository (http://datadryad.org/resource/doi:10.5061/dryad.r8n7c?show=full). The following datasets can be assessed:

1. Contemporary COI sequence alignment

2. Historical COI sequence alignment

3. Historical COI sequence alignment of short sequences

4. Genetic diversity estimates for mitochondrial data

5. Microsatellite data

6. SNP data including STRUCTURE output

7. Morphological measurements

8. Data from temperature preference -and tolerance experiments

9. Data from reciprocal transplant experiment

10. All primer sequences

11. Sample list containing specimen identifiers, museum collection numbers and collection sites

Supplementary figure 1.1 Neighbor joining tree based on Nei`s genetic distance for 16 microsatellite Loci. Native- (yellow) & Invasive (red) European as well as Russian (green) populations are included.

Supplementary table 1.1 Sampling locations for contemporary and historical *Argiope bruennichi* samples, which were used in chapter 1. The specimens are sorted according to the distinct geographic regions, which I distinguish in the chapter.

Identifier/Museum No.	Region	Country	District/state	City	GPS N	GPS E	Year	Collector	Collection
13047-13050 & 13056	Central Asia	Uzbekistan	Samarqand	Kata Kurgan	39.90	66.50	1903	E. Zoffmann	Berlin
13059	Central Asia	Uzbekistan	Surxondaryo	Patta Hissar Termiz	39.77	64.43	1906	Ryssel	Berlin
10724	Central Asia	Iran	Fars	Maharlu See	29.35	52.82	1953	Roewer	Frankfurt
CnXiXA01(Ar6340)	Central Asia	China	Xinjiang	unknown	39.45	75.98	1991		Beijing
3509	East Asia	Japan	Kyushu	Nagasaki	32.78	129.87	1882	Dönitz	Frankfurt
13061	East Asia	China	Shandong	Quingdao	36.07	120.38	1905	Glaue	Berlin
CnGuGA01(Ar5848)	East Asia	China	Guizhou	unknown	26.83	106.83	1978		Beijing
CnYuYA01(Ar5862)	East Asia	China	Yunnan	unknown	24.50	101.50	1983		Beijing
CnHuHA01(Ar6196)	East Asia	China	Hubei	unknown	30.97	112.23	1984		Beijing
CnFuFA01(Ar5458)	East Asia	China	Fujian	unknown	25.90	118.30	1991		Beijing
CnHeHA01(Ar5402)	East Asia	China	Hebei	unknown	39.30	116.70	1998		Beijing
JpKaIA	East Asia	Japan	Kagoshima	Isa-shi	31.60	130.56	2007	T.Tsukada	Plön
JpKaSA	East Asia	Japan	Kagoshima	Satsuma	31.60	130.56	2007	T.Tsukada	Plön
JpKaYA	East Asia	Japan	Kagoshima	unknown	31.60	130.56	2007	T.Tsukada	Plön

Identifier/Museum No.	Region	Country	District/state	City	GPS N	GPS E	Year	Collector	Collection
JpMyEA	East Asia	Japan	Miyazaki	Ebino-shi	32.02	131.35	2007	T.Tsukada	Plön
CnJaJA01(Ar19504)	East Asia	China	Jiangxi	unknown	27.63	115.77	2008		Beijing
Cn	East Asia	China	Fujian	unknown	25.90	118.30	2010	RC. Cheng	Plön
JpHoHA	East Asia	Japan	Hokkaido	Hakodate	41.77	140.73	2010	K. Tanaka	Plön
JpMySA	East Asia	Japan	Miyagi	Sendai	38.27	140.87	2010	K. Tanaka	Plön
JpToToA	East Asia	Japan	Tottori Prefecture	Tottori, Katsurami	35.50	134.23	2010	N. Tsurusaki	Plön
JpEhYA	East Asia	Japan	Ehime	Yuyama	33.83	132.77	2011	N. Tsurusaki	Plön
JpFuFA	East Asia	Japan	Fukuoka	Fukuoka, Tarumaru Lizuka	33.65	130.68	2011		Plön
JpFuFB	East Asia	Japan	Fukuoka	Fukuoka, Yamada Ryokuchi	33.57	130.72	2011		Plön
JpIbTA	East Asia	Japan	Ibaraki	Tsukuba	36.00	140.10	2011	Y. Baba	Plön
JpKaAA	East Asia	Japan	Kagoshima	Amami-oshima Island	28.33	129.32	2011	Y. Baba	Plön
JpKaAB	East Asia	Japan	Kagoshima	Amami-oshima Island	28.28	129.43	2011	Y. Baba	Plön
JpKaAC	East Asia	Japan	Kagoshima	Amami-oshima Island	28.23	129.35	2011	Y. Baba	Plön
JpKaMA	East Asia	Japan	Kagoshima	Hirata	31.60	130.56	2011	T. Maeda	Plön
JpKaCA	East Asia	Japan	Kagoshima	Mihama-chö	31.60	130.56	2011	K. Nakamura	Plön
JpKaKA	East Asia	Japan	Kagoshima	Shimofukmoto-chö	31.60	130.56	2011	T. Maeda	Plön
JpKaHA	East Asia	Japan	Kagoshima	Sogi,Ohkuchi	31.60	130.56	2011	K.Nakamura	Plön
JpNaAA	East Asia	Japan	Nagano	Azusagawa-Azusa	36.23	137.97	2011	N. Tsurusaki	Plön
JpShOA	East Asia	Japan	Shiga	Ozigaoka	35.12	136.07	2011	T. Masumoto	Plön
JpTcUA	East Asia	Japan	Tochigi	Utsunomiya	36.53	139.95	2011	Y. Baba	Plön
JpToKA	East Asia	Japan	Tottori	Kagamiganaru	35.28	133.48	2011	N. Tsurusaki	Plön
JpToSA	East Asia	Japan	Tottori	Katsurami	35.50	134.17	2011	N. Tsurusaki	Plön
3709	East Asia	China	Beijing	Beijing	39.91	116.39	<1900	Möllenhoff	Berlin
13064	East Asia	Japan	Honshu	Tokyo	35.69	139.69	<1900	Hilgendorff	Berlin
2900	East Asia	Japan	unknown	unknown			<1900	Dönitz	Berlin
2693	East Asia	Japan	unknown	unknown			<1900	Hilgendorff	Berlin
JP2	East Asia	Japan	unknown	unknown			>2000		Plön
13065	East Asia	Japan	Hokkaido/Yezo	unknown	43.28	143.08			Berlin
CnLiLA01(Ar4926)	East Asia	China	Liaoning	unknown	41.10	122.30			Beijing
13063	East Asia	Polynesia	Polynesia	Polynesia				Putze	Berlin
13062	East Asia	China	Shandong	Quingdao	36.07	120.38		Redinberg	Berlin
CnZhZA01(Ar4927)	East Asia	China	Zhejiang	unknown	29.20	120.50			Beijing
13085	Invasive	Germany	Berlin	Tegel	52.35	13.17	1897		Berlin
13083	Invasive	Germany	Berlin	Müggelsee	52.26	13.39	1903		Berlin
13078	Invasive	Germany	Brandenburg	Kotzen	52.63	12.52	1906	Zimmermann	Berlin
13084	Invasive	Germany	Brandenburg	Königs Wusterhausen	52.18	13.38	1909	Bäume	Berlin
13086	Invasive	Germany	Berlin	Grunewald Havel	52.29	13.16	1919	Ulrich	Berlin
13079	Invasive	Germany	Brandenburg	Erkener	52.25	13.45	1923	Ude	Berlin
13089	Invasive	Germany	Brandenburg	Erkener	52.25	13.45	1926	Ude	Berlin
13066	Invasive	Germany	Brandenburg	Erkener	52.25	13.45	1927	Ude	Berlin
13088	Invasive	Germany	Brandenburg	Erkener	52.25	13.45	1928	Ude	Berlin
9307-9309	Invasive	Germany	Brandenburg	Erkener	52.25	13.45	1929	Ude	Berlin
61280	Invasive	Germany	Saxony-Anhalt	Dessau Roßlau Dellnau	51.50	12.17	1933	Wiehle	Frankfurt
4337	Invasive	Germany	Berlin	Berlin	52.31	13.24	1934	Roewer	Frankfurt
13091	Invasive	Germany	Brandenburg	Caputh	52.21	13.00	1935	Sick	Berlin

Identifier/Museum No.	Region	Country	District/state	City	GPS N	GPS E	Year	Collector	Collection
13069	Invasive	Germany	Brandenburg	Genshagener Forst	52.19	13.19	1935	Ehlers	Berlin
13092	Invasive	Germany	Saxony	Bötzener Wiesen	51.26	12.34	1935	Preuße	Berlin
13068	Invasive	Germany	Brandenburg	Großbeeren	52.21	13.18	1936	Ehlers	Berlin
13073	Invasive	Germany	Brandenburg	Ruhlsdorf	52.40	13.27	1936	Ehlers	Berlin
13076	Invasive	Germany	Berlin	Krumme Laake Müggeheim	52.25	13.42	1937	Ehlers	Berlin
13074	Invasive	Germany	Brandenburg	Hennigsdorf	52.64	13.20	1937	Ehlers	Berlin
13071	Invasive	Germany	Brandenburg	Höpenberge			1937	Ehlers	Berlin
13072	Invasive	Germany	Brandenburg	Klosterwadle Uckermark	53.07	13.30	1937	Ehlers	Berlin
13077	Invasive	Germany	Brandenburg	Röddelin Mahlgast See	53.07	13.30	1937	Ehlers	Berlin
13075	Invasive	Germany	Brandenburg	Schmöckwitz	52.22	13.38	1937	Ehlers	Berlin
13067	Invasive	Germany	Brandenburg	Summt	52.38	13.23	1937	Ehlers	Berlin
14696	Invasive	Germany	Brandenburg	Oberer Falkensee	52.56	13.09	1960		Berlin
9301	Invasive	Germany	Brandenburg	Straupitz	51.91	14.12	1960	Crome	Berlin
	Invasive	Germany	Mecklenburg-Vorpommern	Thiessow	54.28	13.71	1978	Jaeschke	Berlin
27026	Invasive	Germany	Brandenburg	Neuendorf Lübben	51.95	13.90	1985	Köhler	Berlin
30534	Invasive	Germany	Brandenburg	Müncheberg	52.50	14.14	1992	Wendt	Berlin
30539	Invasive	Germany	Mecklenburg-Vorpommern	Hiddensee	54.54	13.09	1992	Eichler	Berlin
GeNsXA	Invasive	Germany	Lower Saxony	Buxtehude	53.48	9.80	2009		Plön
GeBbBA	Invasive	Germany	Brandenburg	Belzig	52.13	12.53	2010	L. Friman	Plön
GeBbBB	Invasive	Germany	Brandenburg	Belzig	52.13	12.60	2010	L. Friman	Plön
GeBbEA	Invasive	Germany	Brandenburg	Eberswalde	52.73	13.73	2010	H. Krehenwinkel	Plön
GeBbEB	Invasive	Germany	Brandenburg	Eberswalde	52.73	13.75	2010	H. Krehenwinkel	Plön
PlEmOA	Invasive	Poland	Ermland-Masuren	Olsztyn	53.73	20.25	2010	H. Krehenwinkel	Plön
PlEmPA	Invasive	Poland	Ermland-Masuren	Paslek	54.02	20.25	2010	H. Krehenwinkel	Plön
GeHhHA	Invasive	Germany	Hamburg	Nordheide	53.28	10.03	2010	J. Helms, K. Welke, T. Dirks	Plön
CzJkBA	Invasive	Czech Republic	Jihomoravský	Brno	49.20	16.67	2010	S. Winkler, V. Brya	Plön
CzJk?A	Invasive	Czech Republic	Jihomoravský	Brno?	49.20	16.61	2010	S. Winkler	Plön
CzJkMA	Invasive	Czech Republic	Jihomoravský kraj	Moravian Karst	49.28	16.73	2010	V. Hula	Plön
CzJkSA	Invasive	Czech Republic	Jihomoravský kraj	Skřinářov	49.35	16.18	2010	S. Winkler	Plön
SwKlKA	Invasive	Sweden	Kalmar län	Kalmar	56.83	14.15	2010		Plön
LtKlPA	Invasive	Lithuania	Klaipeda	Palanga	55.97	21.10	2010	H. Krehenwinkel	Plön
LvLpLA	Invasive	Latvia	Liepaja District	Liepaja	56.42	21.00	2010	H. Krehenwinkel	Plön
LvLpNA	Invasive	Latvia	Liepaja District	Nidasciems	56.08	21.12	2010	H. Krehenwinkel	Plön
LvLiAA	Invasive	Latvia	Limbazi	Ainazi	57.85	24.35	2010	H. Krehenwinkel	Plön
AuNiVA/B/C	Invasive	Austria	Lower Austria	Sankt Valentin	48.17	14.53	2010	H. Krehenwinkel	Plön
AuNiVD	Invasive	Austria	Lower Austria	Sankt Valentin	48.17	14.53	2010	M. Freudenschuss	Plön
GeNsBA	Invasive	Germany	Lower Saxony	Braunschweig	52.32	10.43	2010	H. Krehenwinkel	Plön
GeNsBB	Invasive	Germany	Lower Saxony	Braunschweig	52.23	10.47	2010	H. Krehenwinkel	Plön
GeNsLA	Invasive	Germany	Lower Saxony	Lehrte	52.37	9.93	2010	H. Krehenwinkel	Plön
GeNsMA	Invasive	Germany	Lower Saxony	Mattierzoll	52.05	10.77	2010	K. Moschütz	Plön
GeNsOA	Invasive	Germany	Lower Saxony	Oldau	52.65	9.92	2010	H. Krehenwinkel	Plön
LtMaPA	Invasive	Lithuania	Marijampole	Pasiekos	54.30	23.12	2010	H. Krehenwinkel	Plön
PlMaCA	Invasive	Poland	Masowien	Cosznow	52.38	20.73	2010	W. Wawer	Plön
PlMaJA	Invasive	Poland	Masowien	Jonina Cosznow	52.38	20.73	2010	W. Wawer	Plön
PlMaPA	Invasive	Poland	Masowien	Pienkow Cosznow	52.37	20.83	2010	W. Wawer	Plön

Identifier/Museum No.	Region	Country	District/state	City	GPS N	GPS E	Year	Collector	Collection
PlMaDA	Invasive	Poland	Masowien	Siedlce	52.13	23.20	2010	S. Longhorn	Plön
PlMaTA	Invasive	Poland	Masowien	Tomna Las	52.38	20.78	2010	W. Wawer	Plön
GeMvSA	Invasive	Germany	Mecklenburg-Vorpommern	Selmsdorf	53.88	10.82	2010	H. Krehenwinkel	Plön
DkMlMA	Invasive	Denmark	Midtjylland	Mols Bjerge	56.22	10.58	2010	S. Toft	Plön
PlPoGA	Invasive	Poland	Pomerania	Gdansk	54.30	18.57	2010	H. Krehenwinkel	Plön
PlPoLA	Invasive	Poland	Pomerania	Lebork	54.55	17.78	2010	H. Krehenwinkel	Plön
GeShAA	Invasive	Germany	Schleswig Holstein	Ascheberg	54.13	10.32	2010	H. Krehenwinkel	Plön
GeShLA	Invasive	Germany	Schleswig Holstein	Blankensee	53.78	10.70	2010	M.Lemke	Plön
GeShGA/B	Invasive	Germany	Schleswig Holstein	Grönauer Heide	53.80	10.72	2010	M.Lemke	Plön
GeShHA/B	Invasive	Germany	Schleswig Holstein	Hasenmoor	53.90	9.98	2010	J. Wolff	Plön
GeShBA	Invasive	Germany	Schleswig Holstein	Negernbötel	53.97	10.27	2010	H. Krehenwinkel	Plön
GeShBB	Invasive	Germany	Schleswig Holstein	Negernbötel	53.98	10.27	2010	H. Krehenwinkel	Plön
GeShNA	Invasive	Germany	Schleswig Holstein	Neumünster	54.07	10.00	2010	H. Krehenwinkel	Plön
GeShSA	Invasive	Germany	Schleswig Holstein	Schleswig	54.52	9.58	2010	H. Krehenwinkel	Plön
GeShRA	Invasive	Germany	Schleswig Holstein	Schwentinental	54.25	10.28	2010	H. Krehenwinkel	Plön
GeShRB	Invasive	Germany	Schleswig Holstein	Schwentinental	54.25	10.27	2010	H. Krehenwinkel	Plön
SwSlAA	Invasive	Sweden	Skåne län	Ahus, Horna	55.95	14.28	2010	L. Jonson	Plön
SwSlLA	Invasive	Sweden	Skåne län	Veberöd, Vaselund	55.67	13.45	2010	L. Jonson	Plön
SwSlVA	Invasive	Sweden	Skåne län	Vittskövle	55.85	14.15	2010	L. Jonson	Plön
FlSbSA	Invasive	Finland	Südösterbotten	Seinäjoki	62.80	22.83	2010	N. Fritzen	Plön
FlSsHA	Invasive	Finland	Südsavo	Hirvensalmi	61.63	26.77	2010	S.Koponen	Plön
DkSyKA	Invasive	Denmark	Syddanmark	Kolding	55.53	9.48	2010	S. Toft	Plön
GeThEA	Invasive	Germany	Thüringia	Ettersberg	51.03	11.27	2010	A. Grabolle	Plön
GeThJA	Invasive	Germany	Thüringia	Jena	50.95	11.57	2010	D. Neubert	Plön
GeThSA	Invasive	Germany	Thüringia	Saalborn	50.89	11.33	2010	A. Grabolle	Plön
GeThWA	Invasive	Germany	Thüringia	Weimar	50.93	11.33	2010	D. Neubert	Plön
SkTkNA	Invasive	Slovakia	Trenčiansky kraj	Novaky	48.72	18.53	2010	S. Pekar	Plön
AuObMA/B	Invasive	Austria	Upper Austria	Linz, Mitterwasser	48.25	14.37	2010	J. Nigl	Plön
AuViLA	Invasive	Austria	Vienna	Vienna	48.18	16.21	2010	H. Krehenwinkel	Plön
AuViVA	Invasive	Austria	Vienna	Vienna	48.20	16.37	2010	H. Krehenwinkel	Plön
GeBeMA	Invasive	Germany	Berlin	Müggelheim	52.40	13.65	2011	H. Krehenwinkel	Plön
SwBlAA	Invasive	Sweden	Blekinge county	Asarum	56.18	14.85	2011	H. Krehenwinkel	Plön
SwBlOA	Invasive	Sweden	Blekinge county	Öljersjö	56.18	15.70	2011	H. Krehenwinkel	Plön
GeBbKA	Invasive	Germany	Brandenburg	Erkner	52.40	13.75	2011	H. Krehenwinkel	Plön
GeBbFA	Invasive	Germany	Brandenburg	Fangschleuse	52.42	13.78	2011	H. Krehenwinkel	Plön
GeBbWA	Invasive	Germany	Brandenburg	Fürstenwalde	52.33	14.07	2011	H. Krehenwinkel	Plön
GeBbGA	Invasive	Germany	Brandenburg	Gosen	52.38	13.70	2011	H. Krehenwinkel	Plön
GeBbHA	Invasive	Germany	Brandenburg	Heidsee	52.30	13.78	2011	H. Krehenwinkel	Plön
AuBuRA	Invasive	Austria	Burgenland	unknown	47.50	16.42	2011		Plön
PlEmYA	Invasive	Poland	Ermland Masuren	Pilchy	53.68	21.90	2011	W. Wawer	Plön
PlGPPA	Invasive	Poland	Greater Poland	Poznan	52.30	17.53	2011	H. Krehenwinkel	Plön
SwKaSA	Invasive	Sweden	Kalmar county	Söderakra	56.43	16.07	2011	H. Krehenwinkel	Plön
LtKaKA	Invasive	Lithuania	Kaunas	Kaunas	55.02	24.20	2011	H. Krehenwinkel	Plön
PlLPZA	Invasive	Poland	Lesser Poland	Zawoja	49.63	19.53	2011	W. Wawer	Plön
LvLiAC	Invasive	Lativa	Limbazi	Ainazi	57.87	24.35	2011	H. Krehenwinkel	Plön

Identifier/Museum No.	Region	Country	District/state	City	GPS N	GPS E	Year	Collector	Collection
PlLuGA	Invasive	Poland	Lubusz	Grodziszcze	52.25	15.53	2011	H. Krehenwinkel	Plön
LtMaNA	Invasive	Lithuania	Marijampole	Naujoji Valia	54.33	23.15	2011	H. Krehenwinkel	Plön
PlMaLA	Invasive	Poland	Masovia	Laski	52.32	20.55	2011	W. Wawer	Plön
PlMaBA	Invasive	Poland	Masovien	Budykierz	52.67	21.62	2011	H. Krehenwinkel	Plön
PlMaSA	Invasive	Poland	Masovien	Sochaczew	52.20	20.25	2011	H. Krehenwinkel	Plön
DkMjSA	Invasive	Denmark	Midtjylland	Skanderborg	56.07	9.97	2011	H. Krehenwinkel	Plön
EsPuMA	Invasive	Estonia	Pärnu	Mereküla	58.28	24.58	2011	H. Krehenwinkel	Plön
PlPlAA	Invasive	Poland	Podlaskie	Augustow	53.90	22.97	2011	H. Krehenwinkel	Plön
PlPlBA	Invasive	Poland	Podlaskie	Bialystok	53.33	23.10	2011	H. Krehenwinkel	Plön
LvSaSA	Invasive	Latvia	Salaspils	Salaspils	56.85	24.38	2011	H. Krehenwinkel	Plön
SwSkKA	Invasive	Sweden	Skane county	Kristianstad	55.95	14.10	2011	H. Krehenwinkel	Plön
LvSNSA	Invasive	Latvia	Skriveru Novads	Skriveri	56.62	25.10	2011	H. Krehenwinkel	Plön
PlScBA	Invasive	Poland	Subcarpathian	Budy	50.18	21.77	2011	W. Wawer	Plön
LtUtUB	Invasive	Lithuania	Utena	Utena	55.50	25.48	2011	H. Krehenwinkel	Plön
LtUtUA	Invasive	Lithuania	Utena	Utena	55.47	25.37	2011	H. Krehenwinkel	Plön
LtUtZA	Invasive	Lithuania	Utena	Zarasai	55.73	26.30	2011	H. Krehenwinkel	Plön
FlVsKA	Invasive	Finland	Varsinais-Suomi	Kaarina, Turku	60.38	22.35	2011	C. Neffling & E. Virta	Plön
13041	Invasive	Germany	Berlin	Berlin	52.31	13.24	<1945	HedIcke	Berlin
13087	Invasive	Germany	Brandenburg	Groß Glienicke	52.47	13.12	<1960	Meise	Berlin
61274	Invasive	Germany	Europe	unknown			<1960	H. Wiehle	Frankfurt
61272	Invasive	Germany	Saxony-Anhalt	Dessau or Berlin			<1960	H. Wiehle	Frankfurt
GeThHA	Invasive	Germany	Thüringia	Hainich	51.10	10.38	>1990	T. Blick	Plön
13030	Native	Italy	Sicily	Taurus	37.34	14.16	1846	Holz	Berlin
3521	Native	Morocco	Grand Casablanca	Casablanca	33.35	-7.37	1872	Fritzsch & Rein	Frankfurt
5589	Native	Germany	Hesse	Frankfurt	50.07	8.41	1873		Frankfurt
3506	Native	Germany	Hesse	Frankfurt	50.07	8.41	1883	Koch	Frankfurt
13027	Native	Turkey	Mersin	Gülek	37.32	34.80	1897	Holtz	Berlin
13022	Native	Croatia	Istria	Rovigno	45.05	13.38	1899	Müggeb.	Berlin
3514	Native	Germany	Hesse	Mainkur Frankfurt	50.08	8.46	1901	Römer	Frankfurt
3515	Native	Germany	Hesse	Schwanheim	50.05	8.35	1901	Knoblauch	Frankfurt
3508	Native	Germany	Hesse	Frankfurt	50.07	8.41	1902	Römer	Frankfurt
13038	Native	Croatia	Istria	Rovigno	45.05	13.38	1902	Büllener	Berlin
3512	Native	Switzerland	Ticino	Lugano	46.01	8.58	1904	Edinger	Frankfurt
13080	Native	Germany	Rhineland-Palatinate	Trier	49.46	6.39	1905	Rübesaamen	Berlin
13031	Native	Italy	Trentino	Trentino	46.04	11.07	1906		Berlin
5594	Native	Germany	Hesse	Michelstadt im Odenwald	49.41	9.00	1908	Neuenburg	Frankfurt
13026	Native	Croatia	Istria	Fiume Buccari	45.32	14.53	1912	Ramme	Berlin
13039	Native	Italy	Sardinia	Monti del Carmagenta	40.02	9.04	1912	Krauß	Berlin
13035	Native	Italy	Sardinien	Sorgonj	40.02	9.04	1912	Krauße	Berlin
3507	Native	Spain	Catalonia	La Fosca de Palemos	41.59	2.49	1914	Haas	Frankfurt
3510	Native	Spain	Catalonia	Flix	41.14	0.32	1915	Haas	Frankfurt
6432	Native	Germany	Hesse	Obertshausen Offenbach	50.06	8.46	1915		Frankfurt
13034	Native	France	Oise	Noyon	49.58	3.00	1916	Welzkow	Berlin
3511	Native	Spain	Catalonia	Pobla de Segur	42.15	0.58	1918	Haas	Frankfurt
7354	Native	Germany	Rhineland-Palatinate	Neustadt	49.21	8.09	1922	Stellwaag	Frankfurt

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815	Native	Greece	Crete	Meskla	35.00	25.00	1926	Rower	Frankfurt
9269	Native	Croatia	Istria	Opatija	45.33	14.30	1926	Enderlein	Berlin
13082	Native	Germany	Hesse	Offenbach	50.06	8.46	1928	Vogt	Berlin
1366	Native	Hungary	Tolna	Tolna	46.42	18.79	1928	Roewer	Frankfurt
14264	Native	France	Corsica	Piarea	42.09	9.05	1930	Schulze	Berlin
13042	Native	Germany	Baden-Würtemberg	Freiburg im Breisgau	48.00	7.51	1931	Kracht	Berlin
5593	Native	Germany	Hesse	Schwanheim	50.05	8.35	1931	Cürten	Frankfurt
3513	Native	Germany	Hesse	Darmstadt	49.52	8.39	1934	Haas	Frankfurt
3522	Native	Germany	Hesse	Offenbach, Luhrwald	50.06	8.46	1935	Zilch	Frankfurt
5597	Native	Germany	Hesse	Darmstadt	49.52	8.39	1936	Donien	Frankfurt
5596	Native	Germany	Hesse	Eppstein	50.08	8.24	1936	Hohorst	Frankfurt
1794	Native	Italy	Sardinia	unknown	43.47	11.15	1938	Roewer	Frankfurt
5658	Native	Italy	Tuscany	Florenz	40.02	9.04	1940	Roewer	Frankfurt
1796	Native	France	Eastern Pyrenees	unknown	42.50	2.75	1942	Roewer	Frankfurt
5595	Native	Germany	Hesse	Vockenhausen	50.09	8.23	1942	Pape	Frankfurt
8992	Native	France	Cote d´Azur	Marseille	43.18	5.23	1943	Roewer	Frankfurt
9009	Native	Macedonia	unknown	Ostrowo	42.00	22.00	1943	Roewer	Frankfurt
6236	Native	Germany	Hesse	Frankfurt	50.07	8.41	1948	Hesse, Herbst	Frankfurt
5772	Native	Germany	Hesse	Frankfurt Eukheimer Ried	50.09	8.45	1948	Schuster	Frankfurt
5773	Native	Germany	Hesse	Hofheim	50.05	8.27	1948	Nothdurfth	Frankfurt
5914	Native	Germany	Hesse	Schwanheim am Main	50.05	8.35	1948	Hesse	Frankfurt
5774	Native	Germany	Hesse	Sprendlingen	49.52	7.59	1948	Neubecker	Frankfurt
6386	Native	Germany	Hesse	Frankfurt Südfriedhof	50.07	8.41	1949	Lerner	Frankfurt
6547	Native	Germany	Hesse	Höchst	50.06	8.33	1949	Bott	Frankfurt
6547	Native	Germany	Hesse	Hofheim	50.05	8.27	1949	Nothdurft	Frankfurt
6548	Native	Germany	Hesse	Loßbach	50.05	8.27	1949	Nothdurft	Frankfurt
6385	Native	Germany	Hesse	Mönchsbruck	49.97	8.51	1949	Hesse	Frankfurt
6545	Native	Germany	Hesse	Wersau	49.47	8.52	1949	Schnellbäcker	Frankfurt
7498	Native	Germany	Hesse	Wisselsheim	50.23	8.46	1949	Scherf	Frankfurt
7504	Native	Germany	Hesse	Hengsten	50.04	8.50	1950	Zilch	Frankfurt
7535	Native	Germany	Hesse	Hofheim	50.05	8.27	1950	Nothdurft	Frankfurt
7507	Native	Germany	Hesse	Taunus Sulzbach	49.54	9.09	1950	Millowitsch	Frankfurt
10143	Native	Germany	Hesse	Wächtersbach	50.27	9.30	1950	Braun	Frankfurt
7509	Native	Germany	Hesse	Wetterau Niederweisel	50.28	8.95	1950	Kraus	Frankfurt
7503	Native	Germany	Rhineland-Palatinate	Pfalz Burrweiler	49.25	8.08	1950	Menges	Frankfurt
10269	Native	Germany	Rhineland-Palatinate	Waldorf	50.49	7.23	1950	Braun	Frankfurt
10190	Native	Germany	Hesse	Lorch am Rhein	50.03	7.48	1951	Braun	Frankfurt
10304	Native	Germany	Rhineland-Palatinate	Kühkopf	49.49	8.26	1951	Braun	Frankfurt
8349	Native	France	Corsica	Calacuccia	42.28	9.12	1952	Schnellb.	Frankfurt
8348 & 8351	Native	France	Corsica	Casamozza	42.31	9.26	1952	Schnellb.	Frankfurt
8355 & 8353	Native	France	Corsica	Corte	42.18	9.09	1952	Schnellb.	Frankfurt
8354	Native	France	Corsica	Col de Prato	42.09	9.05	1952	Schnellb.	Frankfurt
8352	Native	France	Corsica	Ponte Lecchia	42.31	9.32	1952	Schnellb.	Frankfurt
8350	Native	France	Corsica	Lumio Calenzana	42.35	8.49	1952	Schnellb.	Frankfurt
8422	Native	Spain	Catalonia	Barcelona	41.24	2.10	1953	Schnellb.	Frankfurt

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8423	Native	Spain	Catalonia	Castelldefels	41.28	1.97	1953	Schnellb.	Frankfurt
9523	Native	Italy	Sardinien	Oschiri	40.44	8.59	1954	Kraus	Frankfurt
8810	Native	Italy	Sardinien	Olbia	40.55	9.30	1954	Kraus	Frankfurt
8811	Native	Italy	Sardinien	Tempio	40.54	9.06	1954	Kraus	Frankfurt
9931	Native	Germany	Hesse	Mühlberg	50.29	8.37	1957	Braun	Frankfurt
10168	Native	Germany	Hesse	Schwanheim	50.05	8.35	1957	Braun	Frankfurt
10480	Native	Croatia	Istria	Rovinj	45.05	13.38	1957	Kraus	Frankfurt
3596	Native	France	Camargue	unknown	43.53	4.50	1960	Kraus	Frankfurt
TnGaGA	Native	Tunesia	Galita Archipelago	Ile de Galite	37.53	8.93	1972	Maija & J.E. Vesmaris	Bonn
Slovenia	Native	Slovenia					2009	J. Helms, M. Kuntner	Plön
GeNwBA	Native	Germany	North Rhine-Westphalia	Bonn	50.73	7.10	2009	H. Krehenwinkel	Plön
FrAuBA/B	Native	France	Auvergne	Bizeneuille	46.40	2.73	2010	H. Krehenwinkel	Plön
FrAuCA	Native	France	Auvergne	Clermont Ferrand	45.88	3.13	2010	H. Krehenwinkel	Plön
FrAuCB	Native	France	Auvergne	Clermont Ferrand	45.95	2.98	2010	H. Krehenwinkel	Plön
FrAuLA	Native	France	Auvergne	Lindron	46.42	2.78	2010	H. Krehenwinkel	Plön
GeBwFA	Native	Germany	Baden Württemberg	Freiburg	48.00	7.85	2010	C. Hanner	Plön
GeBwRA	Native	Germany	Baden Württemberg	Rielingshausen	48.08	9.25	2010	V. von Wirth	Plön
GeBwZA	Native	Germany	Baden Württemberg	Stuttgart Zuffenhausen-Rot	48.83	9.17	2010	T. Bauer	Plön
GeBwUA	Native	Germany	Baden-Württemberg	Unteruhldingen	47.72	9.23	2010	S. Huber	Plön
SpBIIA	Native	Spain	Balearic Islands	Ibiza	38.95	1.40	2010	B. Hinrichs	Plön
FrBnAA	Native	France	Basse Normandie	Alencon	48.45	0.12	2010	H. Krehenwinkel	Plön
GeBvBA	Native	Germany	Bavaria	Berching	49.10	11.43	2010	R. Samm	Plön
GeBvCA	Native	Germany	Bavaria	Chamerau	49.20	12.75	2010	M. Thierer Lutz	Plön
GeBvTA	Native	Germany	Bavaria	Deining (Bad Tölz)	47.07	11.55	2010	E.Bausbach	Plön
GeBvEA	Native	Germany	Bavaria	Dollnstein	48.87	11.07	2010	E.Bausbach	Plön
GeBvDA	Native	Germany	Bavaria	Dorfen	48.27	12.08	2010	J. Haft	Plön
GeBvLA	Native	Germany	Bavaria	Landsberg am Lech	47.98	10.87	2010	A. Roglmayr	Plön
GeBvMA	Native	Germany	Bavaria	Perlacher Forst	48.07	11.57	2010	E.Bausbach	Plön
GeBvPA	Native	Germany	Bavaria	Pfarrkirchen	48.42	12.93	2010	M. Vogt	Plön
GeBvMB	Native	Germany	Bavaria	Sauerlach	47.97	11.65	2010	E.Bausbach	Plön
GeBvMC	Native	Germany	Bavaria	Straßlach-Digharting	48.00	11.52	2010	E.Bausbach	Plön
GeBvKA	Native	Germany	Bavaria	Trautskirchen	49.45	10.58	2010	M. Hecht	Plön
PoBeCA	Native	Portugal	Beja	Corte Pequena	37.73	-7.85	2010	H. Krehenwinkel	Plön
ChBeHA	Native	Switzerland	Bern	Herzogenbuchsee	47.18	7.70	2010	S. Kumschick	Plön
TuBsBA	Native	Turkey	Black sea region	Black sea region	42.00	33.45	2010	R. Kaya	Plön
FrBuCA	Native	France	Burgundy	Chalon Sur Saone	46.75	4.83	2010	H. Krehenwinkel	Plön
FrBuCB	Native	France	Burgundy	Chalon Sur Saone	46.73	4.83	2010	H. Krehenwinkel	Plön
TuBuBC	Native	Turkey	Bursa	Terzioglu island Bursa	40.18	29.07	2010	R. Kaya	Plön
ItCbAA	Native	Italy	Calabria	Agropoli	40.33	15.00	2010	H. Krehenwinkel	Plön
ItCbBA	Native	Italy	Calabria	Battpaglia	40.61	14.93	2010	H. Krehenwinkel	Plön
ItCbCA	Native	Italy	Calabria	Capaccio	40.47	15.02	2010	H. Krehenwinkel	Plön
ItCbOA	Native	Italy	Calabria	Crosia	39.57	16.72	2010	H. Krehenwinkel	Plön
ItCbMA	Native	Italy	Calabria	Montalto Uffugo	39.40	16.15	2010	H. Krehenwinkel	Plön
ItCbRA	Native	Italy	Calabria	Roseto Capo Spulico	39.97	16.60	2010	H. Krehenwinkel	Plön
ItCaGA	Native	Italy	Campania	Giugliano	40.93	14.05	2010	H. Krehenwinkel	Plön

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ItCaPA	Native	Italy	Campania	Pomigliani Di Arco	40.92	14.37	2010	F. Baldanza	Plön
ItCaSA	Native	Italy	Campania	Sessa Aurunca Caserta	41.23	13.78	2010	H. Krehenwinkel	Plön
SpCaCA	Native	Spain	Catalonia	Cambrils	41.10	1.02	2010	H. Krehenwinkel	Plön
SpCaBA	Native	Spain	Catalonia	Cubelles	41.20	1.67	2010	H. Krehenwinkel	Plön
SpCaMA	Native	Spain	Catalonia	Mont Roig del Camp	41.08	0.97	2010	H. Krehenwinkel	Plön
SpCaPA	Native	Spain	Catalonia	Puigemma	42.10	2.80	2010	H. Krehenwinkel	Plön
SpCaRA	Native	Spain	Catalonia	Reus	41.13	1.13	2010	H. Krehenwinkel	Plön
SpCaRB	Native	Spain	Catalonia	Reus	41.15	1.13	2010	H. Krehenwinkel	Plön
SpCaFA	Native	Spain	Catalonia	Santa Colomna de Farners	41.87	2.65	2010	H. Krehenwinkel	Plön
SpCaVA	Native	Spain	Catalonia	Vic	41.95	2.27	2010	H. Krehenwinkel	Plön
FrCeBA	Native	France	Centre	Bourges	47.08	2.23	2010	H. Krehenwinkel	Plön
FrCeBB	Native	France	Centre	Bourges	47.03	2.33	2010	H. Krehenwinkel	Plön
FrCeTA	Native	France	Centre	Tours	47.32	0.47	2010	H. Krehenwinkel	Plön
GrEpIA/B	Native	Greece	Epirus	Igoumenitsa	39.52	20.18	2010	H. Krehenwinkel	Plön
GrEpIC	Native	Greece	Epirus	Igoumenitsa	39.52	20.23	2010	H. Krehenwinkel	Plön
GrEpID/E	Native	Greece	Epirus	Preveza	38.95	20.73	2010	H. Krehenwinkel	Plön
GrEpIF	Native	Greece	Epirus	unknown			2010	H. Krehenwinkel	Plön
ChGeAA	Native	Switzerland	Geneve	Avusy	46.15	5.98	2010	L. Monod	Plön
CrIsMA	Native	Croatia	Istria	Porec, Muzalik	45.22	13.58	2010	V. von Wirth	Plön
CrIsPA	Native	Croatia	Istria	Pula	44.85	13.58	2010	M. Dieter	Plön
SlJsPA	Native	Slovenia	Jugovzhodna Slovenija	Primostek	45.62	15.29	2010	M. Kuntner, Gregorič, Lokovšek	Plön
FrLrCA	Native	France	Languedoc-Roussillon	Carcassonne	43.25	2.13	2010	H. Krehenwinkel	Plön
FrLrNA	Native	France	Languedoc-Roussillon	Nimes	43.78	4.40	2010	H. Krehenwinkel	Plön
ItLzPA	Native	Italy	Lazio	Puzzali	41.30	13.75	2010	H. Krehenwinkel	Plön
ItLiNA	Native	Italy	Liguria	Noli	44.20	8.40	2010	A. Trotta	Plön
NlLiVA	Native	Netherlands	Limburg	Venlo	51.38	6.17	2010	H. Krehenwinkel	Plön
NlLiVB	Native	Netherlands	Limburg	Venlo	51.40	6.17	2010	H. Krehenwinkel	Plön
FrLiMA	Native	France	Limousin	Mestes	45.50	2.32	2010	H. Krehenwinkel	Plön
FrLiNA	Native	France	Limousin	Nespouls	45.05	1.48	2010	H. Krehenwinkel	Plön
FrMpMA	Native	France	Midi Pyrenees	Montauban	43.98	1.33	2010	H. Krehenwinkel	Plön
FrNcVA	Native	France	Nord Pas de Calais	Valencienne	50.37	3.62	2010	H. Krehenwinkel	Plön
GeNwQA	Native	Germany	North Rhine-Westphalia	Bochum-Querenburg	51.43	7.25	2010	H. Krehenwinkel	Plön
GeNwHA	Native	Germany	North Rhine-Westphalia	Haltern	51.73	7.23	2010	H. Krehenwinkel	Plön
GeNwKA	Native	Germany	North Rhine-Westphalia	Köln	50.93	6.95	2010	M. Krause	Plön
GeNwLA	Native	Germany	North Rhine-Westphalia	Letmathe	51.35	7.62	2010	H. Krehenwinkel	Plön
GeNwWA	Native	Germany	North Rhine-Westphalia	Wulfen	51.72	7.05	2010	H. Krehenwinkel	Plön
GeNwWB	Native	Germany	North Rhine-Westphalia	Wulfen	51.72	7.03	2010	H. Krehenwinkel	Plön
SlOkSA	Native	Slovenia	Obalno-kraška regija	Slavnik	45.54	13.96	2010	M. Kuntner, Lokovšek	Plön
FrPlAA	Native	France	Pays de la Loire	Angers	47.48	-0.48	2010	H. Krehenwinkel	Plön
FrPlAB	Native	France	Pays de la Loire	Angers	47.50	-0.50	2010	H. Krehenwinkel	Plön
FrPlTA	Native	France	Pays de la Loire	Tierce	47.60	-0.47	2010	H. Krehenwinkel	Plön
ItPgTA	Native	Italy	Puglia	Taranto	40.47	17.30	2010	H. Krehenwinkel	Plön
FrRaLA	Native	France	Rhône-Alpes	Lyon	45.48	4.83	2010	H. Krehenwinkel	Plön
HuSoBA/BB	Native	Hungary	Somogy	Balatonbereny	46.70	17.30	2010	M. Freudenschuss	Plön

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NlShOA	Native	Netherlands	Südholland	Oegstgeest	52.18	4.50	2010	P. van Helsdingen	Plön
61135	Native	Lebanon	unknown	unknown			2010	Jäger	Frankfurt
HuVeSB	Native	Hungary	Veszprem	Sümeg	46.85	17.20	2010	M. Freudenschuss	Plön
BeWaNA	Native	Belgium	Wallonia	Namur	50.48	4.72	2010	H. Krehenwinkel	Plön
PoAlBA	Native	Portugal	Alentejo	Borba	38.80	-7.45	2011	H. Krehenwinkel	Plön
GeBwDA	Native	Germany	Baden Würtemberg	Ditzingen	48.83	9.07	2011	H. Krehenwinkel	Plön
GeBwKA	Native	Germany	Baden Würtemberg	Korntal	48.83	9.12	2011	H. Krehenwinkel	Plön
SpBiEA	Native	Spain	Balearic Islands	Cala Lenya	39.02	1.58	2011	H. Krehenwinkel	Plön
SpBiEB	Native	Spain	Balearic Islands	Santa Eulalia	39.00	1.53	2011	H. Krehenwinkel	Plön
SpBaVA	Native	Spain	Basque Country	Vitoria	42.85	-2.68	2011	H. Krehenwinkel	Plön
PoBeCB	Native	Portugal	Beja	Corte Pequena	37.73	-7.85	2011	H. Krehenwinkel	Plön
SpCaAA	Native	Spain	Catalonia	Amposta	40.71	0.58	2011	H. Krehenwinkel	Plön
SpCaMB	Native	Spain	Catalonia	Mont Roig del Camp	41.09	0.96	2011	H. Krehenwinkel	Plön
SpCaTA	Native	Spain	Catalonia	Tarragona	41.12	1.25	2011	H. Krehenwinkel	Plön
PoCeFA	Native	Portugal	Centro	Figueira da Foz	40.15	-8.85	2011	H. Krehenwinkel	Plön
PoCeGA	Native	Portugal	Centro	Guarda	40.54	-7.27	2011	H. Krehenwinkel	Plön
PoCeNA	Native	Portugal	Centro	Nazare	39.61	-9.07	2011	H. Krehenwinkel	Plön
CrDaBA	Native	Croatia	Dalmatia	Brela	43.37	16.92	2011	H. Krehenwinkel	Plön
CrDaCB	Native	Croatia	Dalmatia	Ciovo	43.50	16.31	2011	M. Freudenschuss	Plön
CrDaSA	Native	Croatia	Dalmatia	Ston	42.83	17.73	2011	H. Krehenwinkel	Plön
FrAqDA	Native	France	Dordogne		45.00	0.67	2011	A. Grabolle	Plön
SpExTA	Native	Spain	Extremadura	Trujillanos	38.95	-6.25	2011	H. Krehenwinkel	Plön
GeHeDA	Native	Germany	Hesse	Darmstadt	49.87	8.65	2011	H. Krehenwinkel	Plön
GeHeHA	Native	Germany	Hesse	Frankfurt Hoechst	50.10	8.55	2011	H. Krehenwinkel	Plön
GeHeSA	Native	Germany	Hesse	Frankfurt Schwanheim	50.09	8.58	2011	H. Krehenwinkel	Plön
FrLrCB	Native	France	Languedoc-Roussillon	Carcassonne	43.21	2.35	2011	H. Krehenwinkel	Plön
FrLrCC	Native	France	Languedoc-Roussillon	Carcassonne	43.21	2.13	2011	H. Krehenwinkel	Plön
FrLrCD	Native	France	Languedoc-Roussillon	Carcassonne	43.25	2.13	2011	H. Krehenwinkel	Plön
FrLrCE	Native	France	Languedoc-Roussillon	Carcassonne	43.23	2.28	2011	H. Krehenwinkel	Plön
FrLrCF	Native	France	Languedoc-Roussillon	Carcassonne	43.25	2.18	2011	H. Krehenwinkel	Plön
FrLrBA	Native	France	Languedoc-Roussillon	Narbonne	43.18	3.00	2011	H. Krehenwinkel	Plön
FrLrNB	Native	France	Languedoc-Roussillon	Nimes	43.78	4.40	2011	H. Krehenwinkel	Plön
SpVaCA	Native	Spain	Valencia	Benicarlo	40.42	0.42	2011	H. Krehenwinkel	Plön
SpVaBA	Native	Spain	Valencia	Bunol	39.42	-0.79	2011	H. Krehenwinkel	Plön
ItVeBA	Native	Italy	Venetia	Bibione	45.77	13.00	2011	H. Krehenwinkel	Plön
5591	Native	Germany	Hesse	Frankfurt Eschenheimer Tor	50.07	8.41	~1900	von Heyden	Frankfurt
13021	Native	Turkey	Istanbul	Istanbul	41.17	28.98	~1900	Gottwald	Berlin
13025	Native	France	Meuse	St Mihiel	4.89	5.54	~1915		Berlin
13036	Native	Algeria	Algiers	Algiers	36.77	3.05	<1900	Quedenfeld	Berlin
13043	Native	Germany	Rhineland-Palatinate	Mombach	50.02	8.22	<1900	L. Koch	Berlin
242	Native	Greece	unknown	unknown			<1900	Krüper	Berlin
13019	Native	Greece		Kunami	37.67	21.44	<1900		Berlin
SpCaXA	Native	Spain	Catalonia	Barcelona	41.40	2.17	>2000	S. Nesseler	Plön
SpCaFA	Native	Spain	Catalonia	Flix	41.23	0.53	>2000	S.Nesseler	Plön
SpCaNA	Native	Spain	Catalonia	La Noguera	41.90	0.93	>2000	S. Nesseler	Plön

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SpCaLA	Native	Spain	Catalonia	Lleida	41.62	0.63	>2000	S. Nesseler	Plön
13045 & 484	Native	Syria	unknown	unknown	34.88	35.88	1820-1825	Ehrenberg	Berlin
5592	Native	Germany	Hesse	Schwanheim am Main	50.05	8.35			Frankfurt
13017	Native	Italy	Tuscany	Livorno	43.55	10.32		Weber	Berlin
13032	not assigned	Romania	Southern Carpathians	Mogura Odobesti	45.77	27.07	1918	P. Schulze	Berlin
BuRiRA (40659)	not assigned	Bulgaria	Kyustendil	Rila	42.12	23.15	2005	Jäger, Kunz	Frankfurt
BuKaDA01	not assigned	Bulgaria	Kavarna	Durankulak	43.70	28.52	2010	C. Deltshev	Plön
BuSoVA01	not assigned	Bulgaria	Sofia	Vitosha	42.70	23.32	2010	C. Deltshev	Plön
13033	Russia	Russia	Dagestan	Buynaksk	42.82	47.12	1902	Haymons	Berlin
	Russia	Ukraine	Kiev	Kiev	50.45	30.52	1963	Crome	Berlin
UAQ	Russia	Ukraine	Eastern Ukraine	unknown			2011	N. Polchaninova	Plön
UaKhGA	Russia	Ukraine	Kharkov	Gaidary Village	49.92	36.32	2011	N. Polchaninova	Plön
RuKuKA	Russia	Russia	Kursk	Khytor Stepnoy Village	51.73	36.18	2011	N. Polchaninova	Plön
RuKuSA	Russia	Russia	Kursk	Selikhovy Dvory Village	51.57	36.07	2011	N. Polchaninova	Plön
RuLiDB	Russia	Russia	Lipetsk	8. 8 km NW from Donskoye	52.85	38.78	2011	N. Polchaninova	Plön
RuLiDA	Russia	Russia	Lipetsk	Donskoye Village	52.85	38.78	2011	N. Polchaninova	Plön
RuLiYA	Russia	Russia	Lipetsk	Yablonevoye Village	52.62	39.60	2011	N. Polchaninova	Plön
UaLuAA	Russia	Ukraine	Lugansk	Kalaus Village	48.57	39.30	2011	N. Polchaninova	Plön
UaLuKA/B	Russia	Ukraine	Lugansk	Krynichnoye Village	48.57	39.30	2011	N. Polchaninova	Plön
UaLuSA	Russia	Ukraine	Lugansk	Streltsovka Village	49.30	39.87	2011	N. Polchaninova	Plön
UaLuVA	Russia	Ukraine	Lugansk	Velokotsk Village	48.57	39.30	2011	N. Polchaninova	Plön
13024	Russia	Russia	Volgograd	Sarepta	48.52	44.52	<1900		Berlin
6020/bzw. 1630	Russia	Russia	Volgograd	Sarepta	48.52	44.52	<1900		Berlin
13016	Russia	Ukraine	Zaporizhia	Melitopol	46.83	35.37	<1900		Berlin
13023 & 1320	Southern Caucasus	Azerbaijan	Lankaran	Lankaran	38.75	48.85	1900	Karsch	Berlin
13040	Southern Caucasus	Caucasus	unknown	unknown			1901	Heymons & Sauter	Berlin
GgAdBA (2647)	Southern Caucasus	Georgia	Adjara	Batumi	41.64	41.64	1975	Moritz det.	Berlin
GgKhGA01	Southern Caucasus	Georgia	Khaketi	Gareji	41.75	45.72	2006	C. Deltshev	Plön
GgTiGA/B	Southern Caucasus	Georgia	Tiblisi	Gamarjveba	41.64	45.00	2006	S. Otto	Plön
GgTiGC	Southern Caucasus	Georgia	Tiblisi	Gamarjveba	41.64	45.00	2010	S. Otto	Plön
61138	Southern Caucasus	Iran	Coast of Caspian Sea	unknown	42.12	23.15	>1960	Jäger	Frankfurt

Chapter 2

Chapter 2 will be submitted to the Journal of Biogeography. Alignments as well as SNP - and microsatellite data will be uploaded to the Dryad Digital Repository. Primers for mitochondrial sequences, microsatellites and nuclear SNP loci for the wasp spider are identical with those used in chapter 1.

Supplementary table 2.1 Sampling sites for *Argiope bruennichi* populations, used in chapter 2. The majority of European and East Asian samples are identical with those, used in chapter 1.

Identifier/Museum No.	Region	Country	District	City	GPS N	GPS E	Year	Collector	Collection
13047-13050 & 13056	Central Asia	Uzbekistan	Samarqand	Kata Kurgan	39.90	66.50	1903	E. Zoffmann	Berlin
CnXiXA01(Ar6340)	Central Asia	China	Xinjiang	unknown	39.45	75.98	1991		Beijing
CN Chonquing	East Asia	China	Chonquing	Chonquing	29.55	106.51	2010	Z. Zhang	Plön
3709	East Asia	China	Beijing	Beijing	39.91	116.39	<1900	Möllenhoff	Berlin
CnFuFA01(Ar5458)	East Asia	China	Fujian	unknown	25.90	118.30	1991		Beijing
Cn	East Asia	China	Fujian	unknown	25.90	118.30	2010	RC. Cheng	Plön
CnGuGA01(Ar5848)	East Asia	China	Guizhou	unknown	26.83	106.83	1978		Beijing
CnHeHA01(Ar5402)	East Asia	China	Hebei	unknown	39.30	116.70	1998		Beijing
CnHuHA01(Ar6196)	East Asia	China	Hubei	unknown	30.97	112.23	1984		Beijing
CnJaJA01(Ar19504)	East Asia	China	Jiangxi	unknown	27.63	115.77	2008		Beijing
CnLiLA01(Ar4926)	East Asia	China	Liaoning	unknown	41.10	122.30			Beijing
13061	East Asia	China	Shandong	Quingdao	36.07	120.38	1905	Glaue	Berlin
13062	East Asia	China	Shandong	Quingdao	36.07	120.38		Redinberg	Berlin
CnYuYA01(Ar5862)	East Asia	China	Yunnan	unknown	24.50	101.50	1983		Beijing
CnZhZA01(Ar4927)	East Asia	China	Zhejiang	unknown	29.20	120.50			Beijing
JpEhYA	East Asia	Japan	Ehime	Yuyama	33.83	132.77	2011	N. Tsurusaki	Plön
JpFuFA	East Asia	Japan	Fukuoka	Fukuoka, Tarumaru Lizuka	33.65	130.68	2011		Plön
JpFuFB	East Asia	Japan	Fukuoka	Fukuoka, Yamada Ryokuchi	33.57	130.72	2011		Plön
JpHoHA	East Asia	Japan	Hokkaido	Hakodate	41.77	140.73	2010	K. Tanaka	Plön
JpIbTA	East Asia	Japan	Ibaraki	Tsukuba	36.00	140.10	2011	Y. Baba	Plön
JpKaAA	East Asia	Japan	Kagoshima	Amami-oshima Island	28.33	129.32	2011	Y. Baba	Plön
JpKaAB	East Asia	Japan	Kagoshima	Amami-oshima Island	28.28	129.43	2011	Y. Baba	Plön
JpKaAC	East Asia	Japan	Kagoshima	Amami-oshima Island	28.23	129.35	2011	Y. Baba	Plön
JpKaIA	East Asia	Japan	Kagoshima	Isa-shi	31.60	130.56	2007	T.Tsukada	Plön
JpKaSA	East Asia	Japan	Kagoshima	Satsuma	31.60	130.56	2007	T.Tsukada	Plön
JpKaYA	East Asia	Japan	Kagoshima	unknown	31.60	130.56	2007	T.Tsukada	Plön
JpKaMA	East Asia	Japan	Kagoshima	Hirata	31.60	130.56	2011	T. Maeda	Plön
JpKaCA	East Asia	Japan	Kagoshima	Mihama-chö	31.60	130.56	2011	K. Nakamura	Plön
JpKaKA	East Asia	Japan	Kagoshima	Shimofukmoto-chö	31.60	130.56	2011	T. Maeda	Plön
JpKaHA	East Asia	Japan	Kagoshima	Sogi,Ohkuchi	31.60	130.56	2011	K.Nakamura	Plön
JpMySA	East Asia	Japan	Miyagi	Sendai	38.27	140.87	2010	K. Tanaka	Plön
JpMyEA	East Asia	Japan	Miyazaki	Ebino-shi	32.02	131.35	2007	T.Tsukada	Plön
JpNaAA	East Asia	Japan	Nagano	Azusagawa-Azusa	36.23	137.97	2011	N. Tsurusaki	Plön
JpShOA	East Asia	Japan	Shiga	Ozigaoka	35.12	136.07	2011	T. Masumoto	Plön
JpTcUA	East Asia	Japan	Tochigi	Utsunomiya	36.53	139.95	2011	Y. Baba	Plön
JpToKA	East Asia	Japan	Tottori	Kagamiganaru	35.28	133.48	2011	N. Tsurusaki	Plön
JpToSA	East Asia	Japan	Tottori	Katsurami	35.50	134.17	2011	N. Tsurusaki	Plön
JpToToA	East Asia	Japan	Tottori Prefecture	Tottori, Katsurami	35.50	134.23	2010	N. Tsurusaki	Plön
2693	East Asia	Japan	unknown	unknown			<1900	Hilgendorff	Berlin
JP2 JpMySB	East Asia	Japan	Miyagi	Sendai	38.27	140.87	>2000	K. Tanaka	Plön
CrDaBA	Europe	Croatia	Dalmatia	Brela	43.37	16.92	2011	H. Krehenwinkel	Plön
CrDaCB	Europe	Croatia	Dalmatia	Ciovo	43.50	16.31	2011	M. Freudenschuss	Plön
CrDaSA	Europe	Croatia	Dalmatia	Ston	42.83	17.73	2011	H. Krehenwinkel	Plön

Identifier/Museum No.	Region	Country	District	City	GPS N	GPS E	Year	Collector	Collection
CrIsMA	Europe	Croatia	Istria	Porec, Muzalik	45.22	13.58	2010	V. von Wirth	Plön
FrAuBA/B	Europe	France	Auvergne	Bizeneuille	46.40	2.73	2010	H. Krehenwinkel	Plön
FrBnAA	Europe	France	Basse Normandie	Alencon	48.45	0.12	2010	H. Krehenwinkel	Plön
FrBuCA	Europe	France	Burgundy	Chalon Sur Saone	46.75	4.83	2010	H. Krehenwinkel	Plön
FrLrCA	Europe	France	Languedoc-Roussillon	Carcassonne	43.25	2.13	2010	H. Krehenwinkel	Plön
FrLrNA	Europe	France	Languedoc-Roussillon	Nimes	43.78	4.40	2010	H. Krehenwinkel	Plön
FrMpMA	Europe	France	Midi Pyrenees	Montauban	43.98	1.33	2010	H. Krehenwinkel	Plön
FrPlAB	Europe	France	Pays de la Loire	Angers	47.50	-0.50	2010	H. Krehenwinkel	Plön
FrRaLA	Europe	France	Rhône-Alpes	Lyon	45.48	4.83	2010	H. Krehenwinkel	Plön
GeBwKA	Europe	Germany	Baden Würtemberg	Korntal	48.83	9.12	2011	H. Krehenwinkel	Plön
GeHeDA	Europe	Germany	Hesse	Darmstadt	49.87	8.65	2011	H. Krehenwinkel	Plön
GeHeSA	Europe	Germany	Hesse	Frankfurt Schwanheim	50.09	8.58	2011	H. Krehenwinkel	Plön
GrEpIA/B	Europe	Greece	Epirus	Igoumenitsa	39.52	20.18	2010	H. Krehenwinkel	Plön
GrEpIC	Europe	Greece	Epirus	Igoumenitsa	39.52	20.23	2010	H. Krehenwinkel	Plön
GrEpID/E	Europe	Greece	Epirus	Preveza	38.95	20.73	2010	H. Krehenwinkel	Plön
GrEpIF	Europe	Greece	Epirus	unknown			2010	H. Krehenwinkel	Plön
HuSoBA/BB	Europe	Hungary	Somogy	Balatonbereny	46.70	17.30	2010	M. Freudenschuss	Plön
HuVeSB	Europe	Hungary	Veszprem	Sümeg	46.85	17.20	2010	M. Freudenschuss	Plön
ItCbAA	Europe	Italy	Calabria	Agropoli	40.33	15.00	2010	H. Krehenwinkel	Plön
ItCbBA	Europe	Italy	Calabria	Battpaglia	40.61	14.93	2010	H. Krehenwinkel	Plön
ItCbCA	Europe	Italy	Calabria	Capaccio	40.47	15.02	2010	H. Krehenwinkel	Plön
ItCbOA	Europe	Italy	Calabria	Crosia	39.57	16.72	2010	H. Krehenwinkel	Plön
ItCbMA	Europe	Italy	Calabria	Montalto Uffugo	39.40	16.15	2010	H. Krehenwinkel	Plön
ItCbRA	Europe	Italy	Calabria	Roseto Capo Spulico	39.97	16.60	2010	H. Krehenwinkel	Plön
ItCaGA	Europe	Italy	Campania	Giugliano	40.93	14.05	2010	H. Krehenwinkel	Plön
ItCaPA	Europe	Italy	Campania	Pomigliani Di Arco	40.92	14.37	2010	F. Baldanza	Plön
ItCaSA	Europe	Italy	Campania	Sessa Aurunca Caserta	41.23	13.78	2010	H. Krehenwinkel	Plön
ItLzPA	Europe	Italy	Lazio	Puzzali	41.30	13.75	2010	H. Krehenwinkel	Plön
ItLiNA	Europe	Italy	Liguria	Noli	44.20	8.40	2010	A. Trotta	Plön
ItPgTA	Europe	Italy	Puglia	Taranto	40.47	17.30	2010	H. Krehenwinkel	Plön
ItVeBA	Europe	Italy	Venetia	Bibione	45.77	13.00	2011	H. Krehenwinkel	Plön
3521	Europe	Morocco	Grand Casablanca	Casablanca	33.35	-7.37	1872	Fritzsch & Rein	Frankfurt
PoCeGA	Europe	Portugal	Centro	Guarda	40.54	-7.27	2011	H. Krehenwinkel	Plön
PoBeCA	Europe	Portugal	Beja	Corte Pequena	37.73	-7.85	2010	H. Krehenwinkel	Plön
SpBIIA	Europe	Spain	Balearic Islands	Ibiza	38.95	1.40	2010	B. Hinrichs	Plön
SpCaBA	Europe	Spain	Catalonia	Cubelles	41.20	1.67	2010	H. Krehenwinkel	Plön
SpCaPA	Europe	Spain	Catalonia	Puigemma	42.10	2.80	2010	H. Krehenwinkel	Plön
SpCaFA	Europe	Spain	Catalonia	Santa Colomna de Farners	41.87	2.65	2010	H. Krehenwinkel	Plön
SpVaCA	Europe	Spain	Valencia	Benicarlo	40.42	0.42	2011	H. Krehenwinkel	Plön
SpVaBA	Europe	Spain	Valencia	Bunol	39.42	-0.79	2011	H. Krehenwinkel	Plön
TnGaGA	Europe	Tunesia	Galita Archipelago	Ile de Galite	37.53	8.93	1972	Maija & J.E. Vesmaris	Bonn
TuBsBA	Europe	Turkey	Black sea region	Black sea region	42.00	33.45	2010	R. Kaya	Plön
TuBuBC	Europe	Turkey	Bursa	Terzioglu island Bursa	40.18	29.07	2010	R. Kaya	Plön
13021	Europe	Turkey	Istanbul	Istanbul	41.17	28.98	~1900	Gottwald	Berlin
BuKaDA01	Europe	Bulgaria	Kavarna	Durankulak	43.70	28.52	2010	C. Deltshev	Plön

Identifier/Museum No.	Region	Country	District	City	GPS N	GPS E	Year	Collector	Collection
BuRiRA (40659)	Europe	Bulgaria	Kyustendil	Rila	42.12	23.15	2005	Jäger, Kunz	Frankfurt
BuSoVA01	Europe	Bulgaria	Sofia	Vitosha	42.70	23.32	2010	C. Deltshev	Plön
13032	Europe	Romania	Southern Carpathians	Mogura Odobesti	45.77	27.07	1918	P. Schulze	Berlin
UaKhGA	Russia	Ukraine	Kharkov	Gaidary Village	49.92	36.32	2011	N. Polchaninova	Plön
UaLuAA	Russia	Ukraine	Lugansk	Kalaus Village	48.57	39.30	2011	N. Polchaninova	Plön
UaLuKA/B	Russia	Ukraine	Lugansk	Krynichnoye Village	48.57	39.30	2011	N. Polchaninova	Plön
13023 & 1320	Europe	Azerbaijan	Lankaran	Lankaran	38.75	48.85	1900	Karsch	Berlin
GgAdBA (2647)	Europe	Georgia	Adjara	Batumi	41.64	41.64	1975	Moritz det.	Berlin
GgKhGA01	Europe	Georgia	Khaketi	Gareji	41.75	45.72	2006	C. Deltshev	Plön
GgTiGA/B	Europe	Georgia	Tiblisi	Gamarjveba	41.64	45.00	2006	S. Otto	Plön
GgTiGC	Europe	Georgia	Tiblisi	Gamarjveba	41.64	45.00	2010	S. Otto	Plön
M1	Madeira	Madeira	Madeira	Camacha	32.72	-16.83	2012	H. Krehenwinkel	Plön
M2	Madeira	Madeira	Madeira	Santana	32.80	-16.88	2012	H. Krehenwinkel	Plön
M3	Madeira	Madeira	Madeira	Ribeiria Brava	32.70	-17.05	2012	H. Krehenwinkel	Plön
M4	Madeira	Madeira	Madeira	Santa Maria	32.85	-17.18	2012	H. Krehenwinkel	Plön
M5	Madeira	Madeira	Madeira	Santa Maria	32.85	-17.20	2012	H. Krehenwinkel	Plön
M6	Madeira	Madeira	Madeira	Camacha	32.73	-17.83	2012	H. Krehenwinkel	Plön
AzPiRA	Azores	Azoren	Pico	Sao Roque	38.52	-28.32	2011	H. Krehenwinkel	Plön
AzPiCA	Azores	Azoren	Pico	Sao Caetano	38.42	-28.40	2011	H. Krehenwinkel	Plön
AzPiFA	Azores	Azoren	Pico	Fetais	38.40	-28.07	2011	H. Krehenwinkel	Plön
AzSjVA	Azores	Azoren	Sao Jorge	Velas	38.66	-28.20	2011	H. Krehenwinkel	Plön
AzSjRA	Azores	Azoren	Sao Jorge	Rosais	38.70	-28.23	2011	H. Krehenwinkel	Plön
AzSjTB	Azores	Azoren	Sao Jorge	Topo	38.55	-27.77	2011	H. Krehenwinkel	Plön
AzSmLA	Azores	Azoren	Sao Miguel	Livramento	37.77	-25.58	2011	H. Krehenwinkel	Plön
AzSmNA	Azores	Azoren	Sao Miguel	Nordeste	37.82	-25.13	2011	H. Krehenwinkel	Plön
AzSmPA	Azores	Azoren	Sao Miguel	Povacao	37.75	-25.22	2011	H. Krehenwinkel	Plön
AzSmFA	Azores	Azoren	Sao Miguel	Furnas	37.73	-25.32	2011	H. Krehenwinkel	Plön
AzSmJA	Azores	Azoren	Sao Miguel	Maia	37.82	-25.38	2011	H. Krehenwinkel	Plön
AzSmCA	Azores	Azoren	Sao Miguel	Capelas	37.83	-25.68	2011	H. Krehenwinkel	Plön
AzSmMA	Azores	Azoren	Sao Miguel	Mosteiro	37.88	-25.82	2011	H. Krehenwinkel	Plön
AzSmMB	Azores	Azoren	Sao Miguel	Mosteiro	37.88	-25.82	2011	H. Krehenwinkel	Plön
AzTeNA	Azores	Azoren	Terceira	Negrito	38.65	-27.27	2011	H. Krehenwinkel	Plön
AzFlFA	Azores	Azores	Flores	Faja Grande	39.45	-31.26	2010	N. Fritzen	Plön
AzSjTA	Azores	Azores	Sao Jorge	Topo	38.54	-27.75	2010	N. Fritzen	Plön

Supplementary table 2.2 Sampling sites for *Pisura mirabilis* populations, which were analyzed in chapter 2. The geographic region for each genetic group is shown in column one.

Region	ID	Country	Site	GPS N	GPS E	Date	Collector
Central	Ba6	Latvia	Skulte	57.35	24.45	2012	Krehenwinkel
	Ba9	Estonia	Ainazi	57.90	24.42	2012	Krehenwinkel
	S0	Sweden	Karlshamn	56.16	14.85	2012	Krehenwinkel
	S1	Sweden	Soderakra	56.45	16.08	2012	Krehenwinkel
	S2	Sweden	Kalmar	56.63	16.22	2012	Krehenwinkel
	S3	Sweden	Kristianstad	55.95	14.01	2012	Krehenwinkel
	D	Denmark	Ebeltoft	56.13	10.34	2012	Allen
	H	Germany	Greifswald	54.08	13.44	2012	Meese
	HT	Germany	Greifswald	54.09	13.38	2012	Meese
	Sue	Germany	Sünna	50.79	10.00	2012	Meese
	PP	Germany	Plön	54.16	10.43	2012	Meese, Frehse
	PME1.2	Bulgaria	Blagoewgrad	42.01	23.10	2005	SMF62883
	PME1.4	Montenegro	Ulcinij	41.92	19.20	2006	SMF62887
	PME2.2	Austria	Burgenland	47.50	16.42	2011	M. Freudenschuss
	PME2.5	Hungary	Balatonbereny	46.71	17.32	2010	M. Freudenschuss
	PME2.9	Austria	Sankt Valentin	48.17	14.53	2009	M. Freudenschuss
Balkan	PME1.3	Croatia	Rab	44.77	14.77	2008	SMF62878
	PME2.3	Croatia	Ciovo	43.50	16.28	2011	M. Freudenschuss
	PME2.4	Croatia	Ciovo	43.50	16.28	2011	M. Freudenschuss
South-Western	PME1.1	Spain	Valencian	39.50	-0.75	2010	SMF62877
	M1	Portugal	Camacha	32.72	16.83	2012	Krehenwinkel
	M4	Portugal	Santa Maria	32.85	17.18	2012	Krehenwinkel
	M6	Portugal	Camacha	32.73	16.83	2012	Krehenwinkel

Supplementary table 2.3 Primer sequences for the two nuclear DNA fragments, which were sequenced for *Argiope bruennichi* populations.

Primer Name	454 read ID	Sequence 5´-3	Tm	Exp. Frag. length
SA11F	GD7TUPT01A6MC4	GCAGTTCGGAGGCACTTAAC	59.9	453
SA11R		CGTGCCGAATTTGATTTCTA	58.8	453
SA24F	GD7TUPT01AR4L0	TGTGGGGGTTAATAAGTAAAATGA	58.8	456
SA24R		TTCCTGATTTACTACGTCTTCGT	57.2	456

Supplementary table 2.4 Primer sequences for the nuclear microsatellites, which were genotyped for *Pisaura mirabilis*.

Primer ID	Sequence 5'-3'	Tm °C	Product size (bp)	Repeat motif	454-Fragment ID
P_ms_33_F	GCGGAACTTGTCCCAATAAA	59.9	338	TATC	HKQ7YSY03CUECY
P_ms_33_R	ACGACATGGCCGCTTAAA	60.2			
P_ms_34_F	CATAGGGTAAGGGGCACACA	60.8	350	AAAT	HKQ7YSY03DICMD
P_ms_34_R	AGCTAGCAGACGTTGGTTCG	60.6			
P_ms_37_F	GTGAAACAAGTTCCGCCATT	60	248	AACA	HKQ7YSY03DHR1E
P_ms_37_R	CGCCTACCGATCAAGCTATC	59.8			
P_ms_41_F	TGTACACATTGACATCAAAAATACTTA	57	148	ATCT	HKQ7YSY03CXRPD
P_ms_41_R	TGGAACTTGCCGTCTATCAA	59.3			

Primer ID	Sequence 5'-3'	Tm °C	Product size (bp)	Repeat motif	454-Fragment ID
P_ms_44_F	ATGCTGAACTTTCGCAGTGA	59.6	229	AAT	HKQ7YSY03DI3ER
P_ms_44_R	CTTAAAACCGCAAACCGAAA	60.1			
P_ms_45_F	TGAATGCCTTTCAGACTTACTAAACA	60.5	239	ATT	HKQ7YSY03DGPKH
P_ms_45_R	TTTCCTTCGTCAAGATGTCG	58.8			
P_ms_47_F	AGGCCATGGAAGCATAAGAA	59.7	148	TTA	HKQ7YSY03C8RCK
P_ms_47_R	ATTGCAGCCTAGGACGAAGA	60			
P_ms_49_F	GAAATGGGTTGTCAATTACGG	59.2	390	AC	HKQ7YSY03DC6J8
P_ms_49_R	TGGTATGTGTTGTGTACTTTCTGA	57.3			
P_ms_50_F	GGCAGAACACCGTCTTCATT	60.1	333	AT	HKQ7YSY03DE117
P_ms_50_R	ACTTGTTATTTTGTCAAACCATTTT	57.4			
P_ms_51_F	GCTGTGGCGAAATGAATGTA	59.7	394	AT	HKQ7YSY03DDI30
P_ms_51_R	TCGAAAAATGCAAGACACCA	60.2			
P_ms_56_F	TTTGCATTTCGACTATTATTTATCA	57.2	134	TA	HKQ7YSY03DAFJA
P_ms_56_R	CCGTCTAGCACATAATCACACA	58.7			
P_ms_57_F	CCAAACACAGCGAATGGAT	59.5	129	CA	HKQ7YSY03DBFBU
P_ms_57_R	TCGTAAACGTGAACAAGGAGAA	59.8			
P_ms_59_F	CAAACACGGTTAGAATTTTCAGTG	60	112	AC	HKQ7YSY03CZ0AC
P_ms_59_R	AAATATTGAAACGCAGGATTGT	57.7			

Supplementary table 2.5 Primer sequences for the four nuclear DNA fragments, which were genotyped for *Pisaura mirabilis* populations.

Primer name	454 fragment	Primer sequence 5'-3'	Tm	Product size bp
PM_S_2F	HKQ7YSY03C5NGE	GCCAGATACAGCAGGAATCG	60.8	241
PM_S_2R		CTCAGAACGGCCAGTTTAGC	60	241
PM_S_3F	HKQ7YSY03CZXGG	TCTGGTCCATGGTACTTTTGG	59.8	208
PM_S_3R		AACTGTTGAATGATTATTTGCTTTG	58.7	208
PM_S_4F	HKQ7YSY03C872R	AAGCGATGCTGTATGCAAAA	59.5	265
PM_S_4R		CTGGAATGGGCCACAGTAGT	60	265
PM_S_10F	HKQ7YSY03CVBLU	TCCGTTTTAGTGAAATGCAAA	58.3	315
PM_S_10R		TGACCAGAGACTGTCTTCCAAA	59.9	315

Supplementary table 2.6 Allele - and haplotype frequencies for the four nuclear SNPs and the mitochondrial COI gene in Central and South-Western European *Pisaura mirabilis* populations.

Marker	Region	N	Freq. SW allele	Freq. Central allele
PMS2	Central	22	0.00	1.00
PMS3	Central	14	0.15	0.85
PMS4	Central	21	0.44	0.56
PMS1	Central	10	0.15	0.85
COI	Central	117	0.03	0.97
PMS2	South-Western	11	1.00	0.00
PMS3	South-Western	8	1.00	0.00
PMS4	South-Western	11	1.00	0.00
PMS1	South-Western	10	0.95	0.05
COI	South-Western	22	100	0

Supplementary table 2.7 Mitochondrial - and nuclear nucleotide diversity for *Argiope bruennichi* populations, which were genotyped in chapter 2.

Region	Population	Mitochondrial π	Nuclear π
East Asia	JpKaAA	0.0000	0.0076
East Asia	JpNaAA	0.0044	0.0104
East Asia	JpTcUA	0.0036	0.0109
East Asia	JpIbTA	0.0033	0.0137
East Asia	JpToSA	0.0041	0.0153
East Asia	JpToKA	0.0033	NA
East Asia	JpFuFA	0.0029	NA
East Asia	JpFuFB	0.0043	NA
East Asia	JpShOA	0.0034	NA
East Asia	JpKaYA	0.0050	NA
East Asia	JpKaSA	0.0043	NA
East Asia	Chongqing	0.0052	NA
East Asia	Tsingtau	0.0038	NA
East Asia	JpEhYA	0.0040	NA
Europe	SpBiIA	0.0006	0.0000
Europe	ItCaGA	0.0014	0.0006
Europe	CrLsMA	0.0013	0.0008
Europe	PoBeCA	0.0015	0.0008
Europe	FrLrCA	0.0014	0.0008
Europe	ItPgTA	0.0007	0.0013
Europe	FrRaLA	0.0014	0.0023
Europe	GrEpIC	0.0023	0.0032
Europe	Katta Kurgan	0.0014	NA
Europe	Lankaran	0.0033	NA
Europe	HuSoBA	0.0011	NA
Europe	TnGaGA	0.0014	NA
Europe	ItCaSA	0.0000	NA
Europe	ItLzPA	0.0003	NA
Europe	ItCbBA	0.0003	NA
Europe	PoCeGA	0.0015	NA
Europe	SpCaFA	0.0012	NA
Europe	SpCaPA	0.0013	NA
Europe	SpCaBA	0.0013	NA
Europe	SpVaBA	0.0015	NA
Europe	SpVaCA	0.0010	NA
Europe	FrLrNA	0.0009	NA
Europe	FrMpMA	0.0005	NA
Europe	FrBnAA	0.0007	NA
Europe	FrPlAB	0.0008	NA
Europe	FrAuBA	0.0014	NA
Europe	FrBuCA	0.0019	NA
Europe	GeBwKA	0.0000	NA
Europe	GeHeDA	0.0007	NA
Europe	GeHeSA	0.0007	NA
Europe	UaLuKA	0.0031	NA

Region	Population	Mitochondrial π	Nuclear π
Europe	UaKhGA	0.0029	NA
Europe	UaLuAA	0.0031	NA
Madeira	M6	0.0000	0.0010
Madeira	M1	0.0000	0.0013
Madeira	M2	0.0000	0.0013
Madeira	M4	0.0000	0.0013
Azores	AzTeNA	0.0004	0.0009
Azores	AzSmPA	0.0008	0.0010
Azores	AzSjTB	0.0004	0.0014
Azores	AzPiCA	0.0001	0.0016
Azores	AzSjRA	0.0000	NA
Azores	AzSjVA	0.0007	NA
Azores	AzSmCA	0.0008	NA
Azores	AzSmLA	0.0009	NA
Azores	AzSmMB	0.0003	NA
Azores	AzSmNA	0.0005	NA

Chapter 3

Chapter 3 will be submitted to Molecular Ecology Resources. Alignments and microsatellite data will be uploaded to the Dryad Digital Repository. Microsatellite and mitochondrial primers have been taken from those presented in chapter 1.

Supplementary table 3.1 List of museum specimens, which were tested for genotyping success. The different DNA markers are shown in the first six columns, with 0 indicating no genotype and 1 meaning successfully genotyped. The samples are sorted according to museum collection and collection date.

~150 bp COI	~350 bp COI	MA53 ~150bp	MA60 ~150bp	MA55 ~250bp	MA56 ~250 bp	Museum	Collection date	bodysize (mm)	Collection site	Extraction no.
1	0	0	0	0	0	Berlin	1820	7.5	Syria	11_5
1	0	0	0	0	0	Berlin	1820	7.5	Syria	11_6
1	0	0	0	0	0	Berlin	1820	7.2	Syria	11_7
1	0	0	0	0	0	Berlin	1820		Syria	11_8
1	0	0	0	0	0	Berlin	1825		Syria	8_11
1	0	0	0	0	0	Berlin	1825		Syria	8_27
1	0	0	0	0	0	Berlin	1825		Syria	8_28
1	0	0	0	0	0	Berlin	1825		Syria	8_29
1	1	0	0	1	1	Berlin	1846	5.8	Taurus Sizilien	9_44
1	0	1	1	1	1	Berlin	1897	4	Tegel	11_45
1	0	0	0	0	0	Berlin	1897	5.2	Asia Minor Gülek	7_2
1	1	1	1	1	1	Berlin	1899	3.9	Rovigno Campagne	7_8
1	1	1	1	1	0	Berlin	1899	4	Rovigno Campagne	7_7
1	1	1	1	1	1	Berlin	1900		Lenkorau	7_14

~150 bp COI	~350 bp COI	MA53 ~150bp	MA60 ~150bp	MA55 ~250bp	MA56 ~250 bp	Museum	Collection date	bodysize (mm)	Collection site	Extraction no.
1	1	1	1	1	1	Berlin	1900		Lenkorau	7_11
1	0	1	1	0	0	Berlin	1900		Lenkorau	9_4
1	1	1	1	1	0	Berlin	1900		Lenkorau	7_10
1	0	0	0	0	0	Berlin	1900		Lenkorau	9_2
1	1	1	1	0	0	Berlin	1900		Lenkorau	9_5
1	1	0	1	1	1	Berlin	1900		Lenkorau	7_12
1	1	0	1	1	0	Berlin	1900		Lenkorau	7_13
1	1	1	1	1	1	Berlin	1900		Lenkorau	7_15
1	1	0	1	1	0	Berlin	1900		Lenkorau	7_9
1	1	1	1	1	0	Berlin	1900		Lenkorau	9_1
1	1	1	1	1	1	Berlin	1900		Lenkorau	9_3
1	0	1	1	0	1	Berlin	1901		Transkaspien oder Kaukasus	10_8
1	1	0	1	0	0	Berlin	1901		Transkaspien oder Kaukasus	10_10
1	0	1	1	0	0	Berlin	1901		Transkaspien oder Kaukasus	10_9
1	1	0	0	0	0	Berlin	1902		Kaukasus Tamir Schau Schura	9_40
1	1	1	1	0	1	Berlin	1902		Rovigno	10_46
1	1	0	1	0	0	Berlin	1902		Rovigno	9_45
1	0	0	0	0	0	Berlin	1902	4.6	Rovigno	9_46
1	0	1	1	1	1	Berlin	1903		Kata Kurgan	10_24
1	1	1	1	1	1	Berlin	1903		Kata Kurgan	10_25
1	1	0	1	1	1	Berlin	1903		Kata Kurgan	10_26
1	1	0	1	1	0	Berlin	1903		Kata Kurgan	10_27
1	1	1	1	1	1	Berlin	1903		Kata Kurgan	10_34
1	1	0	0	0	0	Berlin	1903		Kata Kurgan	10_35
1	0	1	1	1	0	Berlin	1903	4.2	Müggelsee	11_44
1	1	1	1	0	1	Berlin	1903		Kata Kurgan	8_18
1	1	1	1	1	1	Berlin	1903		Kata Kurgan	8_19
1	1	1	1	1	1	Berlin	1903		Kata Kurgan	8_20
1	1	0	1	1	0	Berlin	1903		Kata Kurgan	8_21
0	0	0	0	0	0	Berlin	1904		Formosa Pilam	10_15
0	0	0	0	0	0	Berlin	1904		Formosa Pilam	10_16
0	0	0	0	0	0	Berlin	1904		Formosa Pilam	10_17
0	0	0	0	0	0	Berlin	1904		Formosa Pilam	10_18
0	0	0	0	0	0	Berlin	1904		Formosa Pilam	10_21
0	0	0	0	0	0	Berlin	1904		Formosa Pilam	10_22
0	0	0	0	0	0	Berlin	1904		Formosa Pilam	8_15
1	0	0	0	0	0	Berlin	1904		Formosa Pilam	10_19
1	0	0	0	0	0	Berlin	1904		Formosa Pilam	10_20
1	0	0	0	0	0	Berlin	1904		Formosa Pilam	8_13
1	0	0	0	0	0	Berlin	1904		Formosa Pilam	8_14

~150 bp COI	~350 bp COI	MA53 ~150bp	MA60 ~150bp	MA55 ~250bp	MA56 ~250 bp	Museum	Collection date	bodysize (mm)	Collection site	Extraction no.
1	0	0	0	0	0	Berlin	1904		Formosa Pilam	8_16
1	0	0	0	0	0	Berlin	1904		Formosa Pilam	8_17
1	0	1	1	0	0	Berlin	1905	4.4	bei Trier	13_4
1	0	0	0	0	0	Berlin	1905		Kiautschau Tsingtau	10_36
1	1	0	0	0	0	Berlin	1905		Kiautschau Tsingtau	10_37
1	1	0	0	0	0	Berlin	1905		Kiautschau Tsingtau	10_38
1	0	0	0	0	0	Berlin	1905		Kiautschau Tsingtau	10_39
1	1	0	0	0	1	Berlin	1905		Kiautschau Tsingtau	10_40
1	0	0	0	0	0	Berlin	1905		Kiautschau Tsingtau	10_41
1	1	0	0	0	1	Berlin	1905		Kiautschau Tsingtau	10_42
1	1	0	0	0	0	Berlin	1905		Kiautschau Tsingtau	10_43
1	0	0	0	0	0	Berlin	1905		Kiautschau Tsingtau	10_44
1	1	1	1	0	0	Berlin	1905		Kiautschau Tsingtau	10_45
1	1	0	0	0	0	Berlin	1905		Kiautschau Tsingtau	11_1
1	1	0	0	0	0	Berlin	1905		Kiautschau Tsingtau	11_2
1	1	0	0	0	0	Berlin	1905		Kiautschau Tsingtau	11_3
1	1	0	1	0	0	Berlin	1905		Kiautschau Tsingtau	11_4
1	1	0	0	0	0	Berlin	1905		Kiautschau Tsingtau	8_22
1	0	0	0	0	0	Berlin	1905		Kiautschau Tsingtau	8_23
1	0	0	0	0	0	Berlin	1905		Kiautschau Tsingtau	8_24
1	0	0	0	0	0	Berlin	1905		Kiautschau Tsingtau	8_25
1	0	0	0	0	0	Berlin	1905		Kiautschau Tsingtau	8_26
1	0	1	1	1	0	Berlin	1906		Hochbucharaa Patta hissar bei termes	10_23
1	1	0	0	0	0	Berlin	1906		Kotzen	11_34
1	1	1	1	1	0	Berlin	1906	5.9	Trient	9_26
1	1	1	1	1	0	Berlin	1906		Trient	9_27
1	0	0	0	1	1	Berlin	1909	3.3	Königswusterhausen	11_39
1	1	0	1	0	0	Berlin	1912		Sardinien Monti del Carmagenta	9_35
1	1	0	0	0	0	Berlin	1912		Sardinien Monti del Carmagenta	9_37
1	1	0	0	0	0	Berlin	1912		Sardinien Sorgonj	10_11
1	0	0	0	0	0	Berlin	1912	5.4	Sardinien Sorgonj	10_12
1	1	0	0	0	0	Berlin	1912		Sardinien Sorgonj	10_13
1	0	0	0	0	0	Berlin	1912	4.3	Sardinien Sorgonj	10_14
1	1	1	1	1	0	Berlin	1912	4.5	Finn Bucari	7_6
1	1	0	0	0	0	Berlin	1912		Sardinien Monti del Carmagenta	9_28
1	1	0	0	0	0	Berlin	1912		Sardinien Monti del Carmagenta	9_29
1	1	0	0	0	0	Berlin	1912		Sardinien Monti del Carmagenta	9_30
1	1	0	0	0	0	Berlin	1912		Sardinien Monti del Carmagenta	9_31
1	1	1	0	0	0	Berlin	1912		Sardinien Monti del Carmagenta	9_32
1	1	0	0	0	0	Berlin	1912		Sardinien Monti del Carmagenta	9_33

~150 bp COI	~350 bp COI	MA53 ~150bp	MA60 ~150bp	MA55 ~250bp	MA56 ~250 bp	Museum	Collection date	bodysize (mm)	Collection site	Extraction no.
1	1	0	0	0	1	Berlin	1912		Sardinien Monti del Carmagenta	9_34
1	1	0	0	0	0	Berlin	1912		Sardinien Monti del Carmagenta	9_36
1	1	0	0	0	0	Berlin	1912		Sardinien Monti del Carmagenta	9_38
1	1	0	0	0	0	Berlin	1912		Sardinien Monti del Carmagenta	9_39
1	1	0	0	0	0	Berlin	1916	5	Noyen NW Frankreich	10_5
1	1	1	1	1	1	Berlin	1916	4.6	Noyen NW Frankreich	10_6
1	1	0	1	0	1	Berlin	1916		Noyen NW Frankreich	10_7
1	1	0	0	0	0	Berlin	1916		Noyen NW Frankreich	9_43
1	0	0	0	0	1	Berlin	1918		Transsylv. Alpen Mogura Odobesti 500-600m	10_4
1	1	0	0	0	0	Berlin	1918		Transsylv. Alpen Mogura Odobesti 500-600m	10_3
1	0	1	1	1	0	Berlin	1919	4.5	Grunewald Havel	11_33
1	1	1	1	1	1	Berlin	1923	3.2	Erkener Löcknitzer Wiesen	11_17
1	1	1	1	1	1	Berlin	1923		Erkener Löcknitzer Wiesen	11_18
1	1	0	1	0	0	Berlin	1924		Umgebung von Koldsz	13_24
1	0	0	0	0	0	Berlin	1924		Umgebung von Koldsz	13_25
1	1	1	1	0	1	Berlin	1926	4.1	Erkener	13_11
1	1	1	1	1	1	Berlin	1926	3.4	Erkener	13_12
1	1	1	1	0	0	Berlin	1926		Erkener	13_13
1	0	0	0	0	0	Berlin	1926	5.51	Süd Istrien Abbazia	13_22
1	1	0	1	1	0	Berlin	1927	3.6	Erkener bei Berlin	11_27
1	1	0	1	1	1	Berlin	1927		Erkener bei Berlin	11_28
1	1	0	1	0	0	Berlin	1927		Erkener bei Berlin	11_29
1	1	1	1	1	1	Berlin	1928		Erkener	13_19
1	1	1	1	1	0	Berlin	1928	4.1	Offenbach am main	11_19
1	1	1	1	1	1	Berlin	1928	3.7	Erkener	13_14
1	1	1	1	1	1	Berlin	1928		Erkener	13_15
1	1	1	1	1	1	Berlin	1928	3	Erkener	13_16
1	1	0	1	1	0	Berlin	1928		Erkener	13_17
1	1	1	1	1	1	Berlin	1928	3.4	Erkener	13_18
1	1	1	1	0	0	Berlin	1928	3.3	Erkener	13_20
1	1	1	1	1	1	Berlin	1929	3.37	Fangschleuse Erkener	13_33
1	1	1	1	1	1	Berlin	1929	2.89	Fangschleuse Erkener	13_43
1	1	1	1	1	1	Berlin	1929	3.7	Fangschleuse Erkener	13_45
1	1	1	1	1	1	Berlin	1929	3.22	Fangschleuse Erkener	13_30
1	1	1	1	1	1	Berlin	1929	2.89	Fangschleuse Erkener	13_31
1	1	0	1	1	1	Berlin	1929	2.68	Fangschleuse Erkener	13_32
1	1	0	1	1	1	Berlin	1929	3.81	Fangschleuse Erkener	13_34
1	1	1	1	1	1	Berlin	1929	2.97	Fangschleuse Erkener	13_35
1	1	1	1	1	1	Berlin	1929	2.61	Fangschleuse Erkener	13_36
1	1	0	1	1	1	Berlin	1929	3.47	Fangschleuse Erkener	13_37

~150 bp COI	~350 bp COI	MA53 ~150bp	MA60 ~150bp	MA55 ~250bp	MA56 ~250 bp	Museum	Collection date	bodysize (mm)	Collection site	Extraction no.
1	1	1	1	1	1	Berlin	1929	3.35	Fangschleuse Erkener	13_38
1	1	1	1	1	1	Berlin	1929	3	Fangschleuse Erkener	13_39
1	1	1	1	1	1	Berlin	1929	3.31	Fangschleuse Erkener	13_40
1	0	1	1	1	1	Berlin	1929	2.61	Fangschleuse Erkener	13_41
1	1	1	1	1	1	Berlin	1929	3.39	Fangschleuse Erkener	13_42
1	1	0	1	1	1	Berlin	1929	3.1	Fangschleuse Erkener	13_44
1	1	1	1	1	1	Berlin	1929	3.22	Fangschleuse Erkener	13_46
1	1	1	1	1	1	Berlin	1930	4.97	Corsica Piarea	13_26
1	1	0	1	1	1	Berlin	1930	3.81	Corsica Piarea	13_27
1	1	1	1	1	1	Berlin	1930	3.85	Corsica Piarea	13_28
1	1	1	1	1	0	Berlin	1930	3.48	Corsica Piarea	13_29
1	1	0	0	0	0	Berlin	1931	4.8	Freiburg im Breisgau	9_42
1	0	1	1	0	0	Berlin	1935	6.1	Bötzener Wiesen	11_37
1	1	1	1	1	1	Berlin	1935	4.7	Bötzener Wiesen	11_38
1	0	0	0	0	0	Berlin	1935	4.8	Caputh	11_46
1	0	1	1	1	1	Berlin	1935	4	Genshagener Forst	13_1
1	1	1	0	1	1	Berlin	1935	4.1	Genshagener Forst	13_2
1	1	1	1	1	1	Berlin	1935	3.2	Genshagener Forst	13_3
1	1	1	1	1	1	Berlin	1936		Östlich Großbeeren	11_22
1	1	1	1	0	0	Berlin	1936		Östlich Großbeeren	11_23
1	1	1	1	1	1	Berlin	1936		Östlich Großbeeren	11_21
1	1	1	1	1	1	Berlin	1936		Ruhlsdorf	13_10
1	1	1	1	1	1	Berlin	1936		Ruhlsdorf	13_8
1	0	1	1	1	1	Berlin	1936		Ruhlsdorf	13_9
1	1	1	1	1	1	Berlin	1936	3.8	Östlich Großbeeren	11_20
1	1	1	1	1	1	Berlin	1937	4	Krumme Laake Müggeheim	11_31
1	1	0	0	0	0	Berlin	1937		Röddelin Mahlgast See	11_43
1	1	1	1	1	1	Berlin	1937		Summt	11_24
1	1	1	1	1	1	Berlin	1937	2.8	Summt	11_25
1	1	1	1	1	1	Berlin	1937	3	Summt	11_26
1	1	1	1	1	0	Berlin	1937	4	Höpenberge	11_30
1	1	1	1	1	0	Berlin	1937	3.3	Krumme Laake Müggeheim	11_32
1	0	1	1	1	1	Berlin	1937	4.5	Klosterwadle Uckermark	11_35
1	0	0	1	1	1	Berlin	1937	3.2	Klosterwadle Uckermark	11_36
1	0	1	1	1	0	Berlin	1937	3.5	Röddelin Mahlgast See	11_40
1	1	0	0	0	0	Berlin	1937	3.5	Röddelin Mahlgast See	11_41
1	1	1	1	1	1	Berlin	1937		Röddelin Mahlgast See	11_42
1	0	1	1	1	0	Berlin	1937		Schmöckwitz Torfmoor	13_5
1	0	1	1	1	1	Berlin	1937	3	Schmöckwitz Torfmoor	13_6
1	1	0	0	0	1	Berlin	1937		Tegeler Forst Hennigsdorf	13_7

~150 bp COI	~350 bp COI	MA53 ~150bp	MA60 ~150bp	MA55 ~250bp	MA56 ~250 bp	Museum	Collection date	bodysize (mm)	Collection site	Extraction no.
1	1	1	1	0	1	Berlin	1960	4.4	Oberer Finkensee	13_23
1	1	0	0	0	0	Berlin	1963		Kaukasus No.3 Kiew Desna	7_3
1	1	0	1	1	1	Berlin	1963		Kaukasus No.3 Kiew Desna	7_5
1	1	1	1	1	1	Berlin	1963		Kaukasus No.3 Kiew Desna	7_4
0	1	0	0	0	0	Berlin			Konstantinopel	9_25
1	0	0	0	0	0	Berlin			Sarepta	8_1
1	0	0	0	0	0	Berlin			Sarepta	8_2
1	0	0	0	0	0	Berlin			Sarepta	8_3
1	0	0	0	0	0	Berlin			Süd Russland orlow bei Melitopol	9_11
1	0	0	0	0	0	Berlin			Sarepta	9_14
1	0	0	1	1	0	Berlin		6.1	Konstantinopel	9_21
1	1	1	1	1	1	Berlin			Lenkorau	9_8
1	1	0	1	0	0	Berlin		5.6	Berlin	10_1
1	1	0	1	1	0	Berlin		5.4	Berlin	10_2
1	1	1	1	1	0	Berlin		4.8	Groß Glienke	11_16
1	0	0	0	0	0	Berlin			Livorno	7_1
1	0	0	0	0	0	Berlin			Madeira	8_4
1	0	0	0	0	0	Berlin			Algier	8_7
1	0	0	0	0	0	Berlin			Algier	8_8
1	0	1	1	1	1	Berlin			Frankreich St Mihel	9_12
1	0	1	0	0	0	Berlin			Sarepta	9_13
1	1	1	1	0	0	Berlin			Sarepta	9_15
1	1	0	1	0	0	Berlin		6	Konstantinopel	9_22
1	1	1	0	1	0	Berlin			Konstantinopel	9_23
1	1	0	0	0	0	Berlin			Konstantinopel	9_24
1	1	0	0	0	0	Berlin			Nassau Mombach	9_41
1	1	0	0	0	0	Berlin			Kunami Morea	9_6
1	1	1	1	0	0	Berlin			Lenkorau	9_9
1	0	0	0	0	0	Berlin			Polynesien	13_21
1	0	0	0	0	0	Berlin			Azores Terceiro	8_10
1	0	0	1	0	0	Berlin			Azores Terceiro	8_12
1	1	0	1	0	0	Berlin			S. Miguel Fayal	8_5
1	0	0	1	1	1	Berlin			S. Miguel Fayal	8_6
1	1	0	0	0	0	Berlin			Azores Terceiro	8_9
1	1	0	1	0	0	Berlin			Gisetro	9_16
1	1	1	1	1	1	Berlin			Gisetro	9_17
1	1	1	1	0	0	Berlin			Gisetro	9_18
1	1	1	1	1	1	Berlin			Gisetro	9_19
1	1	1	1	0	1	Berlin			Gisetro	9_20
1	1	1	1	1	1	Berlin			Lenkorau	9_10

~150 bp COI	~350 bp COI	MA53 ~150bp	MA60 ~150bp	MA55 ~250bp	MA56 ~250 bp	Museum	Collection date	bodysize (mm)	Collection site	Extraction no.
1	0	0	0	0	0	Berlin			Griechenland	9_7
1	0	0	0	0	0	Frankfurt	7.1926	5	Kreta	2_1
1	0	0	0	0	0	Frankfurt	1871		Russland Sarepta	5_12
1	0	1	1	1	1	Frankfurt	1872	4.9	Marokko Casablanca	2_33
1	1	1	1	1	0	Frankfurt	1872		Marokko Casablanca	3_10
1	1	1	1	1	0	Frankfurt	1872	6.4	Marokko Casablanca	3_8
1	1	0	0	0	0	Frankfurt	1872	6	Marokko Casablanca	3_9
1	0	0	0	0	0	Frankfurt	1873	4.1	Frankfurt	3_11
1	0	0	0	0	0	Frankfurt	1873	4.2	Frankfurt	3_12
1	0	0	0	0	0	Frankfurt	1882		Japan, Nagasaki und Compina	3_1
1	0	0	0	0	0	Frankfurt	1882		Japan, Nagasaki und Compina	3_2
1	0	0	0	0	0	Frankfurt	1882		Japan, Nagasaki und Compina	3_3
1	0	0	0	0	0	Frankfurt	1882		Japan, Nagasaki und Compina	3_4
1	0	0	0	0	0	Frankfurt	1882		Japan, Nagasaki und Compina	6_28
1	0	0	0	0	0	Frankfurt	1882		Japan, Nagasaki und Compina	6_29
1	0	0	0	0	0	Frankfurt	1882		Japan, Nagasaki und Compina	6_30
1	0	0	0	0	0	Frankfurt	1882		Japan, Nagasaki und Compina	6_31
1	0	0	0	0	0	Frankfurt	1883		Frankfurt	2_23
1	0	0	0	0	0	Frankfurt	1883	4.2	Frankfurt	2_24
1	0	0	0	0	0	Frankfurt	1901	3.7	Mainkur Frankfurt	2_39
1	0	0	0	0	0	Frankfurt	1901	4	Schwanheim	2_43
1	0	0	0	0	0	Frankfurt	1901	3.8	Schwanheim	2_44
1	0	0	0	0	0	Frankfurt	1901	4.3	Schwanheim	2_45
1	0	0	0	0	0	Frankfurt	1901	3.4	Schwanheim	2_46
0	0	0	0	0	0	Frankfurt	1902	4.7	Frankfurt	2_26
0	0	0	0	0	0	Frankfurt	1902	3.1	Frankfurt	2_27
0	0	0	0	0	0	Frankfurt	1902	3.8	Frankfurt	2_28
1	0	0	0	0	0	Frankfurt	1902	4.3	Frankfurt	2_25
0	0	0	0	0	0	Frankfurt	1904		Lugano	2_35
0	0	0	0	0	0	Frankfurt	1904		Lugano	2_36
0	0	0	0	0	0	Frankfurt	1904		Lugano	2_37
1	0	0	0	0	0	Frankfurt	1908	4.5	Michelstadt im Odenwald	3_19
1	0	0	0	0	0	Frankfurt	1914	6.5	La Fosca de Palemos Gerona	2_31
1	0	0	0	0	0	Frankfurt	1914	5.4	La Fosca de Palemos Gerona	2_32
1	0	0	0	0	0	Frankfurt	1914	6	La Fosca de Palemos Gerona	6_17
1	0	0	0	0	0	Frankfurt	1914	4.7	La Fosca de Palemos Gerona	6_18
1	0	0	0	0	0	Frankfurt	1914	6.2	La Fosca de Palemos Gerona	6_19
1	0	0	0	0	0	Frankfurt	1914	5.5	La Fosca de Palemos Gerona	6_20
1	0	0	0	0	0	Frankfurt	1914	4.4	La Fosca de Palemos Gerona	6_21
1	0	0	0	0	0	Frankfurt	1914	4.8	La Fosca de Palemos Gerona	6_22

~150 bp COI	~350 bp COI	MA53 ~150bp	MA60 ~150bp	MA55 ~250bp	MA56 ~250 bp	Museum	Collection date	bodysize (mm)	Collection site	Extraction no.
1	0	0	1	0	0	Frankfurt	1914	4.7	La Fosca de Palemos Gerona	6_23
1	0	0	0	0	0	Frankfurt	1914	5.7	La Fosca de Palemos Gerona	6_24
1	0	0	0	0	0	Frankfurt	1914		La Fosca de Palemos Gerona	6_25
1	0	0	0	0	0	Frankfurt	1914		La Fosca de Palemos Gerona	6_26
1	0	0	0	0	0	Frankfurt	1914		La Fosca de Palemos Gerona	6_27
1	0	0	0	0	0	Frankfurt	1915	6.2	Flix Tarragona	2_29
1	0	0	0	0	0	Frankfurt	1915	5.1	Flix Tarragona	2_30
1	0	0	0	0	0	Frankfurt	1915		Obertshausen Offenbach	3_20
1	0	0	0	0	0	Frankfurt	1915		Obertshausen Offenbach	3_21
1	0	0	0	0	0	Frankfurt	1918	6.5	Pobla de Segur Lerida	3_6
1	0	0	0	0	0	Frankfurt	1918	5.4	Pobla de Segur Lerida	3_7
1	0	0	0	0	0	Frankfurt	1922	4	Pfalz Neustadt	3_22
1	0	0	0	0	0	Frankfurt	1926	5.1	Kreta	2_2
1	0	0	0	0	0	Frankfurt	1926	6.5	Kreta	2_3
1	0	0	0	0	0	Frankfurt	1928		Ungarn, Strawatornya	2_4
1	0	0	0	0	0	Frankfurt	1931	3.6	Schwanheim	3_26
1	1	0	1	0	1	Frankfurt	1933	5	Dessau Roßlau Dellnau	5_24
1	0	0	0	0	0	Frankfurt	1934	4.3	Berlin	2_8
1	0	0	0	0	0	Frankfurt	1934	4.6	Messeler Parl, Darmstadt	2_38
1	1	1	1	1	1	Frankfurt	1935	4.5	Offenbach, Luhrwald	2_40
1	0	0	0	0	0	Frankfurt	1935	4.6	Offenbach, Luhrwald	2_41
1	0	1	1	0	1	Frankfurt	1935	4.9	Offenbach, Luhrwald	2_42
1	0	0	0	0	0	Frankfurt	1936		Scheftsheimer Wiese Darmstadt	3_23
1	0	0	0	0	0	Frankfurt	1936	3.9	Scheftsheimer Wiese Darmstadt	3_24
1	1	1	0	1	1	Frankfurt	1936	4.2	Taunus Eppstein	3_25
0	0	0	0	0	0	Frankfurt	1938		Sardinien	6_11
1	0	0	0	0	0	Frankfurt	1938		Sardinien	6_5
1	0	0	1	0	0	Frankfurt	1938	6	Sardinien	2_5
1	0	0	0	0	0	Frankfurt	1938	5.1	Sardinien	2_6
1	0	0	0	0	0	Frankfurt	1938	5.2	Sardinien	2_7
1	0	0	0	0	0	Frankfurt	1938		Sardinien	6_1
1	0	0	0	0	0	Frankfurt	1938		Sardinien	6_10
1	0	0	0	0	0	Frankfurt	1938		Sardinien	6_12
1	0	0	0	0	0	Frankfurt	1938		Sardinien	6_13
1	0	0	0	0	1	Frankfurt	1938		Sardinien	6_14
1	0	1	0	1	1	Frankfurt	1938		Sardinien	6_15
1	0	0	0	0	0	Frankfurt	1938		Sardinien	6_16
1	0	0	1	0	1	Frankfurt	1938		Sardinien	6_2
1	0	1	1	0	0	Frankfurt	1938		Sardinien	6_3
1	0	0	0	0	0	Frankfurt	1938		Sardinien	6_4

~150 bp COI	~350 bp COI	MA53 ~150bp	MA60 ~150bp	MA55 ~250bp	MA56 ~250 bp	Museum	Collection date	bodysize (mm)	Collection site	Extraction no.
1	0	0	0	0	0	Frankfurt	1938		Sardinien	6_6
1	0	0	0	0	0	Frankfurt	1938		Sardinien	6_7
1	0	0	0	0	0	Frankfurt	1938		Sardinien	6_8
1	0	0	0	0	1	Frankfurt	1938		Sardinien	6_9
0	0	0	0	0	0	Frankfurt	1940	4.4	Florenz	2_14
1	0	0	0	0	0	Frankfurt	1940	4.2	Florenz	2_13
1	0	0	0	0	0	Frankfurt	1942	4	Ostpyrenäen	2_10
1	0	0	0	0	0	Frankfurt	1942	3.4	Ostpyrenäen	2_11
1	0	0	0	0	0	Frankfurt	1942	3.6	Ostpyrenäen	2_12
1	0	0	0	0	0	Frankfurt	1942	4.2	Ostpyrenäen	2_9
1	1	0	0	1	0	Frankfurt	1942	4	Taunus Vockenhausen b. Eppstein	3_43
1	1	1	1	1	1	Frankfurt	1943	5.8	Macedonien Ostrow See	2_15
1	0	1	1	1	1	Frankfurt	1943	5.6	Macedonien Ostrow See	2_16
1	1	0	1	1	1	Frankfurt	1943		Marseille	2_19
1	1	0	1	1	1	Frankfurt	1943	4.4	Marseille	2_20
1	1	1	1	1	1	Frankfurt	1943	3.5	Marseille	2_21
1	0	1	1	0	1	Frankfurt	1943	4.5	Marseille	2_22
0	0	0	0	0	0	Frankfurt	1948		Eifel Herchenberg	3_34
1	1	1	1	1	1	Frankfurt	1948	4	Frankfurt	3_29
1	1	1	1	1	1	Frankfurt	1948	4.3	Schwanheim am Main	3_30
1	1	0	1	1	0	Frankfurt	1948	4	Taunus zw Loßbach und Hofheim	3_33
1	1	0	0	0	1	Frankfurt	1948	4.5	Frankfurt Eukheimer Ried	3_38
1	0	0	0	0	0	Frankfurt	1948	3.8	Sprendlingen Offenbach	3_40
1	0	0	0	0	0	Frankfurt	1949	3.2	Taunus Hofheim	3_31
1	1	0	1	1	1	Frankfurt	1949	4.5	Taunus Loßbach	3_32
1	1	0	0	0	0	Frankfurt	1949	2.8	Mönchsbruck	3_35
1	0	1	1	0	1	Frankfurt	1949		Mönchsbruck	3_36
1	0	0	0	0	0	Frankfurt	1949	4.5	Wetterau Wisselsheim	3_37
1	0	0	0	0	0	Frankfurt	1949	4	Frankfurt Südfriedhof	3_39
1	0	0	0	1	0	Frankfurt	1949	3.3	Odenwald Wersau	3_41
1	1	0	1	0	0	Frankfurt	1949	3.3	Höchst a.M.	3_42
1	0	0	0	0	0	Frankfurt	1950	4.8	Taunus Sulzbach	3_44
1	0	0	0	0	0	Frankfurt	1950		Wetterau Niederweisel	3_45
1	1	0	0	0	0	Frankfurt	1950	3.9	Hengsten bei Obertshausen	4_10
1	1	0	0	0	0	Frankfurt	1950	3.7	Hengsten bei Obertshausen	4_11
1	0	1	1	0	1	Frankfurt	1950	4.5	Hengsten bei Obertshausen	4_12
1	1	0	0	0	0	Frankfurt	1950		Hengsten bei Obertshausen	4_13
1	1	1	0	0	1	Frankfurt	1950		Hengsten bei Obertshausen	4_14
1	1	0	0	0	0	Frankfurt	1950	4.6	Pfalz Bürzweiler	4_2
1	1	0	1	0	0	Frankfurt	1950	5	Taunus Lorsbacher Tal bei Hofheim	4_3

~150 bp COI	~350 bp COI	MA53 ~150bp	MA60 ~150bp	MA55 ~250bp	MA56 ~250 bp	Museum	Collection date	bodysize (mm)	Collection site	Extraction no.
1	1	0	1	1	1	Frankfurt	1950	3.8	Hengsten bei Obertshausen	4_4
1	0	0	0	0	0	Frankfurt	1950	4.9	Hengsten bei Obertshausen	4_5
1	0	0	0	0	0	Frankfurt	1950	4.9	Hengsten bei Obertshausen	4_6
1	0	1	0	0	0	Frankfurt	1950	4.6	Hengsten bei Obertshausen	4_7
1	0	1	1	0	1	Frankfurt	1950	3.9	Hengsten bei Obertshausen	4_8
1	1	0	1	1	1	Frankfurt	1950	4.2	Hengsten bei Obertshausen	4_9
1	1	1	1	1	1	Frankfurt	1950		Wächtersbach	5_1
1	0	0	0	0	0	Frankfurt	1950		Waldorf	5_8
1	1	0	0	0	1	Frankfurt	1951	5	Lorch am Rhein	4_28
1	0	0	1	0	0	Frankfurt	1951		Kühkopf	5_10
1	1	1	0	0	1	Frankfurt	1951		Kühkopf	5_11
1	1	0	0	0	0	Frankfurt	1951	3.4	Kühkopf	5_9
1	0	0	0	0	0	Frankfurt	1952	4.5	Corsica Casamozza	4_22
1	0	1	1	0	1	Frankfurt	1952	4.7	Corsica Col de Prato 980 m	5_2
1	1	0	0	0	0	Frankfurt	1952	4.5	Corsica Casamozza	3_46
1	0	1	1	1	1	Frankfurt	1952	5.8	Corsica Calacuccia, 1000-1500m	4_1
1	1	1	1	0	1	Frankfurt	1952	3.7	Corsica Corte 400m	4_15
1	1	0	1	1	1	Frankfurt	1952	4.8	Corsica Corte 400m	4_16
1	1	0	1	1	1	Frankfurt	1952	5.1	Corsica Corte 400m	4_18
1	0	0	0	0	1	Frankfurt	1952	4.1	Corsica Corte 400m	4_19
1	1	1	1	1	1	Frankfurt	1952	3.5	Corsica Corte 400m	4_20
1	1	1	1	1	1	Frankfurt	1952	4.8	Corsica Casamozza	4_21
1	0	1	0	1	1	Frankfurt	1952	3.5	Corsica Ponte Lecchia 185 m	4_23
1	1	1	1	1	1	Frankfurt	1952		Corsica Col de Prato 980 m	5_3
1	1	1	1	1	1	Frankfurt	1952	5.4	Corsica Lumio Calenzana 30 m	5_7
1	0	0	0	0	0	Frankfurt	1953		Iran, Maharlu See	2_17
1	0	0	1	0	0	Frankfurt	1953		Iran, Maharlu See	2_18
1	0	0	0	0	0	Frankfurt	1953		Spanien Casteldefels	4_29
1	1	0	0	0	0	Frankfurt	1953	6.2	Barcelona 500 m	5_6
1	0	1	1	1	0	Frankfurt	1954	6	Sardinien bei Oschiri 400 m	4_17
1	1	1	1	1	0	Frankfurt	1954	6.5	Sardinien Olbia	4_25
1	0	1	1	1	1	Frankfurt	1954	5.7	Sardinien Olbia	4_26
1	0	1	0	0	0	Frankfurt	1954	6	Sardinien Tempio	4_27
1	1	0	0	0	0	Frankfurt	1957		Schwanheim	4_30
1	0	1	1	1	1	Frankfurt	1957	4	Mühlberg bei Niederkleen	5_4
1	1	1	1	0	0	Frankfurt	1957	5.2	Istrien Rovinj	5_5
1	1	0	1	1	1	Frankfurt	1960	6	Süd Frankreich Camargue	3_15
1	0	1	1	1	1	Frankfurt	1960	6.2	Süd Frankreich Camargue	3_16
1	0	1	1	0	0	Frankfurt	1960		Süd Frankreich Camargue	3_17
1	0	0	1	1	1	Frankfurt	1960		Süd Frankreich Camargue	3_18

~150 bp COI	~350 bp COI	MA53 ~150bp	MA60 ~150bp	MA55 ~250bp	MA56 ~250 bp	Museum	Collection date	bodysize (mm)	Collection site	Extraction no.
1	1	1	1	1	1	Frankfurt	1966		Mexico Guerrero	5_13
1	0	0	0	0	0	Frankfurt	<1915	2.9	Frankfurt Eschenheimer Tor	3_13
1	0	0	0	0	0	Frankfurt	<1915	3.9	Frankfurt Eschenheimer Tor	3_14
1	1	0	1	1	1	Frankfurt	1930ies	2.9	Dessau, Berlin	5_15
1	0	1	0	0	0	Frankfurt	1930ies	3.2	Dessau, Berlin	5_16
1	1	0	1	0	1	Frankfurt	1930ies	2.7	Dessau, Berlin	5_21
1	0	0	0	0	0	Frankfurt	1930ies	2.6	Dessau, Berlin	5_17
1	0	1	1	0	1	Frankfurt	1930ies	3	Dessau, Berlin	5_18
1	1	0	0	0	0	Frankfurt	1930ies	2.3	Dessau, Berlin	5_19
1	0	0	1	0	1	Frankfurt	1930ies	2.7	Dessau, Berlin	5_20
1	0	0	0	0	0	Frankfurt	1930ies		Europa	5_25
1	1	1	1	1	1	Frankfurt	1930ies		Europa	5_26
1	1	1	1	1	1	Frankfurt	1930ies		Europa	5_28
1	1	1	0	0	1	Frankfurt	1930ies		Europa	5_29
1	1	0	1	1	1	Frankfurt	1930ies	2.6	Dessau, Berlin	5_22
1	1	0	1	0	0	Frankfurt	1930ies	3.1	Dessau, Berlin	5_23
1	0	0	1	0	0	Frankfurt	1930ies		Europa	5_27
0	0	0	0	0	0	Frankfurt			Frankfurt	3_5
1	0	0	1	1	0	Frankfurt			Java	2_34
1	0	0	0	0	0	Frankfurt			Schwanheim am Main	3_27
1	0	0	0	0	0	Frankfurt			Schwanheim am Main	3_28
1	0	0	1	0	1	Frankfurt			Libanon	5_14
1	0	0	0	0	0	Frankfurt			Iran Coast of Caspian Sea	6_32

Supplementary table 3.2 Genetic diversity (mitochondrial nucleotide diversity and microsatellite allelic richness) of *Argiope bruennichi* populations from the former range edge in Berlin and for three time periods.

Collection date	Mitochondrial π	Nuclear allelic richness
≤ 1930	0.0009	6.9931
$> 1930 \leq 1960$	0.0024	9.2500
> 1960	0.0026	11.1636

Chapter 4

All genome and transcriptome mapping files, as well as assemblies, gene expression data and BLAST results will be uploaded on a publicly available server of the Max Planck Institute for Evolutionary Biology, as soon as the thesis is ready for publication.

Supplementary table 4.1 Sampling sites for *Argiope bruennichi* samples, which were used for whole genome sequencing. The first column denotes sample identifiers in the DNA libraires.

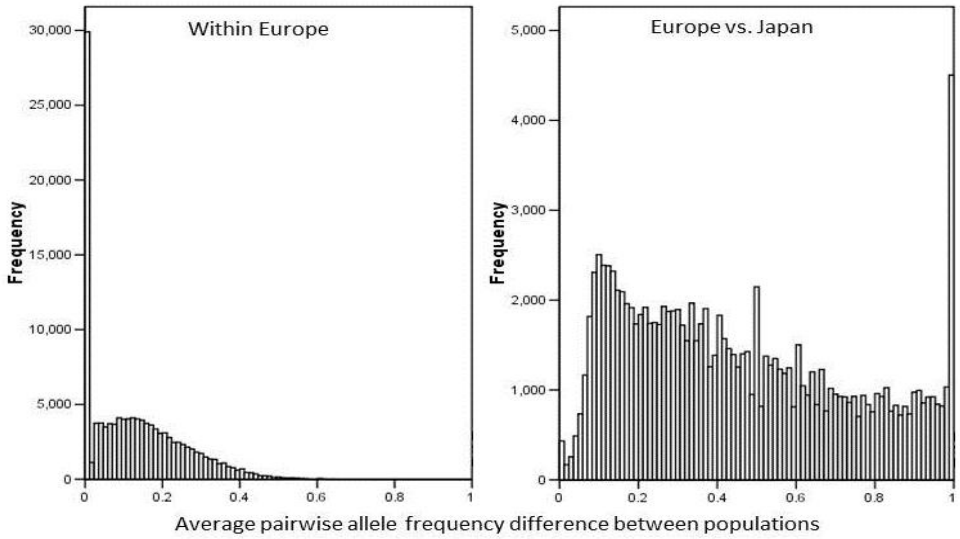
J20	JpIbTA	Japan	Ibaraki	Tsukuba	36.00	140.10	Y. Baba
J22_1	JpKaKA	Japan	Kagoshima	Shimofukmoto-chö	31.60	130.56	T. Maeda
J25_1	JpNaAA	Japan	Nagano	Azusagawa-Azusa	36.23	137.97	N. Tsurusaki
J27_1	JpKaAB	Japan	Kagoshima	Amami-Oshima	28.28	129.43	Y. Baba
J28_1	JpShOA	Japan	Shiga	Ozigaoka	35.12	136.07	T. Masumoto
Pool2	B5	Latvia	Dobele	Annenieki Parish	56.70	23.13	H. Krehenwinkel
	B6	Latvia	Limbazi	Skulte	57.35	24.45	H. Krehenwinkel
	B8	Estonia	Pärnu	Pärnu	58.30	24.62	H. Krehenwinkel
	B10	Latvia	Limbazi	Ainazi	57.87	24.37	H. Krehenwinkel
Pool1	S2	Sweden	Kalmar county	Soderakra	56.45	16.67	H. Krehenwinkel
	S3	Sweden	Kalmar county	Kalmar	56.63	16.22	H. Krehenwinkel
	S4	Sweden	Skane county	Kristianstad	55.95	14.10	H. Krehenwinkel
Pool3	P2	Portugal	Alentejo	Corte Pequena	37.70	-7.85	H. Krehenwinkel
	P3	Portugal	Alentejo	Santo Jao	37.67	-7.83	H. Krehenwinkel
	P4	Portugal	Alentejo	Mertola	37.68	-7.60	H. Krehenwinkel
	P5	Portugal	Alentejo	Mertola	37.65	-7.60	H. Krehenwinkel
	P6	Portugal	Alentejo	Beja	37.80	-7.85	H. Krehenwinkel
Pool4	ItCaGA	Italy	Campania	Giugliano	40.93	14.05	H. Krehenwinkel
	ItCbOA	Italy	Calabria	Crosia	39.57	16.72	H. Krehenwinkel
	ItPgTA	Italy	Puglia	Taranto	40.47	17.30	H. Krehenwinkel
IM4	M1	Portugal	Madeira	Camacha	32.70	-16.80	H. Krehenwinkel

Supplementary table 4.2 Primer sequences for the analysis of genetic clines in different genomic candidate SNPs.

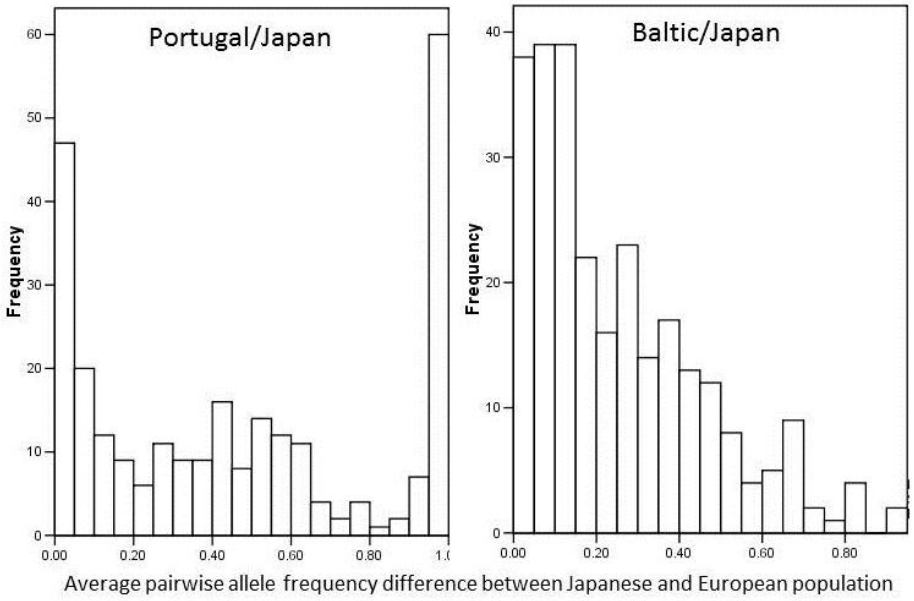
ID	Outlier contig	Primer name	Sequence 5'-3'	Tm	product (bp)	Position in alignment	SNP North/South
G1F1	C609506_contig_52661	C609506_c_52661_1F	CTTTCTTTTTCGCCTCATGTT	58.5	102	34585	A/G
G1R1	C609506_contig_52661	C609506_c_52661_1R	GCTCAGCTGACTTCAAAATCC	59.1	102	34585	A/G
G3F1	s30462_contig_60388	s30462_c_60388_1F	CTTAAGCACTCCGCCAAAAT	59.4	189	14176	A/T
G3R1	s30462_contig_60388	s30462_c_60388_1R	TGAAGTGAAACCAGCAAGTCA	59.5	189	14176	A/T
G4F1	C441899_contig_75705	C441899_c_75705_1F	GGCAGCACATAGATAAAAGCAA	59.4	146	16000	T/C
G4R1	C441899_contig_75705	C441899_c_75705_1R	TCTTTCCAGCAACGTCTATTATTTT	59.6	146	16000	T/C
G7F1	C601208_contig_38174	C601208_c_38174_1F	CCGAACAGATTGAATCACCA	59.5	238	24177	A/G
G7R1	C601208_contig_38174	C601208_c_38174_1R	AGATCAGAATGGAGCAAATGA	57.3	238	24177	A/G

Supplementary table 4.3 SNP frequency data for the four genotyped candidate loci in different European *Argiope bruennichi* populations.

Collection site		GPS coordinates			Frrequency of candidate SNP					Freq. of background SNPs
Country	Region	GPS N	GPS E	Mean NE	G1	G3	G4	G7	Average	
Germany	Berlin	52.52	13.41	32.97	0.34	0.33	0.41	0.49	0.39	0.14
Spain	Catalunia	39.48	-0.37	19.56	0.07	0	0	0	0.02	0.08
France	Nimes	43.84	4.36	24.1	0.06	0	0	0.06	0.03	0.04
Croatia	Istria	44.87	13.84	29.36	0.07	0.06	0.07	0	0.05	0.1
France	Auvergne	45.7	3.3	24.5	0.33	0	0.06	0.13	0.13	0.08
Sweden	Kalmar	56.67	16.36	36.52	0.45	0.63	0.66	0.63	0.59	0.15
Poland	Podlaskie	53.33	23.1	38.22	0.42	0.56	0.5	0.94	0.6	0.21
Poland	Masovia	52.67	21.62	37.15	0.5	0.79	0.6	0.71	0.65	0.19
Batic states	Baltic	57.86	24.36	41.11	0.67	0.77	0.85	0.67	0.74	0.15
Russia	Lipetsk	52.62	39.6	46.11	0.67	0.93	0.92	0.79	0.82	0.18
Czech Republic	Brno	49.2	16.61	32.91	0.18	0.31	0.37	0.5	0.34	0.21
Germany	Wulfen	51.72	7.02	29.37	0.13	0.31	0.29	0.13	0.21	NA
Switzerland	Geneve	46.2	6.15	26.18	0.25	0	0	0.14	0.1	NA
France	Lyon	45.76	4.84	25.3	0.06	0.06	0.06	0.06	0.06	0.08
Germany	Stuttgart	48.78	9.18	28.98	0.06	0.13	0.13	0.31	0.16	0.13
Italy	Puglia	40.47	17.23	28.85	0.08	0.05	0.08	0.06	0.07	0.1
Portugal	Allentejo	37.64	-7.66	14.99	0	0	0	0	0	0.11
Central Asia	Katta Kurgan	39.9	66.26	53.08	NA	NA	NA	NA	NA	0.23
Japan	Ehime	33.83	132.77	83.3	1	1	1	1	1	0.99
Japan	Tottori	35.28	133.48	84.38	1	1	1	1	1	0.98



Supplementary figure 4.1 Distribution of average pairwise allele frequency differences within European populations (left plot) and between European and Japanese populations (right plot). The plots are based on all variable sites in approximately 6,000,000 bp of DNA in 276 genomic contigs. These contigs contain at least one completely fixed difference between Europe and Japan. The mean difference is 0.13 for the within European comparison and 0.45 for the comparison of Europe and Japan. On average, I find one completely fixed SNP between all European and Japanese samples per ~ 1000 bp. European and Japanese populations still share much variation. These shared SNPs could be based on retained ancestral variation.



Supplementary figure 4.2 Distribution of average pairwise allele frequency differences between Portugal and Japan (left plot) and between Baltic States and Japan (right plot). The plots are based on all variable sites in approximately 35,000 bp of DNA in the genomic candidate contig C601208_contig_38174 (see Table 4.2). Baltic populations share distinctively more variation with Japan, than Portuguese ones. On average Portuguese and Japanese populations are divergent by an allele frequency difference of 0.47, and Baltic and Japanese populations by only about half of that. The shared alleles between Portuguese and Japanese population are probably due to common differences towards the mapping reference, which is a single specimen from an isolated population on Madeira.

Chapter 5

All transcriptome mapping files, as well as gene expression data and BLAST results will be uploaded on a publicly available server of the Max Planck Institute for Evolutionary Biology, as soon as the thesis is ready for publication.

Supplementary table 5.1 Sampling sites for wasp spiders, which were used for transcriptome sequencing after thermal stress. The second column denotes the respective library identifiers.

Region	ID	Country	Region	City	GPS N	GPS E	Collector
Baltic	B4	Latvia	Saldus	Viduskrogs	56.68	22.13	H. Krehenwinkel
	B5	Latvia	Dobele	Annenieki Parish	56.70	23.13	H. Krehenwinkel
	B8	Estonia	Pärnu	Pärnu	58.30	24.62	H. Krehenwinkel
Portuguese	P2	Portugal	Alentejo	Corte Pequena	37.70	-7.85	H. Krehenwinkel
	P4	Portugal	Alentejo	Mertola	37.68	-7.60	H. Krehenwinkel
	P5	Portugal	Alentejo	Mertola	37.65	-7.60	H. Krehenwinkel

Supplementary table 5.2 Sampling sites for spiders, used for the cold tolerance experiment. Column 2 denotes sample identifiers.

Region	ID	Country	Region	City	GPS N	GPS E	Collector
Southern	P2	Portugal	Alentejo	Corte Pequena	37.70	-7.85	H. Krehenwinkel
	P4	Portugal	Alentejo	Mertola	37.68	-7.60	H. Krehenwinkel
	P5	Portugal	Alentejo	Mertola	37.65	-7.60	H. Krehenwinkel
Northern	B1	Lithuania	Klaipeda	Klaipeda	55.80	21.15	H. Krehenwinkel
	B2	Latvia	Liepaja	Niedasciems	56.08	21.12	H. Krehenwinkel
	B4	Latvia	Kurzemes	Viduskrogs	56.68	22.13	H. Krehenwinkel
	B5	Latvia	Dobele	Annenieki Parish	56.70	23.13	H. Krehenwinkel
	B7	Estonia	Pärnu	Pärnu	58.30	24.62	H. Krehenwinkel
	B8	Estonia	Pärnu	Pärnu	58.31	24.60	H. Krehenwinkel
	B10	Latvia	Limbazi	Ainazi	57.87	24.37	H. Krehenwinkel
	S2	Sweden	Kalmar county	Soderakra	56.45	16.67	H. Krehenwinkel
	S4	Sweden	Skane county	Kristianstad	55.95	14.10	H. Krehenwinkel

Supplementary table 5.3 Data from the cold tolerance experiment. The table shows the numbers of woken up spiders at certain time after cold shock. The eighth column shows the number of dead or permanently damaged spiders.

Region	Eggsac ID	Woken up animals, minutes after cold shock					Damaged or death after 24 h	Total spiders
		30min	45min	60min	90min	120min		
Baltic	B10.1x5	1	2	3	1	1	2	9
Baltic	B10.2x6	3	5	1	0	1	1	10
Baltic	B10x1	5	1	2	1	1	1	10
Baltic	B1ax9	0	0	1	0	3	2	9
Baltic	B1mn1	6	4	0	0	0	1	10
Baltic	B1x12	0	0	1	2	1	8	10
Baltic	B1x13	7	1	1	0	0	1	10
Baltic	B1x14	0	1	2	3	3	1	9
Baltic	B1x2	0	2	2	2	2	3	10
Baltic	B1X4	3	0	1	2	1	5	10
Baltic	B1x7	0	0	2	1	3	4	10
Baltic	B2ab1	0	1	2	5	1	3	10
Baltic	B2x11	0	0	1	0	0	9	10
Baltic	B2x16	0	1	0	3	4	3	10
Baltic	B2x18	3	5	0	2	0	0	10
Baltic	B2x5	0	0	2	4	3	2	10
Baltic	B2x9?1	0	1	6	2	1	0	10
Baltic	B2x9_2	1	2	2	3	1	1	10
Baltic	B4fz1	8	1	1	0	0	0	10
Baltic	B4x1	0	0	2	1	2	6	10
Baltic	B4x4	0	0	0	6	1	3	10
Baltic	B4x5	0	2	2	2	3	3	10
Baltic	B4x9	0	1	2	4	0	3	10
Baltic	B5.4x12	0	0	0	0	0	9	10
Baltic	B5c1	2	5	2	0	0	1	10
Baltic	B5x2	1	1	0	2	2	7	11
Baltic	B5x5	0	1	0	4	2	6	10
Baltic	B5x7	0	0	0	0	0	8	10
Baltic	B5x8	1	4	3	0	1	0	10
Baltic	B7ab2_2	1	1	3	2	1	5	10
Baltic	B7x12	0	4	0	0	3	5	10
Baltic	B7x3	0	1	1	3	1	2	10
Baltic	B7X4	0	2	0	2	1	6	10
Baltic	B7X6	0	7	2	1	0	1	10
Baltic	B8X2	3	2	2	1	1	2	10
Baltic	B8x3	2	1	1	1	0	6	10

Region	Eggsac ID	Woken up animals, minutes after cold shock					Damaged or death after 24 h	Total spiders
		30min	45min	60min	90min	120min		
Portugal	P2.10x1	0	0	1	5	1	6	10
Portugal	P2.1Ax1	0	0	0	0	3	10	10
Portugal	P2.2Ax2	0	0	1	3	0	9	10
Portugal	P2.4Ax1	0	0	0	0	2	9	10
Portugal	P2.5Ax1	0	0	1	1	3	9	10
Portugal	P2.6Ax2	0	1	1	1	2	6	10
Portugal	P2.6x1	0	2	0	4	1	7	10
Portugal	P2.8x1	0	3	0	0	4	9	10
Portugal	P2.8x2	0	2	2	4	1	4	10
Portugal	P2.xz	0	2	2	4	1	5	9
Portugal	P2x1	0	0	1	2	2	10	10
Portugal	P2x2	0	0	0	0	2	10	10
Portugal	P2xy	0	0	1	1	0	3	8
Portugal	P4.2x1	0	0	1	0	2	10	10
Portugal	P5.1x1	1	1	0	0	1	10	10
Portugal	P5.1x1.2	0	1	0	0	4	8	10
Sweden	S2.3x2.1	5	4	0	1	0	0	10
Sweden	S2x12	0	1	0	1	5	6	10
Sweden	S2x12g	0	1	0	1	3	9	10
Sweden	S2x17yg	0	6	2	0	0	1	9
Sweden	S2X18	0	0	2	2	3	4	10
Sweden	S2x4	0	2	2	4	0	1	8
Sweden	S2X9	1	4	0	2	1	5	10
Sweden	S4.3x20	1	1	0	3	1	5	10
Sweden	S4x11	6	1	2	0	1	0	10
Sweden	S4X12	1	1	1	0	2	9	10
Sweden	S4x13	0	2	1	5	2	1	10
Sweden	S4X14	0	0	0	0	4	7	10
Sweden	S4x18	0	0	0	3	0	9	10
Sweden	S4X4	0	1	2	2	0	4	10
Sweden	S4Xz3	0	0	0	2	3	6	10

Supplementary table 5.4 Data from the heat stress experiment. The left three columns show Northern European and the right three ones Southern European spiders.

Region	Eggsac ID	Lethal temperature (°C)	Region	Eggsac ID	Lethal temperature (°C)
Baltic	LtMaNA03	45.5	Mediterranean	SpBilBI1.1A	50.0
Baltic	LtMaNA08	46.0	Mediterranean	SpBilBI1.2A	47.5
Baltic	LtKaKA11	42.0	Mediterranean	SpBilBI1.6?	52.0
Baltic	LtKaKA03	43.0	Mediterranean	SpBilBI1.7?A	49.0
Baltic	LvSaSA01	49.0	Mediterranean	SpBilBI1.7?B	51.3
Baltic	LvSaSA02	44.0	Mediterranean	SpBilBI1.8?	51.0
Baltic	LvSaSA04	40.0	Mediterranean	SpBilCI2.2	51.0
Baltic	LvSaSA08	42.5	Mediterranean	SpBilCI2.3	48.5
Baltic	LvLiAC04	41.3	Mediterranean	SpBilCI2.4	46.0
Baltic	LvLiAC09	41.5	Mediterranean	SpBilBW	45.0
Baltic	EsPuMA04	42.0	Mediterranean	PoBeCB	49.3
Sweden	SwKaSA01	46.0	Mediterranean	PoAlBA01	47.0
Sweden	SwKaSA15	45.5	Mediterranean	SpVaBA	48.0
Sweden	SwKaSA02	46.0	Mediterranean	SpVaBA05	48.0
Sweden	SwKaSA05	45.0	Mediterranean	SpVaBA55	48.0
Sweden	SwKaSA06	46.5	Mediterranean	SpVaBA04	50.0
Sweden	SwBlAA03	44.5	Mediterranean	SpVaBA09	48.5
Sweden	SwSkKA01	46.5	Mediterranean	SpVaCA05	44.5
Sweden	SwSkKA12	41.0	Mediterranean	SpCaMB01	45.0
Sweden	SwSkKA17	48.5	Mediterranean	SpCaMB	48.0
Sweden	SwSkKA02	44.7	Mediterranean	SpCaTA03	47.0
Sweden	SwSkKA06	43.8	Mediterranean	SpCaTA07	49.5
Sweden	SwSkKA08	41.0	Mediterranean	SpCaTA11	44.0

Supplementary table 5.5 Gene expression values (RPKM) for the three genes, which were differentially expressed between control and cold stress conditions. The RPKM values for each six samples per condition and gene are shown.

Region/Condition	Glucose-6-phophatase	Ubiquitin	Alanine-aminotransferase
	RPKM C598444	RPKM scaffold1784	RPKM scaffold29285
Baltic -10°C	156.533	1198.032	19.491
Baltic -10°C	204.155	1083.685	18.681
Baltic -10°C	216.658	1215.142	24.417
Baltic -10°C	204.384	1225.627	13.583
Baltic -10°C	121.575	1137.610	24.324
Baltic -10°C	137.323	1079.329	15.810
Baltic 15°C	21.077	1265.932	10.440
Baltic 15°C	19.806	1346.151	11.259
Baltic 15°C	28.385	1312.037	10.765
Baltic 15°C	45.561	1194.400	10.331
Baltic 15°C	35.917	984.230	9.149
Baltic 15°C	74.746	1309.411	14.174

Region/Condition	Glucose-6-phophatase	Ubiquitin	Alanine-aminotransferase
	RPKM C598444	RPKM scaffold1784	RPKM scaffold29285
Baltic 40°C	95.526	895.768	9.903
Baltic 40°C	49.356	869.194	7.768
Baltic 40°C	58.235	1040.439	9.189
Baltic 40°C	78.661	859.535	5.758
Baltic 40°C	92.924	831.784	6.848
Baltic 40°C	52.168	958.170	9.134
Portugal -10°C	185.898	1116.592	24.915
Portugal -10°C	142.056	1153.977	29.386
Portugal -10°C	161.273	1085.907	19.970
Portugal -10°C	173.313	845.896	17.351
Portugal -10°C	97.675	1024.804	18.841
Portugal -10°C	169.587	883.910	15.600
Portugal 15°C	24.352	1157.819	7.395
Portugal 15°C	22.237	1056.923	9.590
Portugal 15°C	30.809	1139.523	9.874
Portugal 15°C	12.962	1346.257	8.366
Portugal 15°C	22.072	1049.272	7.892
Portugal 15°C	13.001	1047.876	6.331
Portugal 40°C	70.800	651.994	6.267
Portugal 40°C	49.884	737.363	11.069
Portugal 40°C	41.146	845.561	9.644
Portugal 40°C	39.468	785.627	8.283
Portugal 40°C	64.934	728.827	6.878
Portugal 40°C	59.112	796.195	8.638

Supplementary table 5.6 Mean gene expression values (RPKM) for differentially expressed transcripts between Northern and Southern European wasp spider populations. The table shows data for the cold stress experiment (blue), the control (green) and heat stress (red).

Differentially expressed at -10 ˚C			Differentially expressed at 15 °C			Differentially expressed at 40 °C		
Contig ID	RPKM Baltic	RPKM Portugal	Contig ID	RPKM Baltic	RPKM Portugal	Contig ID	RPKM Baltic	RPKM Portugal
C408562	4.06	13.00	C420849	17.42	7.24	C397354	75.78	56.35
C422786	6.47	0.54	C422271	27.68	41.62	C397684	48.62	116.05
C428988	87.20	236.13	C428988	100.91	243.40	C408562	6.38	13.65
C430705	48.60	151.36	C430705	45.47	143.82	C420849	16.43	6.13
C437766	671.45	1219.33	C437766	678.95	1327.87	C422908	2.14	6.99
C452375	0.00	3.50	C449357	26.49	42.26	C428166	103.27	76.46
C470153	1.71	6.64	C454093	62.91	44.76	C430357	1356.78	989.68
C471259	334.95	253.88	C462948	25.31	13.80	C430705	57.96	162.91
C473781	6.14	14.12	C473781	4.64	15.57	C431061	20.81	10.57

Differentially expressed at -10 ˚C			Differentially expressed at 15 °C			Differentially expressed at 40 °C		
Contig ID	RPKM Baltic	RPKM Portugal	Contig ID	RPKM Baltic	RPKM Portugal	Contig ID	RPKM Baltic	RPKM Portugal
C478133	1385.91	2297.64	C478327	23.84	7.72	C449087	101.53	64.17
C491425	241.64	583.69	C483589	1.04	6.61	C452375	0.00	2.98
C497253	4.52	13.92	C485990	0.92	5.19	C460758	114.87	81.12
C513159	27.66	49.31	C491425	306.67	664.52	C461884	49.35	13.63
C516569	903.22	1347.29	C516569	906.67	1369.53	C478133	1273.08	2624.92
C524020	6.03	13.93	C518583	2.66	0.00	C483589	2.50	10.84
C530662	152.04	395.86	C524020	5.90	15.05	C494991	1853.18	1346.72
C530678	42.30	24.27	C530662	174.74	380.26	C498737	1.32	6.33
C532832	26.97	48.77	C544331	223.88	108.11	C503755	467.69	533.42
C539244	12.26	4.57	C551725	92.41	132.94	C513023	1556.94	2903.89
C544331	226.13	103.46	C554717	81.76	113.37	C513159	28.26	45.17
C550389	686.76	532.23	C554733	1.31	5.76	C516233	222.21	181.04
C551725	93.76	135.32	C557257	70.41	134.42	C516569	849.41	1542.18
C554733	1.26	5.72	C573116	15.57	28.97	C523889	28.23	60.09
C557257	71.35	132.52	C575630	35.24	50.74	C524020	3.83	12.23
C565118	37.48	74.33	C585262	24.34	11.62	C543445	116.06	293.43
C567022	870.26	508.95	C585880	416.52	618.17	C544331	167.81	82.18
C575630	38.41	54.07	C591912	4.20	0.00	C548697	57.43	82.69
C576678	11.59	45.57	C591936	8.31	16.27	C550389	576.78	456.07
C578794	0.31	7.71	C592296	9.43	17.98	C551247	6.83	17.42
C585262	24.36	13.28	C595206	114.66	211.69	C557257	73.36	122.54
C585880	431.67	669.19	C599518	36.18	58.10	C563210	622.63	919.66
C587118	75.25	306.57	C600600	77.23	119.55	C565118	32.57	58.32
C590888	16.63	28.88	C600924	3.00	0.00	C565510	22.31	34.03
C592296	10.91	20.72	C603096	11.39	4.40	C566178	2.99	17.78
C596362	207.85	137.33	C605328	5.72	16.80	C567890	32.65	74.36
C596472	9.89	20.43	C608432	16.68	42.05	C570268	260.47	184.76
C597028	2.00	6.94	C608448	100.10	63.75	C570572	866.52	1135.94
C597864	99.00	72.03	scaffold10510	142.09	90.69	C571050	15.34	27.66
C599518	37.33	67.06	scaffold12736	293.33	346.83	C574014	86.56	56.60
C600600	74.93	121.82	scaffold1455	5.26	0.33	C576678	10.64	39.97
C603096	12.80	4.22	scaffold1457	5.35	0.51	C577774	3.46	13.46
C605328	5.59	20.83	scaffold1458	16.21	1.34	C578794	0.42	6.55
C608432	17.61	43.76	scaffold1784	948.83	741.23	C578924	24.15	14.23
C608558	157.57	81.21	scaffold17865	4.73	19.37	C580504	377.73	255.49
scaffold10510	144.02	86.07	scaffold19587	34.17	18.24	C582750	29.65	17.04
scaffold11590	25.99	48.59	scaffold20228	37.29	65.69	C585880	454.63	937.90
scaffold12056	112.93	77.90	scaffold20480	7.22	15.40	C586314	409.33	1339.27
scaffold13008	0.55	4.04	scaffold20481	5.17	20.70	C587118	60.21	289.11

Differentially expressed at -10 ˚C			Differentially expressed at 15 °C			Differentially expressed at 40 °C		
Contig ID	RPKM Baltic	RPKM Portugal	Contig ID	RPKM Baltic	RPKM Portugal	Contig ID	RPKM Baltic	RPKM Portugal
scaffold13048	7.26	19.98	scaffold22035	33.76	61.74	C591912	6.72	0.00
scaffold13629	288.54	211.31	scaffold22583	7.77	16.53	C594018	584.17	430.94
scaffold14228	11.14	29.74	scaffold229	4.44	0.00	C594244	8.24	16.72
scaffold1455	5.05	0.61	scaffold24855	156.51	0.59	C596148	6.97	24.07
scaffold1457	5.17	0.19	scaffold2517	2.78	23.99	C596362	225.30	161.03
scaffold1458	13.98	1.86	scaffold27303	2.15	9.46	C598126	10.88	22.15
scaffold14915	11.00	31.89	scaffold2757	19.33	32.84	C599518	30.55	52.27
scaffold14916	4.01	14.07	scaffold28600	70.75	93.11	C600692	18.28	8.41
scaffold15117	11.52	23.27	scaffold29335	50.43	28.32	C600924	3.49	0.00
scaffold16424	13.54	25.82	scaffold30086	109.09	74.88	C600952	185.34	143.04
scaffold17219	376.25	266.36	scaffold31115	3.40	15.31	C605328	5.80	18.66
scaffold18366	32.61	15.20	scaffold31581	30.27	12.83	C605410	32.86	48.73
scaffold19587	36.61	21.05	scaffold32648	0.95	4.77	C608432	13.26	31.62
scaffold20133	1.95	6.62	scaffold33027	16.59	29.74	C608558	187.74	99.96
scaffold20481	4.80	18.73	scaffold33034	51.00	87.59	scaffold10341	55.75	39.07
scaffold21043	108.43	41.98	scaffold34950	76.71	100.01	scaffold11414	75.14	52.87
scaffold21046	11.00	22.48	scaffold36946	60.65	98.63	scaffold1228	1.88	7.87
scaffold22035	29.67	65.15	scaffold37821	428.20	549.75	scaffold12335	0.48	3.70
scaffold24855	124.90	0.74	scaffold38017	49.13	90.18	scaffold12538	1.00	6.24
scaffold2517	2.91	21.81	scaffold39566	35.86	55.05	scaffold13008	0.13	2.98
scaffold26374	5.35	17.85	scaffold40311	76.35	106.93	scaffold1316	1117.36	677.82
scaffold2756	34.65	56.29	scaffold42067	32.33	47.34	scaffold1455	3.42	0.36
scaffold28307	58.85	28.71	scaffold42249	76.27	56.86	scaffold14915	10.80	27.20
scaffold29229	15.98	27.90	scaffold42386	9.54	19.92	scaffold14916	4.54	14.33
scaffold29335	42.76	20.18	scaffold42452	28.74	51.32	scaffold15363	2205.16	1006.13
scaffold30086	103.94	72.78	scaffold42576	24.03	46.13	scaffold15365	242.53	112.25
scaffold31115	5.10	16.10	scaffold42587	3.13	10.55	scaffold16705	18.80	9.86
scaffold31581	28.19	11.98	scaffold42687	136.56	69.24	scaffold17222	2.47	0.00
scaffold31590	0.93	6.39	scaffold43622	189.81	93.61	scaffold19044	732.43	1233.04
scaffold32756	2.17	7.71	scaffold43738	0.69	4.93	scaffold19587	74.90	33.14
scaffold36275	4.45	12.79	scaffold44995	24.67	43.05	scaffold20160	14.57	31.31
scaffold36353	18.16	29.27	scaffold45807	31.21	15.17	scaffold21567	143.83	58.50
scaffold36770	41.52	63.58	scaffold47381	3.72	0.03	scaffold22035	20.41	33.49
scaffold38017	53.29	93.77	scaffold47692	42.18	81.38	scaffold22069	3.44	10.02
scaffold39216	50.15	34.28	scaffold48122	3.69	0.04	scaffold229	6.61	0.00
scaffold39980	115.46	70.86	scaffold48324	162.09	131.41	scaffold2321	5.92	15.93
scaffold41507	134.08	89.76	scaffold49338	31.30	47.26	scaffold24855	137.22	0.79
scaffold42067	29.84	47.60	scaffold49566	31.15	53.21	scaffold24856	64.32	186.53
scaffold42249	77.61	55.82	scaffold50169	17.37	33.62	scaffold2517	2.44	18.00

Differentially expressed at -10 ˚C			Differentially expressed at 15 °C			Differentially expressed at 40 °C		
Contig ID	RPKM Baltic	RPKM Portugal	Contig ID	RPKM Baltic	RPKM Portugal	Contig ID	RPKM Baltic	RPKM Portugal
scaffold42386	8.26	20.45	scaffold50328	20.93	9.34	scaffold25956	107.77	81.86
scaffold42452	25.18	48.65	scaffold50460	97.96	138.85	scaffold26522	10.67	4.07
scaffold42576	24.68	46.72	scaffold5139	51.27	22.74	scaffold26888	21.44	7.85
scaffold42587	3.47	10.98	scaffold5213	155.91	102.49	scaffold28531	127.60	245.69
scaffold43177	4.37	12.78	scaffold5250	21.21	35.75	scaffold28626	16.39	32.63
scaffold43622	183.15	95.54	scaffold5476	12.77	23.96	scaffold30456	141.04	180.21
scaffold44901	88.23	119.03	scaffold5499	564.82	368.26	scaffold30927	96.02	42.30
scaffold44995	27.75	42.93	scaffold5503	6.01	1.36	scaffold31115	3.75	11.17
scaffold47692	46.89	84.01	scaffold6005	2.66	13.15	scaffold31136	59.85	32.99
scaffold47990	19.68	32.58	scaffold6164	50.38	19.04	scaffold33588	179.53	103.63
scaffold48340	27.51	48.80	scaffold7244	182.54	128.07	scaffold34950	89.90	118.92
scaffold48517	2.66	8.24	scaffold7682	11.43	22.80	scaffold35083	50.40	72.28
scaffold48637	45.66	29.94	scaffold8606	13.33	32.69	scaffold35207	50.68	30.89
scaffold48900	18.28	29.51	scaffold8843	31.03	75.36	scaffold36353	22.97	35.83
scaffold49392	6.98	16.19	scaffold9213	4.75	11.39	scaffold36984	55.20	37.04
scaffold49566	28.09	51.24	scaffold9867	99.35	77.63	scaffold37404	31.19	19.14
scaffold50328	36.04	16.87				scaffold3761	5.50	12.74
scaffold5139	44.58	18.80				scaffold37821	346.18	458.86
scaffold5213	148.98	99.92				scaffold38017	43.62	65.68
scaffold5250	19.35	35.57				scaffold38612	123.79	149.37
scaffold5373	123.15	151.69				scaffold39030	58.59	140.47
scaffold5476	10.91	24.77				scaffold3971	5.09	14.92
scaffold5499	594.04	361.95				scaffold3973	8.88	2.15
scaffold7244	210.45	135.33				scaffold39980	115.81	69.80
scaffold7682	10.77	23.07				scaffold40081	120.18	95.41
scaffold8541	44.06	20.35				scaffold40205	76.13	55.99
scaffold8606	12.41	31.72				scaffold40655	20.99	53.44
scaffold9213	4.65	11.68				scaffold40812	169.75	117.70
						scaffold41505	12.51	22.03
						scaffold42249	74.07	50.71
						scaffold4233	43.93	24.87
						scaffold42370	14.54	48.21
						scaffold42452	35.36	52.75
						scaffold42587	2.86	8.65
						scaffold42687	65.40	27.79
						scaffold4327	26.68	49.60
						scaffold43355	22.81	11.70
						scaffold43622	166.08	95.22
						scaffold44441	12.87	5.67

Differentially expressed at 40 °C		
Contig ID	Contig ID	Contig ID
scaffold44618	2.24	15.48
scaffold45454	24.86	47.17
scaffold45676	98.06	67.97
scaffold45789	36.74	17.61
scaffold45829	174.39	136.06
scaffold45850	52.63	36.74
scaffold46005	683.87	452.48
scaffold46019	104.03	51.14
scaffold47377	2.37	11.04
scaffold47381	5.74	0.07
scaffold48122	8.96	0.08
scaffold48236	249.16	192.42
scaffold48732	462.17	231.37
scaffold48785	40.28	68.76
scaffold49367	20.03	34.41
scaffold49566	25.31	49.59
scaffold4960	2.19	7.13
scaffold50328	24.90	11.41
scaffold50466	6.65	31.46
scaffold5139	52.34	18.65
scaffold5213	115.64	74.43
scaffold5215	22.10	12.59
scaffold5373	121.04	147.49
scaffold59	3.87	10.76
scaffold5918	23.55	41.65
scaffold6016	86.20	248.46
scaffold6164	52.43	25.78
scaffold6459	116.18	63.55
scaffold661	172.56	125.00
scaffold720	39.52	24.63
scaffold7244	144.55	112.01
scaffold7462	23.95	49.59
scaffold8606	11.84	31.14
scaffold8666	2.08	27.30
scaffold8843	33.43	65.09
scaffold9925	6.31	22.32

Curriculum vitae

Name: Henrik Krehenwinkel
Date of birth: 13.08.1983
Place of birth: West-Berlin
Place of residence: Danziger Strasse 5, 24306 Plön
Nationality: German

School and Military Service

1990 - 1994 Primary school in Dorsten
1994 - 2003 Gymnasium Petrinum in Dorsten
2003 - 2004 Military service in the Wachbataillon in Berlin & Siegburg

University education

October 2004 – February 2010: Studies of Biology at the Ruhr-University in Bochum. Main subjects: Zoology, Ecology, Genetics and Biochemistry

March – October 2008: Studies in Molecular Ecology at the Indian Institute of Science in Bangalore, India

April 2009 – February 2010: Diploma thesis at the Max Planck Institute for Evolutionary Biology in the department of Evolutionary Genetics. Title: "Associations between testes gene expression and fertility in male house mouse hybrids"

Since April 2010: Dissertation at the Max Planck Institute for Evolutionary Biology in the department of Evolutionary Genetics on "A phylogeographic, ecological and genomic analysis of the recent range expansion of the wasp spider *Argiope bruennichi*".

Scholarships

2007-2010 Undergraduate scholarship of the Studienstiftung des Deutschen Volkes
2010-2013 PhD scholarship of the Studienstiftung des Deutschen Volkes

Affidavit

Hiermit erkläre ich, dass die vorliegende Arbeit

- nach Inhalt und Form meine eigene ist, abgesehen von der Beratung durch meinen Betreuer Prof. Diethard Tautz

- an keiner anderen Stelle im Rahmen eines Prüfungsverfahrens vorgelegen hat, noch nicht veröffentlicht ist und auch nicht zur Veröffentlichung eingereicht wurde

- unter Einhaltung der Regeln guter wissenschaftlicher Praxis der Deutschen Forschungsgemeinschaft entstanden ist.

Plön, den 05.08.2013

Henrik Krehenwinkel