Parallel phenotypic evolution in a wolf spider radiation on Galápagos

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Within island archipelagos, repeated ecological settings may lead to radiations wherein similar niches are recurrently occupied. Although it has been shown that species with common habitat requirements share particular traits, it remains relatively unexplored to what extent this may lead to the repeated evolution of almost identical phenotypes (phenocopies) and how this correlates with traits subjected to sexual selection. Exploring divergence patterns of ecological and sexual relevant traits within spiders seem promising to enhance our understanding of the relative role of natural and sexual selection. Here, we conduct a detailed morphological analysis on a large set of genital and non-genital traits (morphometrics, colour pattern) within a radiation of the wolf spider genus Hogna Simon, 1885 on Galápagos and interpret these data, taking into account their known phylogenetic relationship. Our results show that recurrent environmental gradients have led to the parallel evolution of almost identical phenotypes, which not only proves that natural selection has driven morphological divergence, but also suggests that a similar genetic or developmental basis most likely underlies this divergence. Among-species variation in genital traits in contrast rather reflects the phylogenetic relationships on Santa Cruz and San Cristóbal. The combination of these data indicate that speciation in this system is driven by the combined effect of ecological mechanisms and allopatric divergence in sexual traits. © 2012 The Linnean Society of London, Biological Journal of the Linnean Society, 2012, 106, 123–136.


INTRODUCTION

Repeated ecological settings provide ample opportunities to investigate the mechanisms and processes behind population and species divergence. Such replicated environmental settings can be found on true island systems as the Hawaiian, the Caribbean and the Galápagos Islands that bear a great within-island habitat diversity and on ‘continental islands’ as isolated lakes and fragmented habitats (Howarth, 1990; Jackson, 1993; Gillespie & Roderick, 2002; Losos & Ricklefs, 2009). Several studies on species radiations within such island systems showed repeatedly that species inhabiting similar niches on different islands share particular phenotypic traits. Although this suggests species sorting mechanisms, whereby each ecotypic species repeatedly occupies the same niche through inter-island dispersal, species from the same island are often monophyletic. This indicates that similar environmental gradients on each island resulted in repeated and independent ecotypic differentiation. Classical examples of such island radiations include Anolis lizards in the Caribbean (Losos et al., 1998) and Tetragnatha spiders on the Hawaiian Islands (Gillespie, 2004, 2009).

Sharing of particular morphological traits among taxa might be explained by factors such as similar selection pressures (Rundle et al., 2000; Schluter, 2000) or phylogenetic relatedness (Kraft et al., 2007).
Hence, when similar phenotypic traits are found in more distantly related taxa that occupy similar niches, this provides one of the most stringent types of evidence that natural selection has driven divergence (Schluter, 1996; Losos et al., 1998). However, most studies concentrated on a relatively small set of clearly diverged traits and detailed morphological analyses that cover a large suite of different traits and, as such, describe almost the complete phenotype of species that evolved in parallel radiations, still remain relatively scant [but see (Glor et al., 2003; Langerhans, Knouft & Losos, 2006)]. Such studies might reveal some preliminary indications as to what extent non-independent processes underlie this divergence. Indeed, if species belonging to the same ecotype represent almost identical copies of each other, this rather suggests that similar developmental ecotype represent almost identical copies of each other, this rather suggests that similar developmental processes underlie this divergence of traits supposed to be under sexual selection.

Surprisingly, studies of repeated phenotypic evolution mostly do not consider the morphological evolution of traits supposed to be under sexual selection [but see (Salzburger, 2009)], although the differentiation of these traits can be of considerable importance in the first stages of speciation attributable to the evolution of isolating barriers between the incipient species (Hosken & Stockley, 2004). In general, genital traits are predicted to diverge between populations because of mechanisms that are not related to the environment, such as genetic drift and sexual selection (Futuyma, 2005). Indeed, sexual selection is considered to be the main driving force invoking rapid divergence of genital traits (Eberhard, 1993; Arnqvist, 1998; Hosken & Stockley, 2004), although sexual selection might also act upon genital traits without resulting in exuberant genital trait variation through stabilizing sexual selection (Eberhard et al., 1998; Ramos et al., 2005). Hence, we might expect the divergence pattern of genital traits to be uncorrelated with the environmental settings and rather to reflect phylogenetic relatedness. Their general congruence with phylogenetic relationships might furthermore be useful in order to reveal true phylogenetic relationships between species in recently diverged species complexes that are affected by introgression (Shaw, 2002) or when phylogenies based on morphological traits are incongruent with molecular phylogenies as a result of convergence (Yoshizawa & Johnson, 2006). However, sexual selection could also contribute to speciation as a consequence (‘by-product’) of divergent natural selection (Rundle et al., 2000; Schluter, 2000; Rundle & Nosil, 2005). In the latter case, genital traits may diverge in parallel with the ecological gradient.

Exploring spider radiations has broadened our insight in several topics ranging from island diversity (Arnedo, Oromi & Ribera, 2001; Hormiga, 2002), community assembly (Gillespie, 2004) and colour polymorphism (Croucher et al., 2011) to genital evolution (Eberhard, 2004, 2010; Ramos et al., 2005; Kuntner, Coddington & Schneider, 2009). Moreover, within spiders, the opportunity to explore both ecological relevant traits such as, for example, colour pattern (Gillespie, 2004) and web building behaviour (Blackledge & Gillespie, 2004), as well as sexual relevant traits, such as traits involved in the courtship (Miller et al., 1998; Masta & Maddison, 2002), might serve as a good model to enhance our understanding of the relative role of natural and sexual selection.

Here, we conduct a detailed morphometric study on a large set of non-genital (colour and biometrics) and secondary sexual (male palpal organ) phenotypic traits of the seven species of a radiated wolf spider genus *Hogna* Simon 1885 (*Lycosidae*) on Galápagos. These ground-dwelling spiders are distributed on the different main islands and can be subdivided into three ecological groups based on the ecological zone they are occupying (Fig. 1A) (Baert, Maelfait & Hendrickx, 2008). A first ecological group, further referred to as ‘high-elevation species’, occurs above 600 m altitude in the dense humid pampa vegetation dominated by ferns and grasses, and these spiders are generally dark brown coloured. This group comprises *H. galapagoensis* (Banks, 1902) on Santa Cruz, Santiago and Isabela (Sierra Negra and Alcedo), *H. jacquesbreti* Baert & Maelfait 2008 on Isabela (Sierra Negra and Cerro Azul) and *H. junco* Baert & Maelfait 2008 on San Cristóbal (Baert et al., 2008). A second ecological group, hereafter called ‘coastal dry species’, is confined to the dry supralittoral and arid zone along the coast in vegetated dunes and open shrubland. This group is represented by *H. snodgrassi* (Banks, 1902) on San Cristóbal, *H. espanola* Baert & Maelfait 2008 on Española and *H. hendrickxi* Baert & Maelfait 2008 on Santa Cruz (Baert et al., 2008). They are in general characterized by a pale median and sublateral carapace band. High-elevation species are active both during day and night while, in contrast, coastal dry species hide during daytime and leave their retreat at sunset (C. De Busschere, pers. observ.). The third ecological group consists of all populations of the widely distributed species *H. albemarlensis* (Banks, 1902) that mainly occurs in saline habitats along the coast (salt marshes, bays) and in permanent wetlands below 600 m of altitude. In a previous molecular phylogenetic study (De Busschere et al., 2010), we demonstrated that at least the species *H. espanola*, *H. snodgrassi*, *H. junco*, *H. galapagoensis* and *H. hendrickxi* radiated from a single ancestor on Galápagos (Fig. 1B). Furthermore, within-island habitat specialization was demonstrated for *H. snodgrassi* and *H. junco* on San Cristóbal and for *H. galapagoensis*
Figure 1. Geographical distribution (A) and phylogenetic relationship (B) based on mitochondrial COI (~650 bp) and 28S rDNA (~800 bp) of all *Hogna* wolf spiders on the Galápagos (De Busschere et al., 2010) (with node numbers representing posterior probabilities). Panorama of the habitat of *H. galapagoensis* (C) and *H. hendrickxi* (D) on Santa Cruz and of *H. junco* (E) and *H. snodgrassi* (F) on San Cristóbal.
and *H. hendrickxi* on Santa Cruz. For the latter species pair, it was even demonstrated that habitat specialization occurred under low levels of gene flow (De Busschere et al., 2010).

Based on our measurements, we here investigate (1) to what extent habitat specialization results in identical phenotypic changes in a larger suit of morphological traits and (2) if non-genital and genital traits show a distinct divergence pattern, as expected from differential selection modes.

Our data demonstrate that phenotypic variation in non-genital traits is strikingly identical in similar habitats, even between distantly related species. In contrast, morphology of genital traits is less congruent with the ecotypic divergence.

**MATERIAL AND METHODS**

**SAMPLING**

The currently known lycosid fauna of the Galápagos is only represented by members of the genus *Hogna*. Based upon Baert et al. (2008), male and female *Hogna* spiders have, on average, a total body length of 11.32 ± 2 and 14 ± 1.6 mm, respectively. Adult specimens of all known species of the genus *Hogna* from Galápagos were sampled on field trips conducted between 1996 and 2010. Specimens were captured and photographed alive and stored directly in 97% ethanol. All specimens are deposited at the collection of the Entomology Department of the Royal Belgian Institute of Natural Sciences (Brussels, Belgium).

High-elevation specimens were obtained from two populations of *H. galapagoensis*, one on Santa Cruz and one on Isabela (Volcan Alcedo); one population of *H. junco* on San Cristóbal (Volcan El Junco) and two populations of *H. jacquesbreli* on Isabela (Volcan Sierra Negra and Volcan Cerro Azul). Coastal dry specimens were sampled from the only known population of *H. hendrickxi* on Santa Cruz (Las Palmas); two populations of *H. snodgrassi* on San Cristóbal (Punta Bassa and Puerto Grande) and one population of *H. espanola* on Espanola (Bahia Gardner). Three populations of *H. albemarleinsis* were sampled, namely on Isabela (Volcan Cerro Azul), Fernandina (Cabo Hammond) and on Santiago (coast).

**COLOUR ANALYSIS**

Two colour images of the dorsal side of living spiders were taken under standardized conditions using a Canon EOS 1000D digital camera with a 50-mm lens and extension tube. Photographs were taken in a small transparent plastic jar on a white background when the spider was situated motionless in the centre of the jar. Light intensity was standardized by using artificial light (flash mode), constant aperture (F5.6) and shutter speed (1/125 s). On these pictures we quantified the colour pattern by measuring the mean value of the colours red (R), green (G) and blue (B) separately on predefined sections of 21 × 21 pixels on the dorsal side of the carapace and abdomen (Fig. 2). We also obtained an average colour estimate for the total carapace and abdomen (Fig. 2). Colour values were obtained using ImageJ software (Abramoff, Magelhaes & Ram, 2004). The sections were chosen such that they described the observed colour variation as much as possible (Fig. 2, and see also Supporting Information, Table S1). The average RGB background values of eight background sections (bg1-bg8) were used to measure and correct for the overall light intensity of the picture. RGB values were scored for each section for each specimen on two independent pictures. Next, we calculated values per section per specimen that correct for differences in background light intensity. These were obtained by first estimating the common slope of the linear relationship between the two RGB trait and two average RGB background values across all specimens. Then, we calculated the corrected RGB values, i.e. the least square mean (lsmeans), for the average background intensity for each individual. The mean value of a trait was calculated by averaging the lsmeans for the lateral bands (LB = mean of LL_1, LL_2, LR_1 and LR_2); the carapace bands (CB = mean of CL_a, CL_m, CL_p, CR_a, CR_m and CR_p), the anterior and posterior region around the heart spot (AH_a = mean of AH_1, AH_2 and AHp = AH_3 and AH_4), the heart spot (HA = means of H_a, H_m and H_p) and the posterior part of the abdomen (Po = mean of P_l, P_m and P_r). These values were subjected to a principal component analysis (PCA) with 33 traits, eleven for each colour (LB, CB, MB_a, MB_m, MB_p, AH_a, AHp, HA, Po, Car and Abd). With this procedure we could obtain pictures taken from 67 females and 25 males (Table 1; sample size: colour). To visualize the colour variation, mean corrected colour values were used to create a schematic representation for each species in the ordinations. To illustrate the contribution of each trait to the first and second PC axis, the eigenvectors, averaged over the three colours, were plotted on the PCA ordinations.

**BIOMETRICS OF NON-GENITAL TRAITS**

After obtaining photographs, adult individuals were stored in ethanol and measured using an Olympus, type SZX7 stereo microscope with a magnification range of 8×–56× (1×10× objective/ocular) (Table 1; sample size: biometrics). The ocular was provided with an ocular micrometer and the maximal precision of length measurements was 0.018 mm. Length...
**Figure 2.** Schematic representation of quantified sections in colour analysis (A) and landmarks describing shape of terminal apophysis (B) and tip of embolus (C) (for abbreviations see Supporting Information, Table S1).

**Table 1.** Number of *Hogna* individuals used in each analysis with population and species information

<table>
<thead>
<tr>
<th>Species</th>
<th>Habitat</th>
<th>Locality</th>
<th>Island</th>
<th>Sample size</th>
<th>Non-genital</th>
<th>Genital</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Colour</td>
<td>Biometrics</td>
</tr>
<tr>
<td><em>H. albemarlensis</em></td>
<td>Humid grassland</td>
<td>Cerro Azul</td>
<td>Isabela</td>
<td>5 7</td>
<td>M</td>
<td>20</td>
</tr>
<tr>
<td><em>H. albemarlensis</em></td>
<td>Salt</td>
<td>Cabo Hammond</td>
<td>Fernandina</td>
<td>– – 20</td>
<td>M</td>
<td>–</td>
</tr>
<tr>
<td><em>H. albemarlensis</em></td>
<td>Salt</td>
<td>Coast</td>
<td>Santiago</td>
<td>– – 20</td>
<td>M</td>
<td>–</td>
</tr>
<tr>
<td><em>H. snodgrassi</em></td>
<td>Coast</td>
<td>Punta bassa</td>
<td>San Cristóbal</td>
<td>5 10 20 17</td>
<td>M</td>
<td>–</td>
</tr>
<tr>
<td><em>H. snodgrassi</em></td>
<td>Coast</td>
<td>Puerto Grande</td>
<td>San Cristóbal</td>
<td>– – 11 14</td>
<td>M</td>
<td>–</td>
</tr>
<tr>
<td><em>H. hendrickxi</em></td>
<td>Coast</td>
<td>Las palmas</td>
<td>Santa Cruz</td>
<td>2 10 10 14</td>
<td>M</td>
<td>–</td>
</tr>
<tr>
<td><em>H. galapagoensis</em></td>
<td>Pampa</td>
<td>Media Luna to Cerro Crocker</td>
<td>Santa Cruz</td>
<td>5 10 20 20</td>
<td>M</td>
<td>–</td>
</tr>
<tr>
<td><em>H. galapagoensis</em></td>
<td>Pampa</td>
<td>Alcedo</td>
<td>Isabela</td>
<td>– – 7</td>
<td>M</td>
<td>–</td>
</tr>
<tr>
<td><em>H. junco</em></td>
<td>Pampa</td>
<td>El junco</td>
<td>San Cristóbal</td>
<td>3 10 20 20</td>
<td>M</td>
<td>–</td>
</tr>
<tr>
<td><em>H. espanola</em></td>
<td>Coast</td>
<td>Bahia Gardner</td>
<td>Española</td>
<td>5 10 13 20</td>
<td>M</td>
<td>–</td>
</tr>
<tr>
<td><em>H. jacquesbreli</em></td>
<td>Pampa</td>
<td>Sierra Negra</td>
<td>Isabela</td>
<td>– 10 19</td>
<td>M</td>
<td>–</td>
</tr>
<tr>
<td><em>H. jacquesbreli</em></td>
<td>Pampa</td>
<td>Cerro Azul</td>
<td>Isabela</td>
<td>– – 3 20</td>
<td>M</td>
<td>–</td>
</tr>
</tbody>
</table>

TA, terminal apophysis; Emb, embolus; M, male; F, female.

measurements were obtained for 29 morphological traits (for a complete list, see Supporting Information, Table S2). In short, these traits covered measurements of leg segments, width of posterior eyes, carapace, sternum and chelicerae. In order to investigate the repeatability of the measured traits, we performed a repeatability study in which all traits were measured twice for a data set in which each species was represented by three individuals (in total 21 individuals). As an index of repeatability, an intraclass correlation coefficient (ICC) was computed with a generalized linear mixed model (PROC MIXED SAS System, ver. 9.1, 2002–2003; SAS Institute, Cary, NC, USA). Traits with ICC > 0.75 are commonly interpreted as good-to-excellent repeatable traits (Fleiss, 1986). Principal component analyses were performed to describe the biometric variation between the species and the ecotypes.

**SHAPE ANALYSIS OF GENITAL TRAITS**

The terminal apophysis and the embolus are both male genital structures involved in the copulation process of spiders (Foelix, 1996). The former is supposed to have an anchoring function during copulation and the latter serves as a conductor for sperm transfer. Respectively, 59 and 58 scanning electron microscopy (SEM) images were taken from the terminal apophysis and embolus of male specimens and processed using ImageJ software (Abramoff et al., 2004) (Table 1; sample size: genital). Subsequently, a total of, respectively, 13 and six landmarks were collected with TpsDig software. The position of landmarks was chosen such that they describe the variation in the distal tip of the terminal apophysis and of the embolus in a repeatable manner (see Fig. 2B, C). The obtained landmark configurations were aligned using the Generalized Procrustes Analysis and further analysed using the MorphoJ program (Klingenberg, 2011). Sample sizes ranged from seven to ten individuals per species. Shape differences among species were visualized and investigated by principal component analyses.

**COMPARISON OF DIVERGENCE PATTERNS**

The multiple divergences into high-elevation and coastal dry species allow investigation of divergence patterns of phenotypic traits. Our main aim was to investigate the degree to which different selection modes are reflected in the divergence patterns of non-genital and genital traits. Two approaches were assessed: conventional statistic analyses and phylogenetically independent contrast analyses (PIC), which correct for the effect of phylogeny. In the first analysis, the amount of shared and species-specific morphological variations were compared for non-genital male traits and male genital traits for all high elevation and coastal dry species. The non-genital male trait data sets consist of the first PC axis describing colour variation and of the first three PC axes describing biometric variation in non-genital traits. The male genital data set consists of the first two PC axes describing the shape variation in the terminal apophysis and embolus. The explanatory strength of the different variables was investigated by means of a mixed linear model with habitat (coastal vs. high elevation) as a fixed effect and species, nested within habitat as random effect (PROC MIXED, SAS ver. 9.1). The ratio of the variation among species from the same habitat class to the total phenotypic variation within habitats is expressed as the intraclass correlation coefficient (ICC: \( \rho = \sigma^2_{\text{spec}}/\sigma^2_{\text{tot}} \)), and its significance was tested by means of a likelihood ratio test. Traits that are under the influence of natural selection are expected to have a significant effect for the factor habitat, meaning that individuals occurring in the same habitat are morphologically more similar than individuals of the same species. If ICC is significant, then morphological variation might reflect unique evolutionary history. In an additional analysis, only the species pairs from San Cristóbal (SCB) and Santa Cruz (SCZ) were considered to compare divergence patterns, taking into account the phylogenetic relationship. This setting allows exploration of the degree to which traits are influenced by shared selection history (i.e. habitat) or shared ancestry (i.e. island). Similarly, the effects of habitat and island and their interaction were compared for the non-genital and genital traits. The explanatory strength and relative importance of these factors was studied by means of multivariate ANOVA (MANOVA; PROC GLM, SAS ver. 9.1) and is expressed by using the multivariate test statistic Hotelling-Lawley’s trace (Johnson & Wichern, 2002; Langerhans et al., 2006).

Within a previous study (De Busschere et al., 2010), phylogenetic relationships among all Hognia species on Galápagos were analysed based on two molecular markers, allowing us now to take into account the effect of interspecific genetic relatedness. Hence, in the second approach, phylogenetically independent contrasts (PIC) (Felsenstein, 1985; Garland, Harvey & Ives, 1992) were generated based upon the species mean values along the main PC axes for all high-elevation and coastal dry species by means of the PDAP module in the computer program Mesquite (Midford, Garland & Maddison, 2002; Maddison & Maddison, 2004). Habitat occupancy was scored as a categorical variable with ‘0’ for high-elevation habitat and ‘1’ for coastal dry habitat. Branch lengths were obtained from the majority rule consensus Bayesian
The latter tree was generated based upon a concatenated data set of a mitochondrial marker and a nuclear marker (respectively, COI and 28S rDNA) by implementing a general time reversible substitution model, allowing a proportion of invariant sites for the 28S fragment (GTR+I) and additionally allowing a gamma distributed variation in substitution rate among sites (GTR+I+G) for the COI fragment. Branch lengths were expressed in units of mutations per site and were exponentially transformed to meet the assumptions of no significant trend between the absolute values of standardized contrast and their standard deviations. Correlation coefficients between the phenotypic variables and habitat occupancy were investigated.

**RESULTS**

**COLOUR PATTERNS**

All eigenvectors along the first PC axis, which explained 71% of the total variation in female individuals, showed positive scores ranging from 0.045 to 0.198 (Fig. 3, see also Supporting Information, Table S3). Except for the carapace bands (CB), all eigenvectors pointed into the same direction parallel with this first PC axis (Fig. 3), with the highest eigenvector values along PC1 corresponding to colours of the median part of the median band (MBm), the abdomen (Abd) and the lateral bands (LB) (eigenvector values >0.192). Along PC1, Th e eigenvector values of the RGB colours of the carapace bands (CB) were considerably less, ranging from 0.045 to 0.067. Hence, the first PC axis reflects the variation in average darkness of the species, with the exception of variation in carapace bands. Along this axis, high elevation species were associated with negative values, while coastal dry species had positive scores (Fig. 3). Along the second PC axis, individuals of *H. albemarlensis* have paler carapace bands than the other species. In comparison, the PCA ordination for the male individuals depicted a similar interspecific pattern compared with the ordination of the females, with the first PC axis representing a gradient of overall darkness of the species (see also Supporting Information, Fig. S1). In sum, with the exception of the carapace bands, all measured traits responded in a similar direction along a gradient of average darkness, with high-elevation species being considerably darker and almost perfectly separated from the lighter coastal dry species.

**BIOMETRICS OF NON-GENITAL TRAITS**

Twenty-nine and 28 non-genital traits were measured for, respectively, 156 male and 208 female specimens.
In general, these traits could be measured with high accuracy as shown by the high ICC values (\(> 0.75\)) (see also Supporting Information, Table S3). For males and females separately, PCAs were performed to describe the biometric variation of non-genital traits revealing similar interspecific variation (Fig. 4; see also Supporting Information, Table S4). For both data sets, the first three PC axes described more than 90% of the total variation in morphology (see also Supporting Information, Table S4). PC1 explained 86% of the total variance in male and female morphology and was characterized by positive eigenvectors for all traits. Hence, we interpreted PC1 as a body size gradient. Based on the eigenvalues, PC2 was interpreted as a shape variable composed of several traits for the male (2%) and female (5%) data set.

Along these axes the variation between high-elevation species and coastal dry species primarily reflects differences in overall body size (PC1), eyes (PlEye, PMeye, and AMeye) and width of the tibia of the first leg (leg1ti_W) and in length of the chelicerae (CheL) (Fig. 4). However, shape differences appeared not to be independent from size differences within each species. In particular, if individuals with an identical body size are compared, coastal dry species tend to have larger eyes than high-elevation species. Hence, the difference in eye size is attributable to a different allometric relationship between coastal and highland. In sum, high-elevation species tend to be smaller and have relatively smaller eyes and stouter legs compared with the coastal dry species, with the exception of some female individuals of \(H.\) jacquesbreli. Although \(H.\) albemarlensis clusters primarily as a distinct group, it resembles more the high-elevation species.

SHAPE ANALYSIS OF MALE GENITAL TRAITS

Interspecific shape differences in two genital traits (terminal apophysis and embolus) were studied by means of a landmark-based approach and are visualized in Figure 5A and B, respectively. The main shape change for the terminal apophysis is a gradual change from a needle to a more knife-blade-shaped tip of the terminal apophysis along PC1 (73.2%). Large values along the second PC axis (12.54%) are associated with a needle-shaped terminal apophysis, while low values are associated with a broader tip and a slender base of the terminal apophysis. Although there was considerable overlap between some species, the majority of the species showed a fairly distinct shape of this genital trait. In particular, the two San Cristóbal species \(H.\) junco and \(H.\) snodgrassi clearly differed in the shape of the terminal apophysis. This contrasted strongly with \(H.\) hendrickxi and \(H.\) galapagoensis on Santa Cruz, which cannot be delineated based upon the terminal apophysis, which is for both species knife blade shaped.

For the embolus, the first PC axis explained 51.32% of the total variance. Along this first PC axis, species with higher values have a wider tip of
the embolus, for example *H. snodgrassi*, and species with lower values have a slender tip of the embolus, for example *H. albemarlensis*. The second PC axis (28.23%) separates *H. junco* and *H. jacquesbreli* from the other species based upon a more curved tip of the embolus.

**Comparison of Divergence Patterns**

Habitat appeared to have a significant effect on non-genital morphological traits, i.e. colour variation and first PC axis describing the general morphology, but not on variation in genital traits (Table 2). Moreover, colour variation among species within each habitat category appeared to be not significantly higher, as expected from the residual variation within each species. This indicates that the individual colour variation within each species is of comparable magnitude among species within habitat types. For the other traits, significant species-specific morphological variation was present. In sum, male individuals occupying an identical habitat are similar in overall body size and colour pattern and do not share similarities in shape variation of genital traits (Table 2). Multivariate ANOVAs on the restricted set of species from San Cristóbal and Santa Cruz revealed similar results, with habitat type explaining most variation among these four species for colour and general mor-

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**Table 2.** Results of mixed-model analyses on male phenotypic variation in colour, biometric and genital trait variation among high-elevation and coastal dry species

<table>
<thead>
<tr>
<th>Variable</th>
<th>Non-genital</th>
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<th>Genital</th>
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</thead>
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<tr>
<td></td>
<td>Colour</td>
<td>Biometrics</td>
<td>Terminal apophysis</td>
<td>Embolus</td>
</tr>
<tr>
<td></td>
<td>PC1</td>
<td>PC1</td>
<td>PC2</td>
<td>PC3</td>
</tr>
<tr>
<td>Eigenvalue</td>
<td>67%</td>
<td>84%</td>
<td>3%</td>
<td>3%</td>
</tr>
<tr>
<td>Fixed factor</td>
<td>Habitat (P)</td>
<td>0.005*</td>
<td>0.023*</td>
<td>0.58</td>
</tr>
<tr>
<td>Random factor</td>
<td>Species (ρ)</td>
<td>31%</td>
<td>48%**</td>
<td>73%**</td>
</tr>
</tbody>
</table>

**PC**: principal component.

**Figure 5.** Shape differences in two male genital structures: (A) terminal apophysis and (B) embolus with high-elevation species depicted in black [*H. junco* (circles); *H. galapagoensis* (inverted triangles); *H. jacquesbreli* (upright triangles)] and low-elevation species depicted with open symbols [*H. snodgrassi* (circles); *H. hendrickxi* (inverted triangles); *H. espanola* (diamonds)] and *H. albemarlensis* (grey square).
Phenological traits (Table 3, Fig. 6). For genital traits, in contrast, shared ancestry appeared to be the most important factor, as indicated by the high significance of the island effect (Table 3, Fig. 6). Hence, closely related species share more similarities in genital traits, while species residing within the same habitat share a similar colour pattern and general morphology. However, with the exception of colour variation, all different trait sets showed an significant, although smaller, interaction between both factors, which indicates that the within-island differences between high-elevation and coastal dry species are different on Santa Cruz than on San Cristóbal. This is reflected in Figure 6D, where species on Santa Cruz are approximately similar, while species on San Cristóbal are strongly divergent. When accounting explicitly for the genetic relationship among species by means of phylogenetic independent contrasts (PIC), correlation coefficients between phenotypic traits and habitat occupancy were significant for colour variation and general morphology (PC1 and PC2), and marginally significant for the first PC axis describing shape.

Table 3. Hotelling–Lawley trace values indicate different main factors to explain the variation in genital and non-genital male traits

<table>
<thead>
<tr>
<th>Factor</th>
<th>Non-genital</th>
<th>Genital</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Colour</td>
<td>Biometrics</td>
</tr>
<tr>
<td>Island</td>
<td>3.24 (d.f. = 1) NS</td>
<td>16.26 (d.f. = 29)**</td>
</tr>
<tr>
<td>Habitat</td>
<td>19.43 (d.f. = 1)*</td>
<td>29.59 (d.f. = 29)**</td>
</tr>
<tr>
<td>Island × habitat</td>
<td>4.57 (d.f. = 1) NS</td>
<td>9.29 (d.f. = 29)**</td>
</tr>
</tbody>
</table>

**P < 0.0001; *0.0001 < P < 0.05.
d.f., degrees of freedom; NS, non-significant.

Figure 6. Comparison of non-genital (A and B) and genital (C and D) divergence patterns between species radiated on San Cristóbal (circles) and Santa Cruz (triangles) by visualizing species means of high-elevation species [H. junco (black circles); H. galapagoensis (black inverted triangles)] and coastal dry species [H. snodgrassi (white circles); H. hendrickxi (white inverted triangles)].
variation in the terminal apophysis (Table 4). With the exception of the terminal apophysis, these findings are congruent with the above-mentioned results.

DISCUSSION

REPEATED EVOLUTION OF PHENOCOPIES

Our results show that the major component of phenotypic variation of non-genital traits (colour and biometrics) is associated with the ecotypic differentiation among the species. Remarkably, although an extensive number of distinct morphological traits were measured (e.g. colour patterns of different parts of the dorsal side, measurements of eyes, legs, chelicerae, etc.), species occupying the same habitat are phenotypically highly similar. In particular, males of the different high-elevation species (H. jacquesbreli, H. junco and H. galapagoensis) appeared to be virtually indistinguishable in colour pattern and general morphology despite their small genetic relatedness. In contrast, divergence of genital traits was inconsistent with this ecotypic differentiation and primarily reflected the ancestral history of the species on Santa Cruz and San Cristóbal. The observation of identical phenotypes in similar niches strongly indicates that natural selection and adaptation to similar environmental conditions is the main evolutionary force generating this divergence pattern (Rundle et al., 2000; Losos, 2009). Nevertheless, during the last decade there is an increasing number of studies that demonstrate and stress that parallel morphological evolution is often based on similar and often relatively simple developmental or genetic mechanisms (Colosimo et al., 2005; West-Eberhard, 2005; Arendt & Reznick, 2007; Elmer & Meyer, 2011). Hence, given that the divergence in this study apparently results in the recurrence of almost identical phenotypes ['phenocopies' sensu (West-Eberhard, 2003, 2005)], this suggests that the divergence could potentially be facilitated by similar genetic and developmental pathways underlying the trait variation (Arendt & Reznick, 2007).

Indeed, results of the PC analysis of morphological variation showed that particular shape differences, such as relative eye size and leg width, are differentially influenced by an increase in body size. Thus, within both ecotypes, larger individuals have relatively smaller eyes, stouter legs and larger chelicerae. The same set of shape variables distinguishes high-elevation species from coastal dry species when individuals of a given body size are compared. Hence, high-elevation species are characterized by relative smaller eye size, stouter legs and larger chelicerae than coastal dry species. Therefore, the shape variability with respect to body size within a particular ecotype is also reflected in the shape differences between ecotypes, suggesting that similar developmental mechanisms underlie the divergence between high-elevation and coastal dry species. Indeed, as proposed by Emlen & Allen (2004), evolutionary changes in the relationship between adult trait size and body size might result of changes in conserved developmental pathways. Besides this main habitat effect, a significant effect of shared ancestry could still be detected for non-genital biometric traits (Table 3).

Similarly, with the exception of the carapace bands, nearly all colour variation observed within and between species followed a similar gradient from pale to dark. More precisely, all coastal dry species were characterized by a distinct pale dorsal median and lateral carapax band and abdominal colour pattern. Hogna albemarlensis, the only species inhabiting a broader range of habitats, such as salt marshes and humid grasslands, clusters primarily as a distinct group. Until now, little is known of the developmental/genetic pathways controlling coloration in spiders (Oxford & Gillespie, 1998). Coloration in wolf spiders is presumably achieved by pigmentation and hairs. Based upon our current knowledge, we can only hypothesize how these traits are linked to the habitat. The most plausible explanations are camouflage against avian predation and better prey discrimination (eye size) for the nocturnal coastal dry species (Rovner,
1993; Dacke, Doan & O’Carroll, 2001). Although not explicitly studied in the current paper, ecotypic differentiation is also reflected in the species behaviour. Coastal dry species are strictly nocturnal and, under laboratory conditions, they often showed a subterranean behaviour by digging and retreating in holes. In contrast, high-elevation species are active during day and night (C. De Busschere, pers. observ.).

**Genital traits support within-island speciation**

Divergence of genital traits involved in the copulation process could directly contribute to speciation as a pre-zygotic reproductive barrier. For spiders and many arthropods in general, the genitalia are often used to delineate species boundaries (Foelix, 1996) and as parameters to measure the role of sexual selection (Eberhard, 1993). In this study, we compared to what extent genitalia and non-genital traits could be subjected to different modes of selection.

A comparison of the morphological variation of the parallel species pairs from Santa Cruz and San Cristóbal revealed different divergence patterns in genital compared with non-genital traits of the closely related high-elevation and coastal dry species on Santa Cruz and San Cristóbal, indicating that these phenotypic traits are apparently differentially influenced during divergence. Sister species (within island) have similar genital traits, which corroborates the hypothesis that they rather reflect shared ancestry patterns. Taking into account the estimated divergence times (De Busschere et al., 2010), it is rather surprising to observe almost identical genitalia for *H. galapagoensis* and *H. hendrickxi* on Santa Cruz (±0.8 Myr ago) compared to the more divergent genitalia for *H. junco* and *H. snodgrassii* on San Cristóbal (±0.1 Mya). Two scenarios might explain the latter observation. First, disruptive sexual selection (reinforcement) as a consequence of higher rates of secondary contact lead to rapid divergence of genitalia on San Cristóbal and less on Santa Cruz. These higher rates of secondary contact might be a direct consequence of the smaller distance between high-elevation and coastal dry populations on San Cristóbal than on Santa Cruz (respectively, approximately 4 vs. 10 km). Secondly, the estimated divergence time is biased by relatively higher levels of introgression. The latter might be plausible as low levels of gene flow were demonstrated between *H. galapagoensis* and *H. hendrickxi* (De Busschere et al., 2010). Future multi-locus studies might enable more accurate estimates of the amount of gene flow on San Cristóbal and on Santa Cruz. The limited divergence in genital trait variation among the species on Santa Cruz should, however, not be interpreted as evidence that sexual selection has not been involved. Rather, this observation is congruent with strategies such as stabilizing selection on the male genital design (Eberhard et al., 1998; Ramos et al., 2005; Bonduriansky, 2007; Eberhard, 2008), or that sexual selection is acting on non-genital traits such as courtship behaviour. Therefore, future research should address among-species divergence in these other potentially selected sexual traits, although, the main conclusion is that these results strongly indicate that male genital trait variation within the *Hogna* radiation is incongruent with the expectations of divergence under natural selection. In sum, the combined measurement of genital and non-genital traits supports the idea that high speciation rates on islands (Losos & Schluter, 2000) are attributable to a combination of ecological speciation mechanisms acting within a particular island and allopatric processes affecting traits subjected to sexual selection.

**Conclusion**

Natural selection is expected to drive phenotypic evolution in similar directions when similar environmental gradients are occupied by a single ancestral population. Within the *Hogna* radiation, variation in colour and overall body size is in strong concordance with habitat, revealing almost identical phenotypes. This finding suggests that common developmental and/or genetic mechanisms could have facilitated the direction of evolution. Traits involved in sexual selection apparently evolved independently of the environmental gradient, but rather reflect the shared ancestry of the species. These data indicate that speciation along repeated environmental gradients involves the combination of ecological processes, acting primarily within islands, and allopatric divergence in sexual traits among islands, which additionally restrict gene flow among populations of the same ecotype.

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REFERENCES


SUPPORTING INFORMATION

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

Figure 1. Colour variation of male individuals of Hogna species from Galápagos depicted in a PCA ordination.

Table 1. Abbreviations of scored sections in the colour analysis.

Table 2. Description and repeatability of all measured non-genital traits.

Table 3. Eigenvalues and eigenvectors of the first three PC axes describing the colour variation in the female data set.

Table 4. Eigenvalues and eigenvectors for PCA analyses on male and female non-genital traits.

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