

Allozyme characterization of *Hogna* species (Araneae, Lycosidae) of the Galápagos Archipelago

Léon Baert: Royal Belgian Institute of Natural Sciences, Entomology Department, Vautierstraat 29, 1000 Brussels, Belgium. E-mail: leon.baert@naturalsciences.be

Frederik Hendrickx: Royal Belgian Institute of Natural Sciences, Entomology Department, Vautierstraat 29, 1000 Brussels, Belgium; Terrestrial Ecology Unit, Biology Department, Ghent University, K.L. Ledeganckstraat 35, 9000 Gent, Belgium

Jean-Pierre Maelfait: Research Institute for Nature and Forest (INBO), Kliniekstraat 25, 1070 Brussels; Terrestrial Ecology Unit, Biology Department, Ghent University, K.L. Ledeganckstraat 35, 9000 Gent, Belgium

Abstract. The colonization of species on remote islands may result in phenotypic diversification and ultimately speciation. On the Galápagos Archipelago, seven very closely related morpho-species of the wolf spiders genus *Hogna* are distinguishable based on small somatic and genital differences. Based on habitat preference, these species can broadly be categorized into (i) three “high elevation species” occurring on the volcanic highlands, (ii) three “coastal dry” species occurring in dune habitats along the coast, and (iii) one generalist species which is chiefly found in wet coastal habitats such as salt marshes but also in wet habitats at higher altitudes. To determine the degree of reproductive isolation among these morpho-species, we investigated gene flow among populations and species based on nine allozyme loci. Genetic analysis by means of genetic distance estimates and cluster agglomerative analyses confirmed the status of the defined morpho-species. Allele frequencies were highly similar among populations within a species but differed profoundly among species. Genetic differentiation within the generalist species was generally very low. There were no constant differences between high elevation and coastal populations for this species. Neutral genetic divergence between species appeared to correspond more to geographic distribution rather than to a clear separation of the two different ecological groups within an island. This suggests that a parallel parapatric divergence between high elevation and coastal dry species may have taken place on the oldest islands of San Cristobal and Santa Cruz.

Keywords: Wolf spiders, speciation, island biogeography, ecological speciation

Archipelagos are among the world’s great natural laboratories of evolution, as many studies on the Galápagos, Hawaiian, Canary Islands, and other island groups have shown. The Galápagos are of particular interest for the following reasons: they are truly volcanic, well isolated (between 900 and 1000 km west of the Ecuadorian mainland), and of known age (Simkin 1984). There is no evidence of the existence of land bridges so all terrestrial organisms had to cross an oceanic barrier by dispersal from the mainland.

The Galápagos Archipelago consists of 13 large islands and a great number of islets and rocks, all of volcanic origin (Fig. 1). The southeastern islands are the oldest (3–5 million years) while the northern and western islands are the youngest (< 0.7 million years) (Simkin 1984).

Due to geographic isolation, many endemic animal (e.g., Darwin’s finches, giant tortoises, lava lizards, mockingbirds) and plant (e.g., *Opuntia* cacti, *Scalesia* trees) groups have radiated. Evolutionary research on these islands has mainly focused on vertebrate species such as Darwin’s finches, giant tortoises, lava lizards, mockingbirds, and on plant species such as *Opuntia* cacti and *Scalesia* trees (Grant 1981; Fritts 1984; Snell et al. 1984; Stern & Grant 1996; Rassmann et al. 1997; Cacone et al. 2002). Speciation patterns of invertebrates have been, in contrast, much less studied, and only recently have genetic studies been conducted on Coleoptera such as the tenebrionid *Stomium* (Finston & Peck 1995), the chrysomelid *Nesaecrepidia* (Verdyck & Desender 1999), the carabid *Calosoma* (Desender & Verdyck 2000; Verdyck et al. 2003, 2004), the weevil *Galapaganus* (Sequeira et al. 2000, 2008), and the land

snail genus *Bulimulus* (Parent & Crespi, 2006). Genetic studies on Galápagos spiders are presently lacking, while such studies in other locations have revealed adaptive radiations on other archipelagos (e.g., Hawaiian *Tetragnatha* spiders, Gillespie 2004; Hawaiian *Dysdera* spiders, Arnedo 2001).

Previous studies on the spider genus *Hogna*, the only wolf spider genus occurring on the archipelago (Maelfait & Baert 1986; Baert & Maelfait 1997), revealed that this genus consists of several closely related, or even cryptic, species. Based on somatic and small genital differences, a total of seven morpho-species is suggested with a distinct distribution on the islands (Baert et al. unpublished data) (Fig. 1). At least three groups of morpho-species can be distinguished that differ distinctly.

A first group of ecologically and morphologically similar morpho-species occur at higher altitudes on the islands in the pampa vegetation zone and are hereafter referred to as “high elevation species.” Based on differences in morphology of the genital organs, different species can be distinguished: *Hogna* species 1 (H1), living on both southern volcanoes Cerro Azul and Sierra Negra of Isabela, one of the youngest islands, *Hogna* species 4 (H4) which occurs on islands of intermediate age (Santa Cruz, Santiago, and Volcan Alcedo of Isabela), and *Hogna* species 2 (H2) which occurs on the oldest island of San Cristóbal.

A second group, hereafter referred to as “coastal dry species,” lives in the dry arid zone along the coast in vegetated dunes and in the *Opuntia* cactus zone. These morpho-species can only be found on the oldest islands of San Cristóbal (*Hogna* species 5 (H5)), Española (*Hogna* species 7 (H7)), and Santa Cruz (*Hogna* species 6 (H6)). The San Cristóbal species

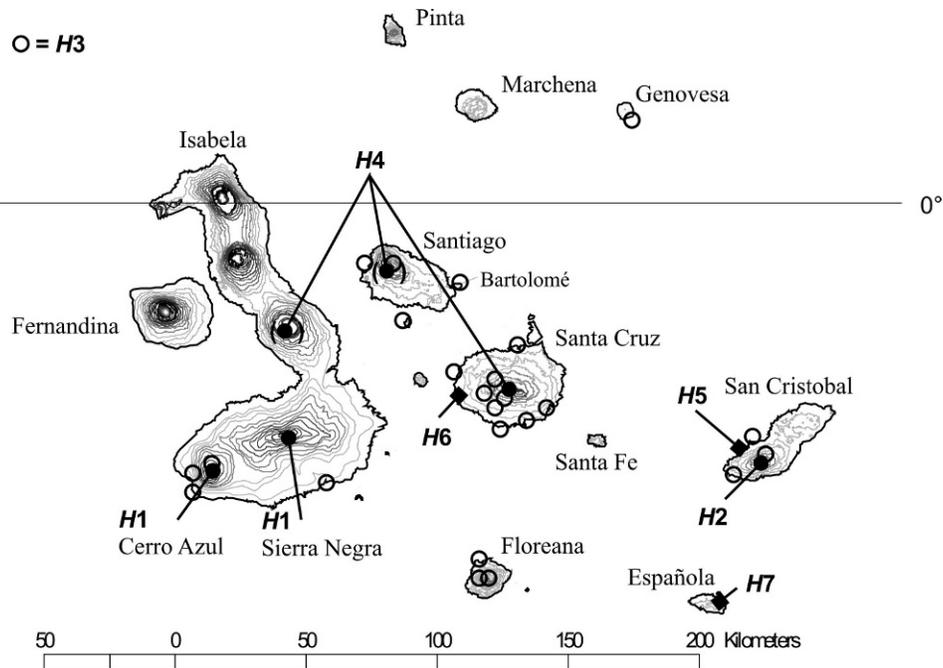


Figure 1.—Map of the sampling locations of the *Hogna* species from Galápagos. Nearby sampling locations are presented as one dot due to space restrictions. Dots between brackets for H4 indicate sites where the species is present but that were not included in this study.

(H5) is mostly found in the depressions overgrown with sea grass (*Sporobolus virginicus*) located behind the shore or on low vegetated dunes, while H7 is found in tall vegetation of the dry arid zone directly adjacent to the littoral zone, but never in the adjacent depressions with salt grass. The Santa Cruz species (H6) is found in the *Opuntia* cactus zone in between dune and pure rocky soil.

The third group comprises populations of the generalist species *Hogna* species 3 that lives in saline habitats along the coast (salt marshes, bays), along permanent pools (e.g., El Chato on Isla Santa Cruz) and in permanent wetlands below 600 m of altitude (e.g., Los Gemelos on Isla Santa Cruz). Scattered populations can also be found above the vegetation inversion zone in wet conditions during El Niño years (characterized by very heavy rainfall giving rise to temporary pools) (Baert & Maelfait 2000). They reach, however, their highest densities in the salt marshes. All these populations have very similar genital organs and are at present interpreted as belonging to a single species. It is widespread over the whole archipelago, with the exception of the northern island Pinta and the southeastern island Española (Baert & Maelfait 1997).

In this paper, we test whether the separation of this genus into seven morpho-species on the Galápagos is justified. By means of cellulose acetate gel electrophoresis, wherein 8 allozyme loci (FUM, G6PDH, GOT, IDH, LDH, MPI, PGI, and PGM) were studied, we investigate whether the genetic variation among species is larger compared to the variation among populations within species and indicative of reproductive isolation among the species.

METHODS

Sampling collection.—In the period between 1996 and 2002, we sampled a total of 43 known *Hogna* populations (see Table 1) from 9 islands (Santa Cruz, Isabela Volcán Sierra

Negra, Isabela Volcán Cerro Azul, San Cristóbal, Floreana, Rábida, Genovesa, Bartolomé, Santiago, and Española) and seven morpho-species. In three localities, the high elevation species occurred sympatrically with H3, [e.g., Cerro Gavillan (populations 40 & 41), El Junco (populations 1 & 2), and Los Gemelos (population 23) (Fig. 1)]. Populations of H4 occurring on the tops of Isabela and Santiago were preserved in ethanol and could therefore not be included in this allozyme study.

Individuals were caught by hand, mostly at sunset with an electric torch worn on the forehead. They were stored and transported in a Taylor-Wharton cryogenic shipper saturated with liquid nitrogen. In the laboratory, the material was stored in an ultra-cold freezer at -80°C . The aim was to investigate at least 40 individuals for each population if possible. In some localities, their densities were so low that this number could not be reached. Some localities were sampled several times but in different years. Voucher specimens are deposited at the Royal Belgian Institute of Natural Sciences.

Allozymes.—Parts of the legs were homogenized in distilled water for performing the cellulose acetate gel electrophoresis, following the procedures of Hebert & Beaton (1989). Eight enzymes (9 loci) were tested for polymorphism: fumarate hydratase (FUM), aspartate aminotransferase (GOT1, GOT2), isocitrate dehydrogenase (IDH), lactate dehydrogenase (LDH), mannose phosphate isomerase (MPI), phosphoglucose isomerase (PGI), phosphoglucumutase (PGM), and 6-phosphoglucuronate dehydrogenase (6PGDH).

Allele frequencies were obtained for each population and species. Deviations from Hardy-Weinberg equilibrium were tested by means of an exact test. Genetic divergence between populations and species were estimated based on Nei's unbiased genetic distance (Nei 1978). Based on this distance metric, divergence among populations within species was

Table 1.—List of the 43 sampled *Hogna* populations on Galápagos during the years 1996, 1998, 2000, and 2002. Situation and number of caught specimens per sample. Abbreviations: SCB = Isla San Cristobal; ESP = Española; GEN = Genovesa; SCZ = Santa Cruz; FLO = Floreana; BAR = Bartolomé; RAB = Rabida; SAN = Santiago; ISN = Isabela, Volcan Sierra Negra; ICA = Isabela, Volcán Cerro Azul.

Code	Island	Locality	Vegetation zone	Elevation	Morpho-species	1996	1998	2000	2002
1	SCB	El junco	pampa	675m	H3	8			36
2	SCB	El junco	pampa	675m	H2	3			44
3	SCB	Cerro San Joaquin	pampa	700m	H2				46
4	SCB	Punto Baso	dune	5m	H5				41
5	SCB	Punto Baso (fregat nesting)	dune	5m	H5				36
6	SCB	Punto Baso (<i>Sesuvium</i>)	littoral zone	1m	H3				27
7	SCB	Caleta de la Tortuga	littoral zone	5m	H3				10
8	SCB	La Lobería	littoral zone	1m	H3				42
9	SCB	Caleta Sapho (Spirobolus)	salt grass	1m	H5	28			55
10	ESP	Punta Cevallos	dry arid zone	2m	H7			40	
11	ESP	Bahía Gardner	dry arid zone	1m	H7			31	
12	ESP	Isla Gardner	dry arid zone	2m	H7			41	
13	GEN	Lago Arcturus	littoral zone (lagoon)	60m	H3			18	
14	SCZ	Laguna Andreas	littoral zone (lagoon)	1m	H3	35		42	40
15	SCZ	Bowdich	littoral zone (lagoon)		H3				31
16	SCZ	El Garapatero	littoral zone (lagoon)		H3			58	22
17	SCZ	Las Palmas	dry arid zone		H6				28
18	SCZ	Meteo Station	littoral zone (lagoon)	5m	H3		40		
19	SCZ	Bahía Tortuga	littoral zone (lagoon)	1m	H3		41	27	53
20	SCZ	Playa Bachas	littoral zone (lagoon)	1m	H3		43	40	44
21	SCZ	El Chato	around permanent pool		H3			40	40
22	SCZ	El Carmen	temporary pool (El Niño)	350m	H3		42		
23	SCZ	Los Gemelos, open	pampa	570m	H3				43
24	SCZ	Los Gemelos, Scalesia	<i>Scalesia</i> forest		H3			47	40
25	SCZ	Media Luna	pampa	600m	H3		51	37	27
26	SCZ	Tss ML & Cpunt	pampa		H4				13
27	SCZ	Cerro Crocker	pampa	875m	H4		42	23	47
28	FLO	Punta Cormoran	littoral zone (lagoon)	1m	H3			40	
29	FLO	Finca Cruz	pampa	200m	H3			15	
30	FLO	Highland	pampa	350m	H3			50	
31	BAR		littoral zone (lagoon)	1m	H3		31		
32	RAB		littoral zone (lagoon)	1m	H3		38		
33	SAN	Playa Espumila	littoral zone (lagoon)	1m	H3		45		40
34	SAN	Aguacate	transition zone (El Niño)	500m	H3		40		41
35	SAN	La Central	pampa (El Niño)	700m	H3		38		40
36	SAN	Jaboncillo	pampa (El Niño)	820m	H3		40		40
37	ISN	Laguna de Villamil	littoral zone (lagoon)	1m	H3	41			
38	ISN	Top	pampa		H1	42			
39	ICA	Caleta Iguana	littoral zone/dry arid zone	2m	H3		32		
40	ICA	Cerro Gavilan	pampa	700m	H3		12		
41	ICA	Cerro Gavilan	pampa	700m	H1		30		
42	ICA	1100m	dry arid zone (El Niño)	1100m	H3		11		
43	ICA	Top	dry arid zone (El Niño)	1530m	H3		30		
Annual total no. specimens						157	606	549	926

compared with the distance among populations of different species. These analyses were performed with the computer packages TFPGA (Miller 1997) and GenAlEx (Peakall and Smouse 2006). Genetic distances were visualized by means of principal component analysis (PCA), designed for ordination of allelic frequency data, by means of the computer package PCA-Gen (Goudet 1999).

RESULTS

Allelic variation and heterozygosity were very low within each species, but differed clearly among species, with one or a few alleles that were fixed within the morpho-species. The low genetic variability among populations within species, compared to the variability among species, is clearly

depicted when genetic distances are compared among populations (Table 2). The genetic distance between populations belonging to the same morpho-species ranged from 0 to maximum 0.031 (H3), demonstrating that the allele frequencies of the different populations within a given morpho-species were highly similar. Differences in allele frequency of populations belonging to a different morpho-species were, in contrast, considerably higher and ranged from 0.118 to 2.277. The smallest genetic distances were observed between H2 (“high elevation” San Cristobal) and H7 (“coastal dry” Española) and between H5 (“coastal dry” San Cristobal) and H7.

Allele frequencies for all loci were near to fixation for almost all morpho-species (Table 3). None of the species

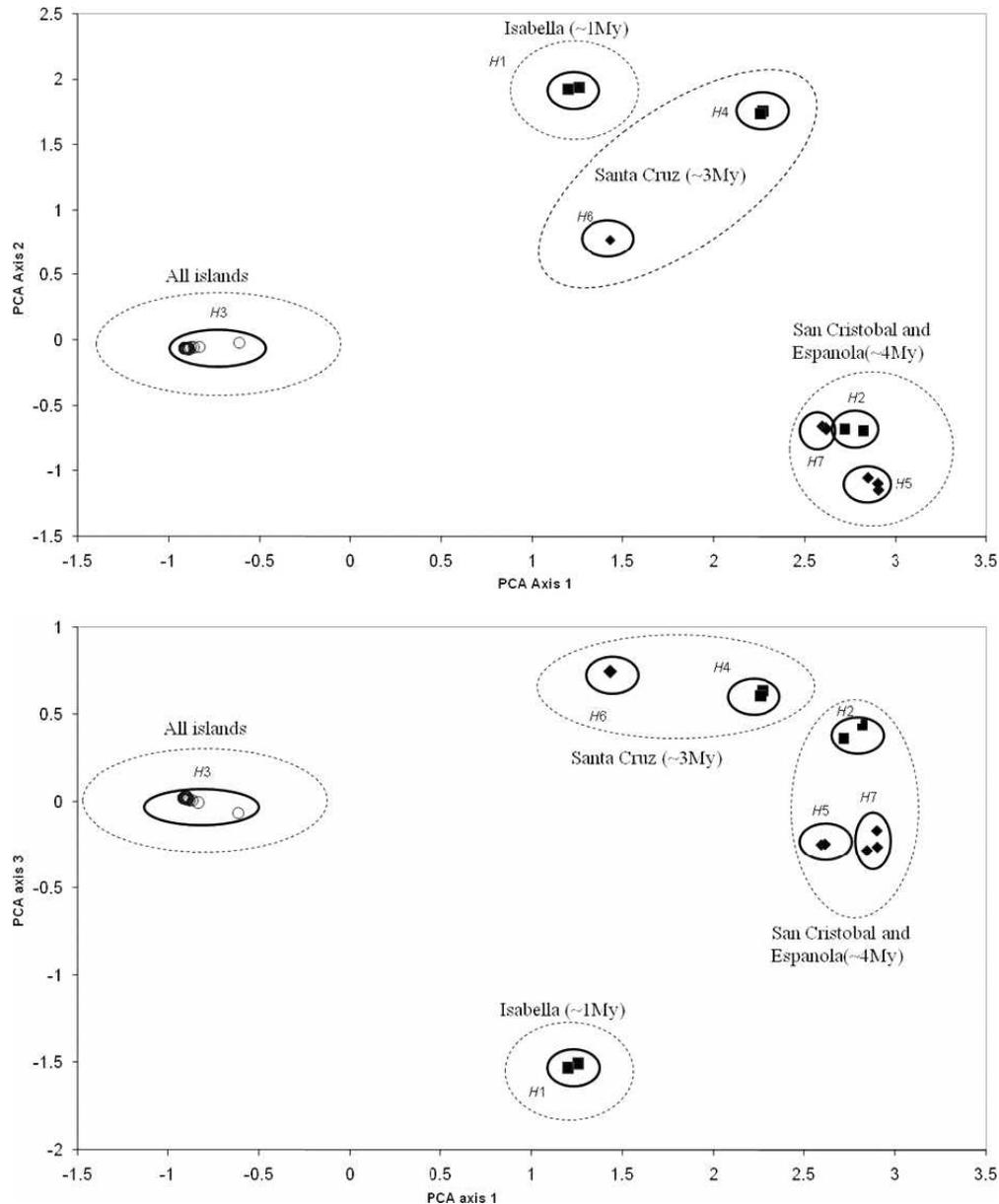


Figure 2.—Results of principal component analysis (axes 1, 2 and 3). (Filled squares = high elevation species; filled diamonds = coastal dry species; open circles = generalist species.)

this axis and the second axis corresponded to their geographic position and age of the islands rather than to their habitat preference. The three species of the oldest islands – H7 from Española and H2 and H5 from San Cristobal – were clustered in the PCA, followed by H4 and H6 from Santa Cruz and H1 from the volcanoes Cerro Azul and Sierra Negra on Isabela.

DISCUSSION

Our results show that there is a clear and very high degree of genetic divergence between the previously defined morpho-species. Moreover, genetic divergence among populations within species was much lower (Table 2, Fig. 2). These two findings indicate that these morpho-species likely represent distinct reproductively isolated species.

Although the validity of allozymes for phylogenetic inferences is questionable since the historical and genealogical relationship between the different alleles remains unknown (Lowe et al. 2004), a few suggestions concerning historical patterns of divergence can be deduced.

First, these results suggest that *Hogna* speciation on the Galápagos is likely due the combined effect of geographic isolation and ecological specialization within the different climatological and vegetation zones present on the different islands. Except for H3, species from the same or proximate island tend to be genetically more similar to each other. Species living on the same islands but in different habitats are, however, genetically fixed for a few loci, clearly indicating a lack of gene flow and hence strong reproductive isolation. Combining these results suggests that ecological specialization

on the islands Santa Cruz and San Cristobal occurred repeatedly in association with speciation events rather than a diversification of a habitat adapted lineage with secondary colonization of the specialized forms to the different islands. According to the second scenario, species living in the same habitat would then be expected to be genetically more similar to each other.

Similar patterns of species diversification in terms of geographic position and ecological specialization have been confirmed by more thorough genetic analyses of *Tetragnatha* spiders from Hawaii (Gillespie 2004). In the Galápagos, this speciation pattern has been observed in other terrestrial invertebrates such as weevils and snails (Parent & Crespi 2006; Sequiera et al. 2008).

Whether the generalist species H3 can be regarded as closely related to the ancestral species, as suggested by Baert & Maelfait (2000), however, cannot be confirmed by these data. The smaller genetic distance between the generalist H3 and the species from younger islands (H1) compared to those of older islands (H2, H5, and H7) is in accordance with this hypothesis. Surprisingly however, H3 showed a very low degree of genetic variation within populations. Moreover, distant populations as well as populations living in different habitats all appeared to be genetically very homogenous. These observations suggest that this generalist and apparently highly dispersive species may have colonized the archipelago independently.

The results can only be interpreted as preliminary as they are based on allozyme data and only a few loci were scored. Moreover, the selective neutrality of allozymes has often been questioned. Our ongoing work aims to add more variable loci such as mitochondrial DNA (Cytochrome Oxidase I) so that more well founded phylogenetic inferences can be made. Also, future work should include *Hogna* species from the South American mainland to better understand the phylogenetic relationships between the species and the colonization history of *Hogna* in Galápagos.

ACKNOWLEDGMENTS

Excellent cooperation and field logistic support were provided by the Charles Darwin Research Station (CDRS, Isla Santa Cruz, Galápagos, Ecuador), the directors F. Koestner, G. Recek, D. Evans, C. Blanton, R. Bensted-Smith, M. Cifuentes and their staff; the Galápagos National Park Service (SPNG Superintendents M. Cifuentes, IR. H. Ochoa, F. Cepeda, A. Izurieta, and E. Cruz), Department of Forestry, Ministry of Agriculture of Ecuador; TAME airline kindly issued a reduced price for travel tickets. Our investigations and field work were financially supported by (1) BELSPO (former Belgian DWTC), (2) the Fund for Scientific Research (FWO-Vlaanderen; research project G.0202.06), and (3) the Léopold III Foundation. Help in the field was provided by K. Desender, L. Roque, and P. Verdyck. Help with electrophoresis was provided by K. Desender, K. Loosveldt, and V. Versteirt. Constructive comments on a previous version were given by Marshal Hedin and an anonymous referee.

LITERATURE CITED

Arnedo, M.A., P. Oromi & C. Ribera. 2001. Radiation of the spider genus *Dysdera* (Araneae, Dysderidae) in the Canary Islands: cladistic assessment based on multiple data sets. *Cladistics* 17:313–353.

- Baert, L. & J.-P. Maelfait. 1997. Taxonomy, distribution and ecology of the lycosid spiders occurring on the Santa Cruz Island, Galápagos Archipelago, Ecuador. Pp. 1–11. *In* Proceedings of the 16th European Colloquium on Arachnology. (M. Zabka, ed.). Wyzsza Szkoła Rolniko-Pedagogiczna, Siedlce, Poland.
- Baert, L. & J.-P. Maelfait. 2000. The influence of the 1997–1998 El Niño upon the Galápagos lycosid populations, and a possible role in speciation. *European Arachnology* 2000:51–56.
- Caccone, A., G. Gentile, J.P. Gibbs, T.H. Fritts, H.L. Snell, J. Betts & J.R. Powell. 2002. Phylogeography and history of giant Galápagos tortoises. *Evolution* 56:2052–2066.
- Desender, K. & P. Verdyck. 2000. Genetic differentiation in the Galápagos caterpillar hunter *Calosoma granatense* (Coleoptera, Carabidae). Pp. 25–34. *In* Natural History and Applied Ecology of Carabid Beetles. (P. Brandmayer, G. Lövei, T.Z. Brandmayer, A. Casale & A.V. Taglianti, eds.). Pensoft Publishers, Sofia & Moscow.
- Finston, T.L. & S. Peck. 1995. Population structure and gene flow in *Stomium*: a species swarm of flightless beetles of the Galápagos islands. *Heredity* 75:390–397.
- Fritts, T.H. 1984. Evolutionary divergence of giant tortoises in Galápagos. *Biological Journal of the Linnean Society* 61:165–176.
- Gillespie, R. 2004. Community assembly through adaptive radiation in Hawaiian spiders. *Science* 303:356–359.
- Goudet, J. 1999. PCA-Gen. Principal Component Analysis using gene frequency data. Online at <http://www2.unil.ch/popgen/softwares/pcagen.htm>.
- Grant, P.R. 1981. Speciation and the adaptive radiation of Darwin's finches. *American Scientist* 69:653–663.
- Hebert, P.D.N. & M.J. Beaton. 1989. Methodologies for Allozyme Analysis Using Cellulose Acetate Electrophoresis. Helena Laboratories, Beaumont, Texas. 32 pp.
- Lowe, A., S. Harris & P. Ashton. 2004. *Ecological Genetics: Design, Analysis, and Application*. Blackwell Publishing, Malden, Massachusetts. 326 pp.
- Maelfait, J.-P. & L. Baert. 1986. Observations sur les lycosides des îles Galápagos. *Mémoires de la Société royal belge d'Entomologie* 33:139–142.
- Miller, M.P. 1997. *Tools for Population Genetic Analyses (TFPGA)*. Northern Arizona University, Flagstaff, Arizona. 30 pp.
- Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89:583–590.
- Parent, C.E. & B.J. Crespi. 2006. Sequential colonization and diversification of Galápagos endemic land snail genus *Bulimulus*. *Evolution* 60:2311–2328.
- Peakall, R. & P.E. Smouse. 2006. GenAlEx: genetic analysis in Excel. Population genetic software for research and education. *Molecular Ecology Notes* 6:288–295.
- Rassmann, K., D. Tautz, F. Trillmich & C. Gliddon. 1997. The microevolution of the Galápagos marine iguana *Amblyrhynchus cristatus* assessed by nuclear and mitochondrial genetic analysis. *Molecular Ecology* 6:437–452.
- Sequeira, A.S., A.A. Lanteri, L. Roque Albelo, S. Bhattacharya & M. Sijapati. 2008. Colonization history, ecological shifts and diversification in the evolution of endemic Galápagos weevils. *Molecular Ecology* 17:1089–1107.
- Sequeira, A.S., A.A. Lanteri, M.A. Scataglini, V.A. Confalonieri & B.D. Farrell. 2000. Are flightless *Galapaganus* weevils older than the Galápagos Islands they inhabit? *Heredity* 85:20–29.
- Simkin, T. 1984. Geology of Galápagos Islands. Pp. 15–41. *In* Key Environments: Galápagos. (R. Perry, ed.). Pergamon Press, Oxford, UK.
- Snell, H.I., H.M. Snell & C.R. Tracy. 1984. Variation among populations of Galápagos land iguanas (*Conolophus*): contrasts of phylogeny and ecology. *Biological Journal of the Linnean Society* 21:185–207.

- Stern, D.L. & P.R. Grant. 1996. A phylogenetic reanalysis of allozyme variation among populations of Galápagos finches. *Zoological Journal of the Linnean Society* 118:119–134.
- Verdyck, P. & K. Desender. 1999. Hierarchical population genetic analysis reveals metapopulation structure in a phytophagous Galápagos beetle. *Belgian Journal of Zoology* 129:95–104.
- Verdyck, P., K. Desender & H. Dhuyvetter. 2003. Genetic diversity of the phytophagous beetle *Docema darwini* Mutchler, endemic to the Galápagos Islands. Pp. 295–301. *In* Special Topics in Leaf Beetle Biology. (D.G. Furth, ed.). Proceedings of the Fifth International Symposium on Chrysomelidae. Pensoft Publishers, Sofia & Moscow.
- Verdyck, P., H. Dhuyvetter & K. Desender. 2004. Genetic differentiation and population structure in *Metachroma labrale* Blair, 1933, a Galápagos leaf beetle (Chrysomelidae). Pp. 131–136. *In* New Developments in the Biology of Chrysomelidae. (P. Jolivet, J.A. Santiago-Blay & M. Schmitt, eds.). SPB Academic Publishing bv, The Hague, The Netherlands.

Manuscript received 27 November 2007, revised 15 July 2008.