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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

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Abstract

Poleward range expansions are observed for an increasing number of species, which may be an effect of global warming during the past decades. However, it is still not clear in how far these expansions reflect simple geographical shifts of species ranges, or whether new genetic adaptations play a role as well. Here, we analyse the expansion of the wasp spider *Argiope bruennichi* into Northern Europe during the last century. We have used a range-wide sampling of contemporary populations and historical specimens from museums to trace the phylogeography and genetic changes associated with the range shift. Based on the analysis of mitochondrial, microsatellite and SNP markers, we observe a higher level of genetic diversity in the expanding populations, apparently due to admixture of formerly isolated lineages. Using reciprocal transplant experiments for testing overwintering tolerance, as well as temperature preference and tolerance tests in the laboratory, we find that the invading spiders have possibly shifted their temperature niche. This may be a key adaptation for survival in Northern latitudes. The museum samples allow a reconstruction of the invasion's genetic history. A first, small-scale range shift started around 1930, in parallel with the onset of global warming. A more massive invasion of Northern Europe associated with genetic admixture and morphological changes occurred in later decades. We suggest that the latter range expansion into far Northern latitudes may be a consequence of the admixture that provided the genetic material for adaptations to new environmental regimes. Hence, global warming could have facilitated the initial admixture of populations and this resulted in genetic lineages with new habitat preferences.

Keywords: adaptation, climate change, genetic admixture, historical DNA, range expansion

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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; Walther *et al.* 2002; Hickling *et al.* 2006; Willis & Bhagwat 2009; 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 (Walther *et al.* 2002; Parmesan & Yohe 2003; 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 time

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frames, 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 interpopulational admixture (Dowling & Secor 1997; de Carvalho *et al.* 2010). 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 analyse the recent range expansion of the European wasp spider *Argiope bruennichi* (Scopoli 1772), a well-studied model organism in behavioural ecology (e.g., Welke & Schneider 2010). The species' original European range comprised the whole Mediterranean and warm Oceanic climate regions (Fig. 1). Apart from a single isolated occurrence around Berlin, it was absent from the Northern Continental climate region of Europe. From ~1930 onwards, the species started slowly expanding its range into increasingly Continental climate regions in, for example, 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 kilometres (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.

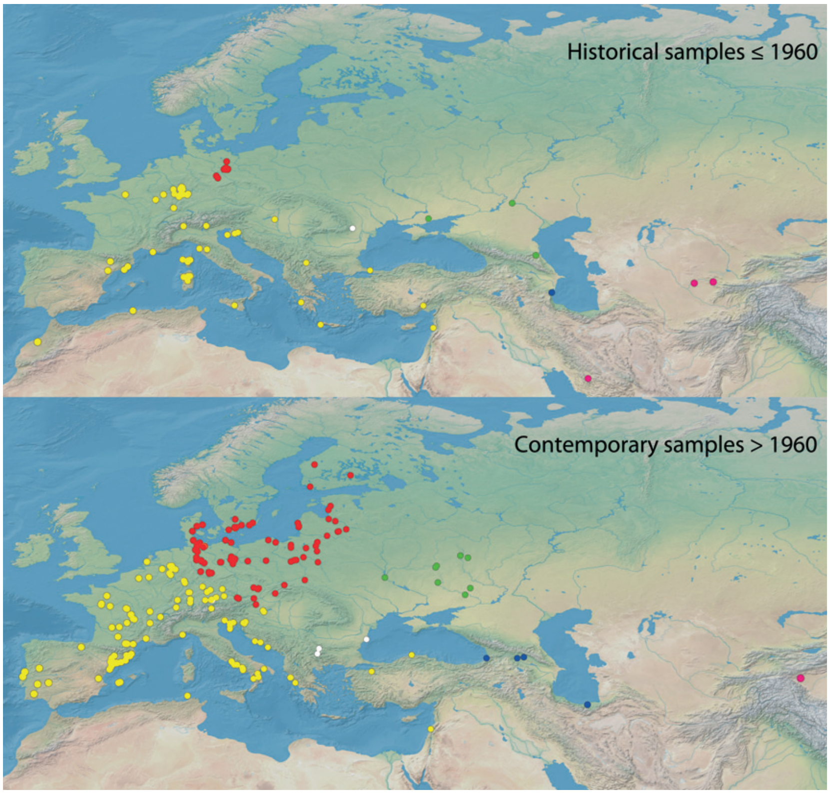

Fig. 1 Sampling locations for historical (upper panel) and contemporary (lower panel) specimens. The colours represent geographical regions distinguished in the haplotype network in Fig. 2. Historical specimens refer to samples until 1960, the time before and at the early phase of the range expansion. Contemporary samples represent spiders during the more massive range shift in the past decades. For an overview of far Eastern Asian sampling sites, see Table S1 (Supporting information). 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 North-Eastern Europe (red dots).

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 analysed ~2000 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. Secondly, 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. Last, 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. As these adaptations correlate with the admixture of lineages, we speculate that hybridization of gene pools may have played a causal role in this.

Material and methods

Sample collection and morphological analysis

About 2000 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 Table S1. 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 geographical locations across the Palearctic (see Fig. 1 and Table S1, Supporting information). 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, for example, France and South-Western 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 1930s.

This distinction of native and invasive spiders is blurred at the species' former distributional range limit in South-Western Germany and Southern Austria. We thus fine-scaled the geographical division based on differences in our mitochondrial and nuclear genetic data (see Figs 2–5 and Fig. S1, Supporting information), 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

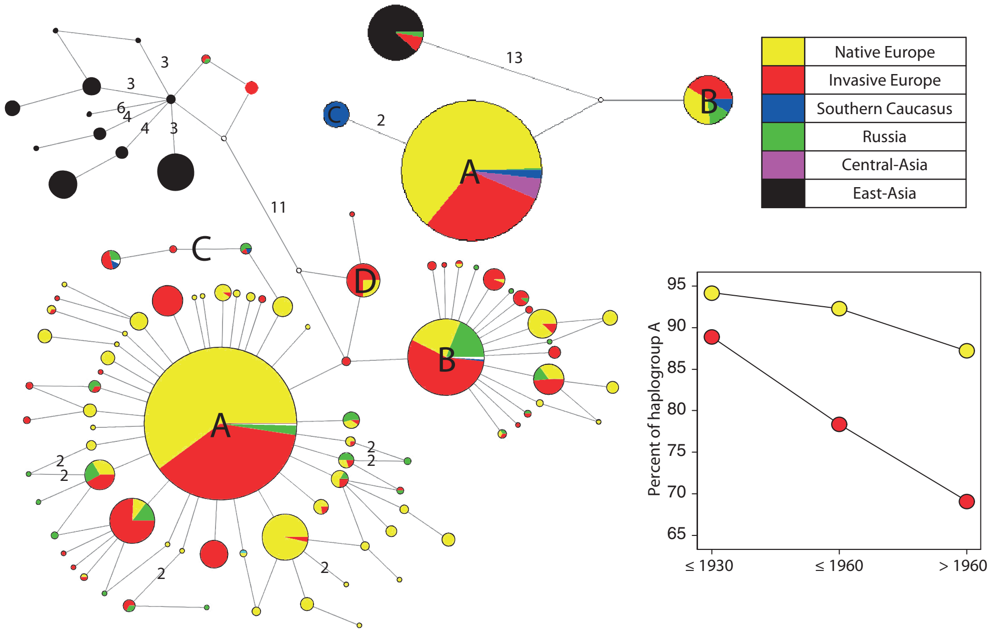

Fig. 2 Median-joining networks of mitochondrial sequences from Palearctic wasp spiders. The lower left network shows contemporary samples (*N* = 1966), based on 1200 bp of the COI gene. The simplified upper right network is based on historical samples (*N* = 368) from before 1960. Colours represent the geographical origin of sampled specimens (corresponding to the colours in Fig. 1). The respective location designations (inset upper right) are used throughout the text. The white fractions in haplogroup A, B and C refer to seven samples from Bulgaria and Romania, which we could not unequivocally assign to a geographical region. Branches represent single mutational steps, if not indicated otherwise by numbers. Major haplotype groups discussed in the text are labelled 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.

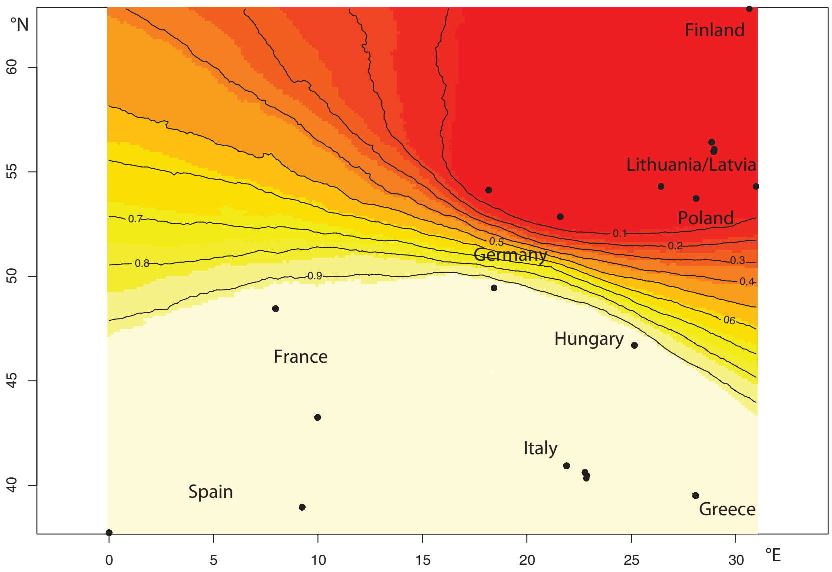

Fig. 3 Geographical clustering of European wasp spider populations according to Geneland analysis. The map represents the corresponding sampling sites, indicated by black dots. Axes represent geographical coordinates. The longitudinal coordinates shifted by 7.85° to the east. The Geneland map shows wasp spider populations forming a distinct genetic cluster in the invasive range in Continental Europe with a high posterior probability. The coloured 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).

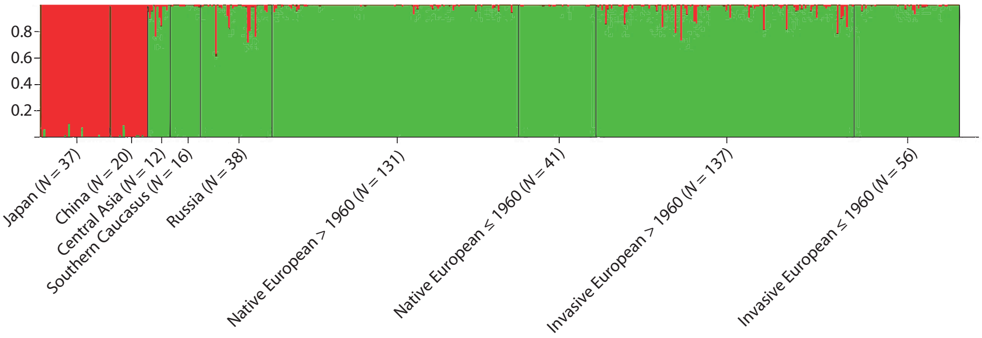

Fig. 4 STRUCTURE analysis for the SNP data set (k = 2). Green bars correspond to Western Palearctic, red ones to East Asian genetic background. Bars represent single specimens from the regions depicted at the bottom.

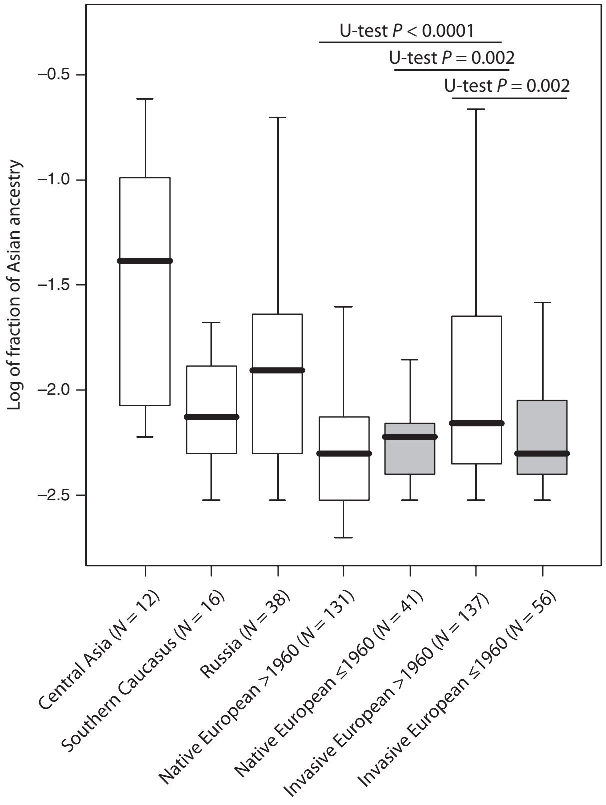

Fig. 5 Box plots of the fraction of Asian ancestry in different Western Palearctic populations according to STRUCTURE (logarithmic scale). The boxes depict median (black bar) and upper and lower quartiles. Distinction of geographical regions is based upon the classification in Fig. 1. Native and invasive 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.

laboratory equipment were used. Extractions were carried out with leg tissue and the 5 PRIME ArchivePure 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 (20 mg/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 1200-bp fragment of the mitochondrial COI gene were amplified for contemporary samples. Ten microlitre PCRs were run in ABI verity fast thermal cyclers (Applied Biosystems, Foster City, USA), using the Quiagen Multiplex PCR kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol. A published partial mitochondrial genome sequence of the species (Hassanin *et al.* 2005) served as the template for primer design. Five microlitre PCR product was purified in an 8 μL reaction by adding 0.12 μL exonuclease I (20 000 units/mL) (NEB, Ipswitch, MA, USA), 0.45 μL shrimp alkaline phosphatase (1 U/μL) (Fermentas, St. Leon-Rot, Germany) and 2.33 μL water. This reaction mixture 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, MA, USA) and then aligned with MEGA (Tamura *et al.* 2007) under default parameters. Based on sequence variation, we designed PCR primers for a short 135-bp COI fragment, which contained diagnostic SNPs to distinguish all major phylogeographic groups within the species. 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 (microsatellites and SNPs), we performed a single-lane 454 shotgun sequencing run (454 Life Sciences, Branford, CT, USA) of genomic DNA. This yielded over 80 000 fragments, of which nearly 1000 included microsatellites. Microsatellite primers were designed for 16 polymorphic loci, which were typed for a selection of contemporary populations. As 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 ~400 bp (derived from the 454 reads) for the 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 and 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, UK). 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 analysed populations and population-wise distance matrices (Nei's standard genetic distance). The PHYLIP package served to construct a neighbour joining tree (Felsenstein 1989), based on the distance matrix. In addition to the distance-based approach, we analysed 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 100th iteration. A noncorrelated allele frequency model with enabled spatial model was used. The individual ancestry for the SNP data set 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 3 months (Köhler & Schäller 1987). This prolonged diapause constitutes a considerable part of the spider's whole lifespan 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 probably 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). 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 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 × 86.5 × 11.75 mm). The plate contained eight channels (each 104 × 5.5 mm), which each spanned the temperature gradient. We allowed the gradient to establish for 30 min 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 min. We thus noted their position after 30 min 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 10 min. Confronted with raised temperatures, the spiderlings increase their activity until they reach their tolerance limit and fall into rigour. After each round of heating, the spiders in rigour were identified and the temperature noted. We tried also to test lower temperature tolerance, but as 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 × 57 × 42 cm). 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, December 3rd, 2011) and two in a Garden near Santa Eulalia on the Spanish island of Ibiza (38.99°N, 1.53°E, December 1st, 2011). Egg sacs for the Northern European treatment were gradually adapted to the cooler climate by decreasing their ambient temperature from 20 to 6 °C over a 14-day period. The egg sacs were then overwintered on their respective sites until 1 March 2012 (Ibiza) and 3 March 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 vs. live animals was performed only for egg sacs that could not be clearly assigned to one of the two categories.

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 analysed mitochondrial sequences, as well as nuclear markers. The results will be presented in turn in the following.

Mitochondrial data

One thousand two hundred base pair of the mitochondrial COI gene was sequenced from 1966 contemporary and 181 historical specimens from all across the species' range. In addition, we sequenced a short COI fragment of ~ 100 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 (Fig. 2).

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 six 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 (Fig. 2), accounting for the majority of haplotypes observed in the region. We refer to these radiations as haplogroups 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 haplogroups 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 (χ^2 test, d.f. = 2, $\chi^2 = 132.60$, $P < 0.0001$). In addition, Russian populations harbour 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 Figs 1 and 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 haplotypic 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 of 21 and 34 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 haplotypic composition of invasive populations. This results in significantly higher nucleotide diversity in invasive compared with native populations ($\pi = 0.0011$ ($N = 56$) vs. $\pi = 0.0017$ ($N = 60$) on average, U-test, $U = 1041.5$, $Z = -3.48$, $P < 0.0001$).

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 (Fig. 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 in 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 and 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 (Fig. 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 data set, based on Nei's genetic distance, confirms this clustering of populations into two different ecological zones (Fig. S1, Supporting information). The inclusion of 79 Russian samples into the microsatellite analysis shows these populations to be distantly related to European ones (Fig. S1, Supporting information). 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.

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 data set confirmed the genetic distinctness between East Asian and Western Palearctic populations for these markers in the larger population sample (Fig. 4). East Asian populations are largely fixed for their respective genotypes (99% Asian background on average, Fig. 4). These clusters correspond also very well to the mitochondrial network (Fig. 2).

These data allow now to assess the degree of introgression of Asian alleles into Western Palearctic groups (Fig. 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 in 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.

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, because the opisthosoma size is plastic and depends, for example, on the nutritional status. We find that Mediterranean spiders have a significantly larger prosoma width than the invasive ones from the Continental climate zones (Fig. 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 and 5.25 mm in historical and contemporary Mediterranean vs. 4.11 and 3.84 mm in Oceanic, vs. 3.44 and 3.38 mm in invasive material (ANOVA, $F = 214.613$, d.f. 895, $P < 0.0001$, Bonferroni *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 Kolmogorov–Smirnov test). Invasive as well as native spiders did not significantly change their mean body size within their respective groups in the past 100 years (Fig. 6a). However, invasive spiders show a significant decrease in 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 to 6.10 mm and today from 2.22 to 4.68 mm, a variance change that is not seen in the other samples (Fig. 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 upper thermal tolerance and thermal preference for spiderlings in the laboratory. Spiderlings from invasive Northern European egg sacs show indeed a significantly decreased thermal tolerance compared with Mediterranean ones (44.7 vs. 48.3 °C on average, $N = 83$, t-test, equal variances assumed, $t = 6.99$, d.f. = 81, $P < 0.0001$) (Fig. 6b) as well as lower preferred temperatures (10.4 vs. 14.6 °C on average, $N = 61$, t-test, equal variances assumed, $t = 4.72$, d.f. = 58, $P < 0.0001$) (Fig. 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 Material and methods). We found a significant association between overwintering locality and survival frequency within egg sacs (χ^2 test, $\chi^2 = 8.22$, d.f. = 3, $P = 0.04$) (Fig. 6d). Interestingly, invasive and native populations are equally affected by reverted overwintering conditions. Forty-five percent of Mediterranean and 42% of the Continental egg sacs had <50% survival of spiderlings under reversed overwintering conditions, while only 27% of the Mediterranean and 20% of the Continental egg sacs had <50% surviving

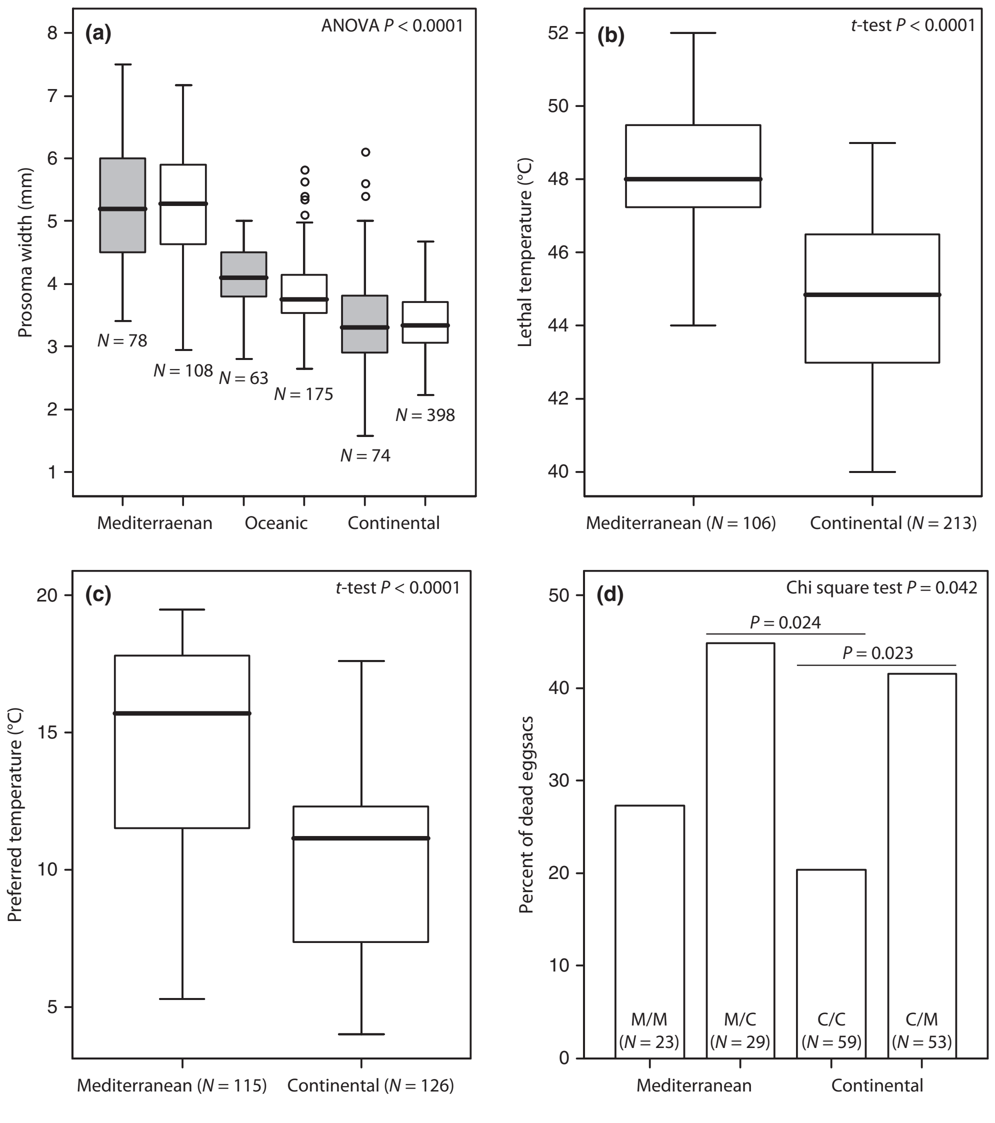

Fig. 6 Morphological and physiological differences between native and invasive European populations. (a) Prosoma width (representing body size) of adult females from native Mediterranean, native Oceanic climate and invaded Continental climate regions of Europe. Historical (≤1960, grey boxes) and contemporary 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 (a–c) show median (black bar), upper and lower quartiles and outliers. (d) Fraction of dead egg sacs (<50% surviving offspring) after reciprocal overwintering of Mediterranean and Continental animals. The letters correspond to origin and overwintering location, for example, C/C represent Continental population overwintered in Continental Climate.

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).

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 & 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 Figs 2 and 4). The genetic distance of 1.1% between these lineages corresponds to ~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 and C) present, we suggest the Southern Caucasus as this refugial area. From here, a single South-Western route could have led to the postglacial recolonization of the Mediterranean region. The South-Western 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, for example, Russian populations. The star-like topologies of the haplotype networks indicate a recent recolonization from the species' glacial refugium (see Fig. 2). A more detailed treatment of the phylogeny and Palearctic-wide phylogeography of the species will be published elsewhere (H. Krehenwinkel *et al.* unpublished data).

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 South-Western Germany (Fig. 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 geographical ranges of many species are limited to a certain ecosystem and an invasion into new habitat is not easy (Bridle & Vines 2007; Hoffmann & 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 (Castenholz 1973; Rehfeldt *et al.* 1999). 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 of 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 (Brönnimann 2009; Stelkens *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 Figs 2, 4 and 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 (see Fig. S1, Supporting information).

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 Figs 2 and 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 (Burton *et al.* 2006; Lee *et al.* 2008). This would lead to a trade-off 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 (Mao *et al.* 2010; Croucher *et al.* 2011), 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 probably 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 Fig. 6b, 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 (Castenholz 1973; Rehfeldt *et al.* 1999). 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 Fig. 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 <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, for example, wing size in insects or leg length in cane toads (Thomas *et al.* 2001; Phillips *et al.* 2006; Hill *et al.* 2011). 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 to be able to reproduce before the onset of autumn (see Fig. 6a). 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 & 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 probably 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 (Hoffmann *et al.* 2002; Edgar 2006; 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 in 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 behaviour 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 kilometres (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 in 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 ~1930 (see Fig. 2 inset). This period approximates the initial onset of climate change (Delworth & Knutson 2000). Additionally, we find a strong increase in 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 historical 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).

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.

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H.K. and D.T. developed the project. H.K. sampled the populations, developed the markers, typed the specimens, conducted the reciprocal transplant experiments and temperature preference tests and analysed all data. H.K. and D.T. wrote the manuscript.

Data accessibility

In Dryad: doi:10.5061/dryad.r8n7c

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

In GenBank: accession nos KC193787y–K195752

1 Single COI sequences

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Neighbour joining tree based on Nei's genetic distance for 16 microsatellite Loci.

Table S1 Sampling localities for wasp spider populations, which were used in this study.