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The odd couple: Contrasting phylogeographic patterns in two sympatric sibling species of woodlouse-hunter spiders in the Canary Islands

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Abstract:	Comparative phylogeography seeks for commonalities in the spatial demographic history of sympatric organisms to characterize the mechanisms that shaped such patterns. The unveiling of incongruent phylogeographic patterns in co-occurring species, on the other hand, may hint to overlooked differences in their life histories or microhabitat preferences. The woodlouse-hunter spiders of the genus Dysdera have undergone a major diversification on the Canary Islands. The species pair Dysdera alegranzaensis and Dysdera nesiotes are endemic to the island of Lanzarote and nearby islets, where they co-occur at most of their known localities. The two species stand in sharp contrast to other sympatric endemic Dysdera in showing no evidence of somatic (non-genitalic) differentiation. Phylogenetic and population genetic analyses of mitochondrial cox1 sequences from an exhaustive sample of D. alegranzaensis and D. nesiotes specimens, and additional mitochondrial (16S, L1, nad1) and nuclear genes (28S, H3) were analysed to reveal their phylogeographic patterns and clarify their phylogenetic relationships. Relaxed molecular clock models using five calibration points were further used to estimate divergence times between species and populations. Striking differences in phylogeography and population structure between the two species were observed. Dysdera nesiotes displayed a metapopulation-like structure, while D. alegranzaensis was characterised by a weaker geographical structure but greater genetic divergences among its main haplotype lineages, suggesting more complex population dynamics. Our study confirms that co-distributed, sibling species may exhibit contrasting phylogeographic patterns in the absence of somatic

differentiation. Further ecological studies, however, will be necessary to clarify if the contrasting phylogeographies may hint at an overlooked niche partitioning between the two species. In addition, further comparisons with available phylogeographic data of other eastern Canarian Dysdera endemics, confirm the key role of lava flows in structuring local populations in oceanic islands, and identify localities that acted as refugia during volcanic eruptions.

ORIGINAL ARTICLE

The odd couple: Contrasting phylogeographic patterns in two sympatric sibling species of woodlouse-hunter spiders in the Canary Islands

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ABSTRACT

Comparative phylogeography seeks for commonalities in the spatial demographic history of sympatric organisms to characterize the mechanisms that shaped such patterns. The unveiling of incongruent phylogeographic patterns in co-occurring species, on the other hand, may hint to overlooked differences in their life histories or microhabitat preferences. The woodlouse-hunter spiders of the genus *Dysdera* have undergone a major diversification on the Canary Islands. The species pair *Dysdera alegranzaensis* and *Dysdera nesiotes* are endemic to the island of Lanzarote and nearby islets, where they co-occur at most of their known localities. The two species stand in sharp contrast to other sympatric endemic *Dysdera* in showing no evidence of somatic (non-genitalic) differentiation. Phylogenetic and population genetic analyses of mitochondrial *cox1* sequences from an exhaustive sample of *D. alegranzaensis* and *D. nesiotes* specimens, and additional mitochondrial (*16S, L1*, *nad1*) and nuclear genes (*28S*, *H3*) were analysed to reveal their phylogeographic patterns and clarify their phylogenetic relationships. Relaxed molecular clock models using five calibration points were further used to estimate divergence times between species and populations. Striking differences in phylogeography and population structure between the two species were observed. *Dysdera nesiotes* displayed a metapopulation-like structure, while *D. alegranzaensis* was characterised by a weaker geographical structure but greater genetic divergences among its main haplotype lineages, suggesting more complex population dynamics. Our study confirms that co-distributed, sibling species may exhibit contrasting phylogeographic patterns in the absence of somatic differentiation. Further ecological studies, however, will be necessary to clarify if the contrasting phylogeographies may hint at an overlooked niche partitioning between the two species. In addition, further comparisons with available phylogeographic data of other eastern Canarian *Dysdera* endemics, confirm the key role of lava flows in structuring local populations in oceanic islands, and identify localities that acted as refugia during volcanic eruptions.

INTRODUCTION

Sympatric species that share ecological requirements are expected to respond similarly to local environmental changes and geographic barriers (Riddle et al. 2000; Irwin 2002; Garrick et al. 2008). Conversely, contrasting population patterns in co-distributed species may indicate underlying species-specific features such as habitat preference, behaviour, life history or dispersal ability (Taberlet et al. 1998; Hodges et al. 2007). Alternatively, co-distributed species may not have been similarly co-distributed in the past. Hence, they may have responded differently to distinct evolutionary events occurring at dissimilar times, resulting in incongruent geographic patterns of genetic diversity (Donoghue and Moore 2003; Carstens and Corinne 2007).

The deeply eroded, dry and relatively species-poor eastern Canary islands, which include Fuerteventura, Lanzarote and the northern islets of La Graciosa, Montaña Clara and Alegranza (the three latter also known as the Chinijo Archipelago), have received little attention from evolutionary biologists compared with their western counterparts. However, the dynamic volcanism, the dramatic climatic changes and episodic island connections, make the eastern Canaries an excellent setting to investigate the role of geological and climatic barriers in structuring local populations and shaping island diversity (Juan et al. 1998; Bidegaray-Batista et al. 2007; Bloor et al. 2008). These islands are the emergent summits of a continuous volcanic edifice that dates back to about 20 million years ago (Ma) (Coello et al. 1992). All of these islands and islets have been connected on several occasions during glacial periods by eustatic sea-level changes (Fernández-Palacios 2011), offering ample opportunities for gene flow among populations on different islands. Lanzarote emerged during the Miocene as two independent islands, corresponding to the present day massifs of Ajaches (15.5-13.5 Ma) on the southwest, and Famara (10.2-3.8 Ma) on the northeast (Coello et al. 1992; Carracedo and Rodríguez-Badiola 1993). Shield stage volcanism was followed by a major gap in activity from 3.8 to 1.8 Ma, leading to extensive erosion of the old edifices, which may have already been connected by sand dunes (Coello et al. 1992; Carracedo and Rodríguez-Badiola 1993). Volcanic activity resumed about 1.6 Ma (post-erosional stage or rejuvenated volcanism) definitively connecting the two former islands about 0.8 Ma (Carracedo and Rodríguez-Badiola 1993), and it has persisted more or less continuously to the present.

The woodlouse-hunter spider genus *Dysdera* is remarkable among spiders in showing a wide range of variation in body size and mouthparts size and shape

(Deeleman-Reinhold and Deeleman 1988; Riddle et al. 2000; Arnedo et al. 2001), which has been attributed to both prey specialisation and capture strategy (Rezac et al. 2008). The genus has undergone a major diversification in the Canary Islands, where more than 40 endemics have been reported (Arnedo et al. 2001). Canarian *Dysdera* species occurring in sympatry often show morphological differences in body size and/or chelicerae shape (Arnedo et al. 2001) which has been used as evidence for the key role of prey segregation in shaping these communities (Arnedo et al. 2007). However, the species pair *Dysdera nesiotes* Simon, 1907 and *D. alegranzaensis* Wunderlich, 1991 challenge the former observations. They co-occur at most of their known localities on Lanzarote and the Chinijo archipelago, but hardly differ in either morphological, other than genitalia, or habitat preferences (Arnedo et al. 2000; Macías-Hernández et al. 2008).

The eastern Canaries are inhabited by two lineages of woodlouse-hunter spiders that colonized the archipelago independently. One of the lineages includes, along *D. nesiotes* and *D. alegranzaensis,* six additional species endemic to Fuerteventura (4), Lanzarote (1) and the Salvage Islands (1) (Macías-Hernández et al. 2010). The second lineage present in the eastern Canaries comprises the single species *Dysdera lancerotensis* Simon, 1907, which is morphologically divergent and has its closest relatives in northern Africa. Unlike the remaining eastern Canarian *Dysdera*, whose distributions are mostly restricted to higher elevation, humid slopes (and in one case to intertidal zone in pebbled beaches) on single islands, *D. lancerotensis* is found in a wider selection of habitat types, including xerophilous, sea-shore and anthropised habitats, distributed throughout Fuerteventura, Lanzarote and the islets (Arnedo et al. 2000). The phylogeographic and demographic patterns of *D. lancerotensis* have been the subject of a recent study, which highlighted the importance of volcanism in shaping the distribution of terrestrial organisms on the eastern Canaries (Bidegaray-Batista et al. 2007).

The species *D. nesiotes*, *D. alegranzaensis* and *D. lancerotensis* provide an interesting model to investigate similarities in the underlying phylogeographic patterns of co-occurring species with contrasting levels of relatedness and phenotypic similarity. In the present study we aim to reveal the phylogeographic patterns of *D. nesiotes* and *D. alegranzaensis*, and further compare them with those recovered for *D. lancerotensis*, to gain insights into the commonalities of such patterns. We hypothesize that co-occurring, phenotypically similar, close relatives, as exemplified by the species pair *D.*

alegranzaensis and *D. nesiotes*, will display similar phylogeographic patterns as a result of their similar responses to the same geological and environmental changes. Alternatively, contrasting phylogeographies in the two close relatives may hint at an overlooked niche partitioning, which would support the suggestion that ecological segregation may be driving species coexistence in Canarian *Dysdera* (Arnedo et al. 2007). Furthermore, comparisons of the phylogeographic patterns of the two close relatives with the co-occurring but morphologically and phylogenetically divergent *D. lancerotensis* will shed light on the existence of common biogeographic patterns in the eastern Canaries.

MATERIALS AND METHODS

Sampling

Specimens of *D. alegranzaensis* and *D. nesiotes* were collected from 20 localities across Lanzarote and its neighbouring islets (Fig. 1, and Appendix S1). Specimens of both species were collected together at most localities, except for La Graciosa and a few localities on Lanzarote, where *D. nesiotes* was not found. Samples were stored in absolute ethanol at -20ºC until DNA extractions were performed.

Specimens of all other *Dysdera* species reported in Fuerteventura and Lanzarote were also included in the analyses. Additional species from the western Canaries and from the continent were included as calibration points for divergence time estimates. All analyses were rooted using the continental species *D. scabricula* Simon, 1882 as outgroup (Macías-Hernández et al. 2008) (see Appendix S1).

DNA extractions, PCR amplifications and sequencing

Protocols for DNA extractions, amplifications and sequencing of the genes included in the study followed Bidegaray-Batista et al. (2007). Approximately 1100 bp of the mitochondrial gene cytocrome c oxidase subunit I (*cox1*) were amplified for all the specimens. Information on additional mitochondrial [16S rRNA (*16S*), tRNA leu UAG (*L1*) and NADH dehydrogenase subunit I (*nad1*)] and nuclear genes [28S rRNA (*28S*) and Histone H3 (*H3*)] was retrieved for a subsample of taxa to resolve phylogenetic relationships and lineage age estimations (see Macías-Hernández et al. 2008 for primer information). PCR products were cycle-sequenced in both directions using BigDye terminator version 3.1 (Applied Biosystems) and sequenced in an ABI 3700 automated sequencer at the Scientific and Technical Services of the University of Barcelona (http://www.sct.ub.es). The DNA sequences obtained were edited using the PREGAP and GAP4 programs included in the Staden Package (http://staden.sourceforge.net/) software and assembled and preliminary manual alignments built using BIOEDIT (Hall 1999).

Phylogenetic analyses

We performed maximum likelihood (ML) and Bayesian inference (BI) analyses to resolve the phylogenetic relationships among *D. alegranzaensis* and *D. nesiotes.* The matrix analysed included 37 specimens (six *D. nesiotes*, six *D. alegranzaensis* and 25 representatives of the remaining species, see Appendix S1) and concatenated sequence information for the mitochondrial genes *cox1, 16S, L1*, *nad1* and the nuclear genes *28S* and *H3*. Ribosomal gene sequences were aligned with the online version of the automatic alignment program MAFFT v. 5.8 (http://align.bmr.kyushu-u.ac.jp/mafft/online/server/), using the manual strategy option set to Q-INS-I with default options. Gaps were scored as presence/absence characters following Simmons & Ochoterena (Simmons and Ochoterena 2000) using the simple method as implemented in the program GAPCODER (Young and Healy 2003). Bayesian inference analyses were conducted with MrBayes v.3.1.2 (Ronquist and Huelsenbeck 2003) and run remotely at the Bioportal computer resources of the University of Oslo (http://www.bioportal.uio.no). Unlinked nucleotide substitution models selected by jMODELTEST were specified for each gene fragment, and a standard discrete model was implemented for the gaps scored as presence/absence data (Nylander et al. 2004). Substitution rates were allowed to vary independently between partitions. The analyses were run for 10 million generations. The temperature was decreased to 0.15 to facilitate the convergence of the chains. The program TRACER v. 1.4 (Rambaut and Drummond 2007) was used to ensure that the Markov chains had reached stationarity by examining the effective sample size (ESS) values and also to determine the correct number of generations to discard as a *burn-in* for the analysis (first 10%). Maximum likelihood analyses were conducted using the software RAxML v. 7.0.4 (Stamatakis 2006). Independent GTR+I+G substitution models were set for each data fragment. The best likelihood tree was selected from 10 iterations of random taxon addition. Non-parametric bootstrap support values were drawn from 100 resampled matrices, and confidence values were mapped onto the best topology.

Divergence time estimation

Divergence times and *cox1* substitution rates were estimated from the preferred topology obtained from previous analysis (after removal of two terminals with close to zero branch lengths) using the program BEAST v.1.4.8 (Drummond and Rambaut 2007), which implements an MCMC framework to infer time-measured phylogenies and to test for alternative molecular-clock models (Drummond et al. 2006). Unlinked evolutionary models retrieved by jMODELTEST were defined for each gene partition, and the prior on the node height distribution was set to follow a birth-death process.

Time constraints on selected nodes were defined based on available biogeographic information. The opening of the Strait of Gibraltar (5.3 Ma) was used as a fixed calibration point corresponding to the divergence between the Iberian *Dysdera inermis* and its Moroccan sister lineage (hereafter referred as *Dysdera cf. inermis*). The times of emergence of several Canary Islands provided hard maximum time bounds for additional nodes. The emergence of La Palma (2 Ma) (Carracedo and Day 2002) provided a maximum estimated age for the divergence between the lineages of *Dysdera calderensis* and *Dysdera silvatica* found on La Palma and La Gomera populations, and the age of El Hierro (1.2 Ma) (Carracedo and Day 2002) provided a maximum estimated age for the divergence between the lineages of *D. gomerensis* and *D. silvatica* found on El Hierro and La Gomera.

A preliminary tree that included the selected time constraints was calculated using the program R8S to ensure that the starting tree for the MCMC chains satisfied our calibration constraints. Analyses were run under various clock models (strict clock, relaxed uncorrelated lognormal and relaxed uncorrelated exponential), and the best model was selected based on Bayes Factors. Two independent runs of 50 million generations each were conducted for each analysis, sampling every 1000 generations and removing the first 10% of samples as burn-in. The results were visualised and convergence and mixing were assessed using TRACER.

Genetic diversity, population structure and demographic analyses

Population genetic analyses were conducted on *cox1* gene sequences obtained from 53 *D. alegranzaensis* and 43 *D. nesiotes* individuals. Diversity indices including nucleotide diversity (π_n) and haplotype diversity (h) were calculated using the software ARLEQUIN 3.01 (Excoffier et al. 2005). The F_{ST} sequence-based statistic and the analysis of molecular variance (AMOVA) (Excoffier et al. 1992) were used to assess population structure, as implemented in ARLEQUIN 3.01. Significance was computed by performing 10,000 permutations. We also calculated the *Snn* statistic (Hudson et al. 1992) implemented in DNAsp 5.0 (Librado and Rozas 2009) to assess overall genetic differentiation within each species. Populations represented by single sequenced individuals were excluded from estimations (localities Z, FA, PM, F and MA for *D. alegranzaensis*; see Table 1). The AMOVA analysis was conducted on different sets of populations defined on the basis of geographical and geological information (see Table 2 for grouping details). The grouping that maximised the variance among groups (Φ_{ct}), minimised the variance within groups (Φ_{sc}) and gave statistically significant Φ_{st}, Φ_{sc} and Φ_{ct} values was assumed to be the most plausible geographical subdivision.

Haplotype network

Haplotype networks were constructed using statistical parsimony (Templeton et al. 1992) with a confidence limit of 95% in the program TCS version 1.21 (Clement et al. 2000) and using median-joining methods (Bandelt et al. 1999) as implemented in the program NETWORK v.4.2.0.1 (www.fluxusengineering.com).

RESULTS

Phylogenetic analyses

Specimens and sequences analysed in the present study, with corresponding GenBank accession numbers, are listed in Appendix S1. Bayesian inference and maximum likelihood analyses converged on similar topologies (Fig. 2). They all agreed that (1) the populations of *D. alegranzaensis* and *D. nesiotes* form monophyletic groups and (2) the two species are not sister taxa, although both are part of a clade that includes all eastern Canary endemic *Dysdera* species except *D. lancerotensis*.

Lineage age estimates

Bayes Factors (BF) indicated that the relaxed lognormal clock model (ln -20025.023) provided a better fit to the data than the relaxed exponential model (ln -20028.677; BF=1.5) or the strict clock model (ln -20056.812; BF=13.8) with low and very strong support, respectively. The *cox1* substitution rate estimate was 0.03 per lineage/million years (My) (confidence interval 0.028-0.032). This value corresponds to a pairwise sequence divergence of 6.1% per My, about three times faster than the 2.3% standard mtDNA substitution rate obtained for arthropods (Brower 1994), but slower than the substitution rate of 0.051 per lineage/My (10% pairwise divergence) obtained for the eastern Canarian *Dysdera lancerotensis*. Divergence time estimates are shown in Fig. 2. *Dysdera alegranzaensis* split from its sister species about 2.7 Ma (1.6-3.9 Ma) and the time of the most recent common ancestor (TMRCA) of its extant haplotypes ~0.8 Ma (0.53-1.1 Ma). The time of split of *D. nesiotes* and its sister lineage was 2.6 Ma (1.1-2.4 Ma) and the TMRCA of its haplotypes was ~0.6 Ma (0.4-0.83 Ma).

Haplotype networks

Statistical parsimony and the median joining method produced the same *cox1* haplotype network for *D. nesiotes* (Fig. 3). Haplotypes from the same locality clustered together or were separated by few mutations, while many mutational steps separated haplotype clusters from different localities. The most frequent haplotype (10N) was the only one shared among localities (three localities on northern Lanzarote). According to coalescent theory (Crandall and Templeton 1993; Posada and Crandall 2001), 10N could be considered the most ancestral haplotype, suggesting that present day *D. nesiotes* haplotypes originated in northern Lanzarote.

Statistical parsimony yielded four independent haplotype networks separated by 18-23 mutational steps (Fig. 3) in *D. alegranzaensis*. This network differed from the median-joining network only in the positions of the Montaña Clara and La Graciosa haplotypes and the number of mutational steps and connection points of the four independent networks (see Fig. 3). The most frequent haplotype (21A) was shared among five localities distributed on southern and central Lanzarote. Other haplotypes were shared among localities within islets (e.g., 3A in Alegranza and 8A and 9A in La Graciosa). The remaining haplotypes were restricted to single localities. The statistical network 1 included two haplotypes from Lanzarote (Zonzamas) and Alegranza; network 2 included four haplotypes from Alegranza and one from La Graciosa; network 3 included four haplotypes from three localities on northern Lanzarote; and network 4 included 18 haplotypes from Lanzarote and the islets (except Alegranza). The complexity of the networks and the uncertainty of their connections precluded assigning the source of colonisation.

Genetic diversity, population structure and demographic analyses

Dysdera nesiotes and *D. alegranzaensis* showed high levels of haplotype diversity (0.9524±0.0198 and 0.9296± 0.0261, respectively) and low levels of nucleotide diversity

(0.012085±0.006151 and 0.021184±0.010506, respectively), although the latter values were almost twofold higher in *D. alegranzaensis* than in *D. nesiotes*.

Pairwise F_{ST} values between localities revealed contrasting patterns of gene flow in the two species. In *D. nesiotes* pairwise F_{ST} values among all populations were greater than 0.7, and most values were significant ($P > 0.05$, except M, MR and FO; see Table 1) indicating strong geographic structure in this species. In *D. alegranzaensis* significant values of F_{ST} were concentrated in comparisons involving the central-south part and north of Lanzarote and the northern islets. F_{ST} values revealed lesser and more localised population structure in *D. alegranzaensis* than in *D. nesiotes*. The *Snn* statistic also provided lower values for *D. alegranzaensis* (*Snn*= 0.397; $P < 0.05$) than *D. nesiotes* (*Snn*= 0.843; $P < 0.05$) (*Snn* values close to one suggest that populations are highly differentiated, while values around 0.5 indicate that localities belong to a panmitic population (Hudson et al. 1992)).

In all population groups defined for *D. nesiotes* the differences among populations within groups explained most of the genetic variance (52-73%), corroborating the high levels of population structure in this species (Table 2). A small percentage of genetic variance was allocated to intra-population variation (7.35-9.5%). The greatest variance among groups (40.23%) was observed when populations were grouped between the northern islets and Lanzarote (two groups). For the remaining groupings (four or five groups), variance was lower and not significant ($P<0.05$). In *D. alegranzaensis*, the variance among populations within groups was 28-37% (Table 2), much lower than the corresponding values for *D. nesiotes* (52-73%), while within population genetic variance (25-31%) was much higher than in *D. nesiotes* (7.35-9.5%) regardless of the grouping, indicating weaker population structure and higher population diversity in this species. The highest genetic variance observed was attributed to differences among groups (47%) when populations were divided between the northern islets and Lanzarote (two groups).

DISCUSSION

Contrasting phylogeographic patterns in co-distributed sibling species

The two species examined in the present study co-occur at most of their known localities across the island of Lanzarote and the northern islets, show no clear differences in life-history traits and are almost identical in somatic morphology (Arnedo et al. 2000; Macías-Hernández et al. 2008). Consequently, we hypothesised that they would have undergone similar historical processes (e.g., population extinction/isolation due to volcanic activity or changes in climatic and environmental conditions) and that they would therefore show similar phylogeographic and demographic patterns. However, our results reveal striking differences in phylogeography and population structure between the two species. *Dysdera nesiotes* display an almost metapopulation-like structure (Levins 1969; Hanski 1991). The pairwise-F_{ST} comparisons indicate high levels of population differentiation. Similarly, AMOVA reveals that most of the genetic variance could be explained by among population and within group comparisons, while differences within populations are small (7-9%). The haplotype network indicates high levels of philopatry, with most haplotype clades being exclusive to particular localities. *Dysdera alegranzaensis,* on the other hand, displays little population structure. The pairwise-F_{ST} values are low, and many of them are not significant. AMOVA results show generally high levels of genetic variance within populations; in two of the three groupings tested, most of the genetic variance corresponds to among group comparisons.

The haplotype network for *D. alegranzaensis* indicates weak geographical structure and greater genetic divergences among the main haplotype clades than those found for *D. nesiotes* (four independent networks versus one single network, respectively). These results are reflected by the TMRCA estimates, which are older for *D. alegranzaensis* haplotypes (~0.8 Ma, 0.53-1.1 Ma) than for *D. nesiotes* haplotypes (~0.6 Ma, 0.4-0.83 Ma), although their confidence intervals partially overlap. Overall, *D. alegranzaensis* shows evidence of more complex population dynamics, possibly including allopatric fragmentations (involving the northern islets), demographic expansions of local populations and high levels of gene flow among most populations.

Causes of incongruent phylogeographic and demographic patterns

Contrasting phylogeographic and demographic patterns in sympatric species have been reported in a variety of organisms. Such differences have been attributed to differences in dispersal abilities (Pizzo et al. 2006; Steele et al. 2009; Kuntner and Agnarsson 2011), habitat preferences (Hodges et al. 2007; Papadopoulou et al. 2009), responses to climatic changes (Taberlet et al. 1998; Sullivan et al. 2000), life history attributes (Zink 1996; Hewitt 1999), niche segregation (Beavis et al. 2011), prey specialisation (Adams and Rohlf 2000; Carmichael et al. 2001) and ecological strategies (e.g. generalist versus specialist) (Vandergast et al. 2004). None of these explanations seems to explain the differences between the two *Dysdera* species, which hardly differ in any of these features.

Like other ground-dwelling spiders, members of the family Dysderidae perform cursorial dispersal, a method that might restrict gene flow and generate population structure (Bond et al. 2001; Woodman et al. 2006). In spiders, larger bodies and greater leg lengths increase running performance (Foelix 1996; Moya-Laraño et al. 2008); thus, body size differences may result in different cursorial capabilities. There are no direct observations or experimental data on the cursorial capabilities of *Dysdera*, although the similarity in body size and leg length between *D. alegranzaensis* and *D. nesiotes* (Macías-Hernández et al. 2008) suggests that the two species have similar roving performance.

Life history traits such as sex-biased philopatry and specificity of reproductive timing may affect dispersal ability and ultimately patterns of phylogeographic subdivision (Avise 2004). For example, sex-biased dispersal with female philopatry has been proposed to explain strong phylogeographic structure in the absence of geographical barriers to dispersal in some reptilians (Thorpe and Richard 2001; Gübitz et al. 2005; Bloor et al. 2008). A comparison of collections of the two *Dysdera* species from 1995 to 2006 (285 adult specimens; 36 male and 75 female *D. nesiotes*, 24 male and 57 female *D. alegranzaensis* and 143 juveniles) failed to show any relevant difference between species in the distribution of the numbers of males and females throughout the year.

Differences in the timing and source of colonisation of the ancestral populations could also explain incongruent phylogeographic patterns. The two species differ in the location of their respective sister groups. While the sister species of *D. alegranzaensis* is also a Lanzarote endemism, the closest relatives of *D. nesiotes* are found in Fuerteventura. A biogeographic reconstruction suggests that the ancestors of the two species originated on different islands (Macías-Hernández et al. 2008): *D. alegranzaensis* was already a resident lineage on Lanzarote, while the ancestors of *D. nesiotes* colonised the island from Fuerteventura. The older TMRCA of the haplotypes and the presence of independent haplotype networks corroborate the longer residence of *D. alegranzaensis* on Lanzarote. The complex geological history of Lanzarote, which originated as two independent islands and endured several cycles of volcanic activity, could have facilitated the survival of both species on different parts of the island, where they would have evolved in isolation until a connection was established between the protoislands and volcanic activity decreased. Although our data suggest that *D. nesiotes* haplotypes probably originated in north Lanzarote and colonised the island southwards, they do not enable us to determine the ancestral source of *D. alegranzaensis* haplotypes.

As stated above, the TMRCA estimates for the present-day haplotypes of the two species are slightly different: *D. alegranzaensis* appears to be older than *D. nesiotes* (~0.8 and ~0.6, respectively) although their confidence intervals overlap. The difference in the TMRCA of the two species, however, is probably a consequence rather than a cause of their contrasting phylogeographic and demographic patterns. The younger age of *D. nesiotes* haplotypes could be the result of historically smaller population sizes and migration rates (Kingman 1982), as suggested by the high level of philopatry of its populations and the close relatedness of local haplotypes.

The lack of morphological differentiation between *D. nesiotes* and *D. alegranzaensis* (Macías-Hernández et al. 2008, 2010) is particularly striking given the pattern of size and mouthparts differentiation among co-occurring *Dysdera* species reported in the Canaries (Arnedo et al. 2001, 2007). More detailed ecological studies specifically aimed to investigate differences between the species in features such as microhabitat preferences, prey composition or life history traits will be conducted to gain a deeper insight on the factors promoting coexistence of close relatives with slight morphological differentiation.

Emerging phylogeographic patterns in the eastern Canary Islands

Recurrent connectivity among islands, dramatic climatic changes and episodic volcanism have provided ample opportunities for the generation of deep phylogeographic patterns in local species of the eastern Canaries. The availability of several studies on different species enables investigation of the commonalities of these patterns.

The population structure of *D. alegranzaensis* closely resembles that of the distantly related *D. lancerotensis*, although the TMRCA estimates for the mtDNA of *D. lancerotensis* is older than those found in *D. alegranzaensis*. Both species exhibit several independent mtDNA haplotype networks and show high levels of within population differentiation.

Shallow waters surround the northern islets and Lanzarote, and they were probably joined on several occasions during glacial periods (Fernández-Palacios 2011). Evidence for recent contact between *Purpuraria* grasshopper populations from

Lanzarote and Montaña Clara has been attributed to land bridge connections due to Pleistocene sea level oscillations (López et al. 2007). Despite the recurrent opportunities for gene flow, the three *Dysdera* species show high levels of genetic differentiation between the northern islets and Lanzarote. Clusters of related haplotypes exclusive to each of the northern islets, in some cases forming independent networks, are found in all three species. The relationships among populations on each islet, however, differ among the three species. Alegranza populations are mostly isolated, though there is evidence of secondary gene flow from north Lanzarote in *D. lancerotensis*. Alegranza was most likely the source of colonists for the extant populations of *D. lancerotensis* on La Graciosa, while populations of *D. alegranzaensis* seem to be an admixture from north Lanzarote and Alegranza. Present-day populations of *D. lancerotensis* and *D. alegranzaensis* from Montaña Clara probably originated in La Graciosa, while *D. nesiotes* haplotypes are more closely related to haplotypes from north Lanzarote. These results corroborate the evolutionary distinctiveness of the Chinijo Archipelago populations, emphasizing its relevance as wildlife refuge, while reflecting the idiosyncratic nature of inter-island colonisation.

The pervasive impact of recurrent volcanic activity on the phylogeographic and demographic patterns of island taxa has been well documented (Carson et al. 1990; Beheregaray et al. 2003; Vandergast et al. 2004). A pattern of population expansion during periods of volcanic quiescence following population extinction and fragmentation by lava flows has been identified in *D. lancerotensis* (Bidegaray-Batista et al. 2007) and the endemic lizard *Gallotia atlantica* (Bloor et al. 2008), although the inferred patterns for these species overlap neither in time nor in space. A star-like network around haplotype 21A in *D. alegranzaensis* may also be indicative of a recent demographic expansion. The fact that these expansions seem to coincide neither in time nor in space may indicate that these phenomena are frequent but easily erased by additional processes, such as the admixture of new migrants with surviving local populations, limiting detection of the pattern to a handful of nonrelated cases.

Of particular interest for the phylogeography of Lanzarote is the finding of an isolated, divergent *D. alegranzaensis* haplotype in the central-east region of the island (Zonzamas). A similar pattern has been found in *D. lancerotensis* and in the endemic lizard *Gallotia atlantica* (Bloor et al. 2008) and has been interpreted as evidence for the survival of local populations in small isolated refugia during Lanzarote's recent and subhistoric phase of volcanism (Carracedo and Rodríguez-Badiola 1993). These multiple lines of evidence confirm the relevance of the area around Zonzamas as a volcanic refugium. Further studies should be conducted to closely delimit the refugial area and assess its relevance across taxonomically and ecologically unrelated taxa.

In conclusion our study confirms that co-distributed, sibling species may exhibit contrasting phylogeographic patterns without somatic differentiation. Further ecological studies are required to demonstrate the existence of cryptic niche partitioning. In addition, our results corroborate earlier suggestions that, in spite of the ample opportunities for gene flow through land bridges induced by sea-level changes, the northern islets (particularly Alegranza) have remained mostly isolated and constitute an important reservoir of haplotype diversity. Similarly, localities on east-central Lanzarote (Zonzamas) seem to have acted as a refugium during volcanic eruptions. Our data also suggest instances of population expansion, probably related to population extirpation by lava flows, as shown for other Lanzarote taxa. Further studies on endemic taxa with limited vagility may offer more detailed insights on the impact of lava flows on demography and population structure and the limits and locations of additional volcanic refugia.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Summary of specimens and sequences included in the study.

TABLES

Table 1. F_{ST} values for mtDNA *cox1* of *D. nesiotes* and *D. alegranzaensis* based on the Tamura & Nei model. Population codes are given in Appendix S1. Values in bold represent significant comparisons ($P<0.05$). Localities represented by single individuals were excluded from the analysis.

Fst_D. nesiotes

	ML	**MC**	**CES**	**BH**	**M**	**MR**	**FO**	**MT**	**MB**	**VF**	**AF**
ML	0.0000										
MC	**0.9572***	0.0000									
CES	**0.9861***	**0.988***	0.0000								
BH	**0.8890***	**0.9056***	**0.9791***	0.0000							
M	0.9678	0.9727	1.0000	0.9411	0.0000						
MR	**0.9784***	**0.9804***	**1.0000***	**0.9643***	0.0000	0.0000					
FO	0.9678	**0.9727***	1.0000	0.9411	0.0000	0.0000	0.0000				
MT	**0.9177***	**0.9444***	**0.9092***	**0.9193***	**0.6771***	**0.7619***	**0.6771***	0.0000			
MB	**0.8077***	**0.8606***	**0.8136***	**0.7321***	0.3471	**0.5023***	0.3471	**0.7340***	0.0000		
VF	**0.9467***	**0.9507***	**0.9343***	**0.9245***	**0.8550***	**0.894***	**0.8550***	**0.8504***	**0.7260***	0.0000	
AF	**0.9340***	**0.9526***	**0.5991***	**0.9229***	0.8170	**0.8751***	**0.8170**	**0.8397***	**0.7262***	**0.8539***	0.0000

Fst _D. alegranzaensis

	ML	**MCA**	**MC**	**MBG**	**MM**	**BH**	**M**	**MR**	**BT**	**MT**	**MB**	**VF**	**AF**
ML	0.0000												
MCA	0.1246	0.0000											
MC	**0.7086***	**0.98147***	0.0000										
MBG	-0.2990	0.0647	**0.5519***	0.0000									
MM	0.6357	**0.97925***	**0.8551***	0.2751	0.0000								
BH	0.7580	**0.98016***	**0.9700***	0.6691	0.9658	0.0000							
M	0.6008	0.9837	**0.9757***	0.5384	0.9681	0.9510	0.0000						
MR	**0.3450***	**0.59171***	0.2619	0.2814	0.2194	0.3573	0.0021	0.0000					
BT	0.6413	0.9927	**0.9856***	0.5651	0.9824	0.9660	0.4008	0.0413	0.0000				
MT	**0.4857***	**0.7395***	**0.6323***	**0.4805***	**0.5753***	**0.6963***	0.0276	0.0330	0.0493	0.0000			
MB	**0.8063***	**0.99367***	**0.9883***	**0.8077***	**0.9879***	**0.9797***	0.6232	**0.3152***	-0.2903	**0.3184***	0.0000		
VF	**0.5903***	**0.84268***	**0.7724***	**0.5575***	**0.7464***	**0.7787***	-0.0174	0.0405	-0.0303	-0.1404	0.2483	0.0000	
AF	**0.7718***	**0.99511***	**0.9897***	0.7632	**0.9894***	**0.9795***	0.6625	0.2455	0.0000	0.2524	-0.0526	0.1829	0.0000

Table 2. AMOVA for mtDNA *cox1* of *D. nesiotes* and *D. alegranzaensis* based on pairwise nucleotide differences, showing significant tests (*P*< 0.05) after 10,000 permutations. F_{SC}, F_{ST} and F_{CT} are the *F*-statistics. (AL: Alegranza; MC: Montaña Clara; GR: La Graciosa; L: Lanzarote; LN: northern Lanzarote; LC: central Lanzarote; LS: southern Lanzarote).

AMOVA_*D. nesiotes*

Source of variation	df	Sum of squares	Variance components	% of variation	Fixation indices
AMOVA 2 Groups (AL+MC / L)					
Among groups	1	131.39	6.49460 Va	40.23*	*Fct:* 0.40233
Among populations within groups	9	301.138	8.46162 Vb	52.42*	*Fsc*: 0.87703
Within populations	32	37.965	1.18642 Vc	7.35*	*Fst*: 0.92650
Total	42	470.497	16.14264		
AMOVA 4 Groups (AL+MC / LN / LC / LS)					
Among groups	3	191.104	2.13841 Va	17.21	*Fct*: 0.17213
Among populations within groups	7	241.427	9.09842 Vb	73.24*	*Fsc*: 0.88464
Within populations	32	37.965	1.18642 Vc	9.55*	*Fst*: 0.90450
Total	42	470.497	12.42325		
AMOVA 5 Groups (AL/ MC / LN / LC / LS)					
Among groups	4	243.704	2.69397 Va	21.62	*Fct*: 0.21620
Among populations within groups	6	188.828	8.58018 Vb	68.86*	*Fsc*: 0.87852
Within populations	32	37.965	1.18642 Vc	9.52*	*Fst*: 0.90479
Total	42	470.497	12.46056		

AMOVA_*D. alegranzaensis*

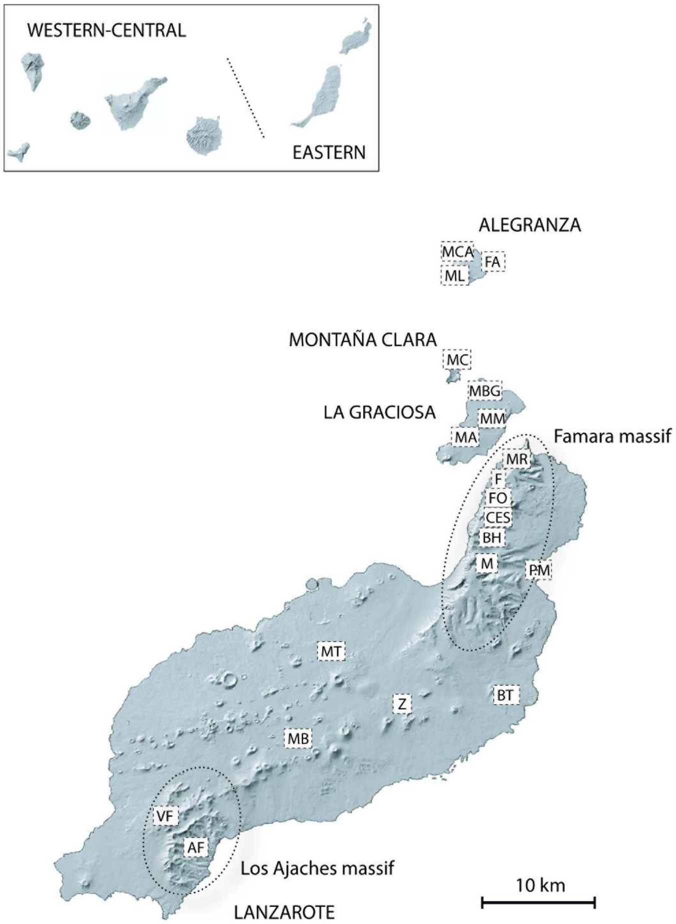
Source of variation	df	Sum of squares	Variance components	% of variation	Fixation indices
AMOVA 2 Groups (AL+MC+GR / L)					
Among groups	1	587.509	21.70167 Va	47*	*Fct*: 0.46997
Among populations within groups	16	774.244	12.93616 Vb	28.01*	*Fsc*: 0.52854
Within populations	35	403.872	11.53920 Vc	24.99*	*Fst*: 0.75011
Total	52	1765.624	46.17703		
AMOVA 4 Groups (AL+ MC+GR/ LN / LC / LS)					
Among groups	3	687.632	12.62614 Va	33.29*	*Fct*: 0.33290
Among populations within groups	14	674.12	13.76245 Vb	36.29*	*Fsc*: 0.54393
Within populations	35	403.872	11.53920 Vc	30.42*	*Fst*: 0.69576
Total	52	1765.624	37.92779		
AMOVA 6 Groups (AL/ MC /GR/ LN / LC / LS)					
Among groups	5	899.517	14.74483 Va	39.8*	*Fct*: 0.39801
Among populations within groups	12	462.236	10.76279 Vb	29.05*	*Fsc*: 0.48259
Within populations	35	403.872	11.53920 Vc	31.15*	*Fst*: 0.68852
Total	52	1765.624	37.04683		

FIGURE LEGENDS

Figure 1. Map of Lanzarote and the Chinijo Archipelago. Letters indicate the localities where specimens of *D. alegranzaensis* and *D. nesiotes* were collected. See Appendix S1 for locality codes.

Fig. 2. Chronogram obtained using the relaxed lognormal clock model of the concatenate matrix. Numbers on nodes are estimated lineage ages for *D. alegranzaensis* and *D. nesiotes*, and bars indicate 95% HPD intervals. Bars on branches denote levels of support for each clade with Maximum likelihood and Bayesian inference, respectively (Black= ML bootstraps>70% and PP>0.95; grey=clade recovered but ML bootstrap <70% and PP<0.95; white= clade not recovered). Black dotes on nodes are the fixed calibration point and white dots refers to maximum age constrains. The *x*-axis scale is in million years.

Fig. 3. Statistical parsimony and median-joining mtDNA haplotype network for *D. alegranzaensis* and *D. nesiotes.* Haplotype numbers as in Appendix S1, and small white circles indicate missing or extinct haplotypes. The size of each circle is proportional to the haplotype frequency. Squared haplotypes represent two alternative arrangements obtained with statistical parsimony and median-joining methods, respectively. Discontinuous lines indicate alternative network connections according to statistical parsimony (black lines) and median-joining (blue lines), or median-joining loops (red dots).



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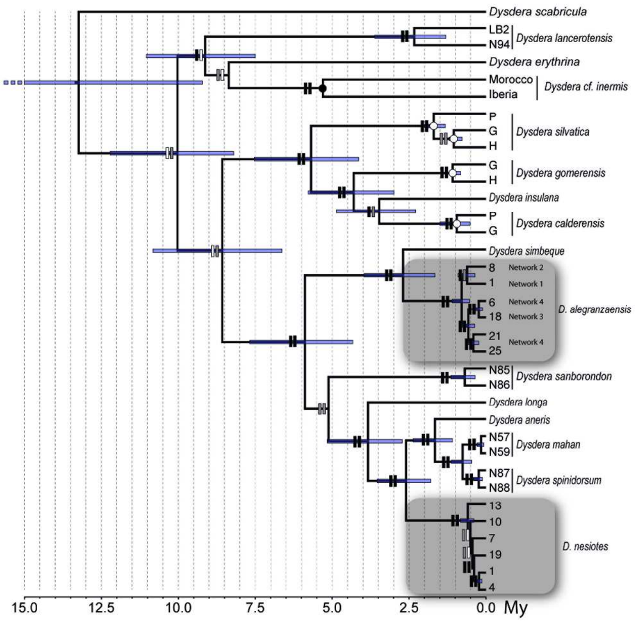

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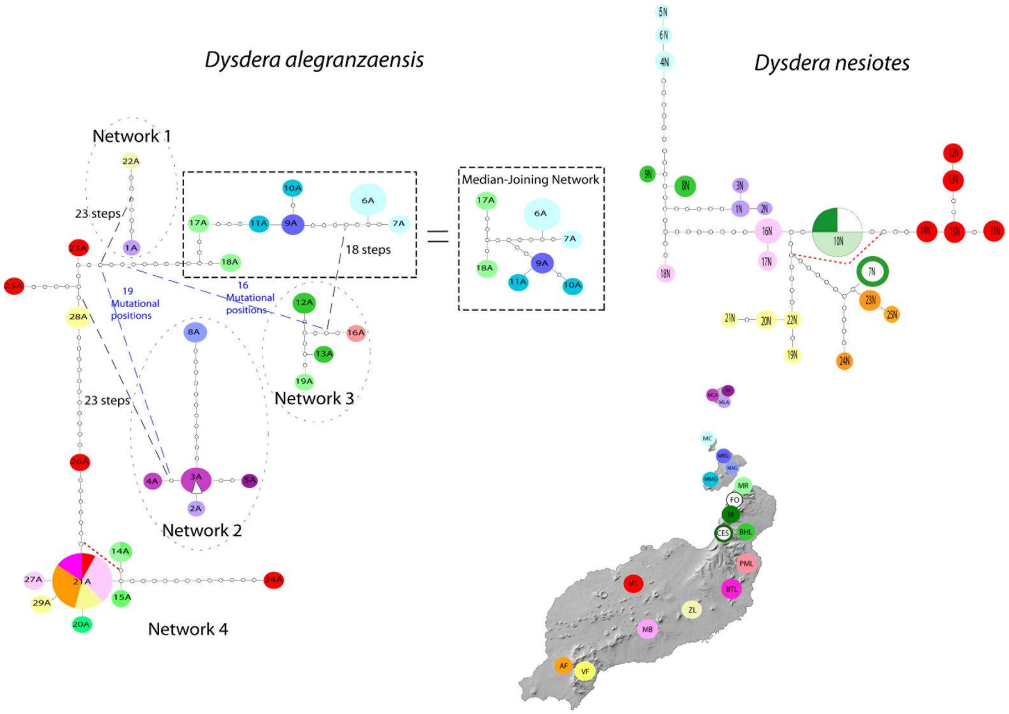

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