Belg. J. Zool. - Volume 129 (1999) - issue 1 - pages 95-104 - Brussels 1999

HIERARCHICAL POPULATION GENETIC ANALYSIS REVEALS METAPOPULATION STRUCTURE IN A PHYTOPHAGOUS GALÁPAGOS BEETLE

PETER VERDYCK ([1,2]) AND KONJEV DESENDER ([1])
([1]) KBIN, Department of Entomology, Vautierstraat 29, B-1000 Brussels, (Belgium)
([2]) Evolutionary Biology Group, Department Biology, University of Antwerp (RUCA), Groenenborgerlaan 171, B-2020 Antwerpen (Belgium)
e-mail : Verdyck@kbinirsnb.be

Abstract. The Galápagos Archipelago has long been considered a living laboratory for the study of evolution. Due to geographic isolation and speciation many endemic animal and plant groups have radiated on the islands. Although the vertebrate fauna of these islands (*e.g.* giant tortoises, Darwin's finches) has been studied in great detail, little is known about invertebrates and especially insects. Results are given of a population genetic study on the phytophagous beetle *Nesaecrepida darwini*. This small alticine beetle is present on all major islands but shows a discontinuous population distribution. To obtain population genetic information we used cellulose acetate gel electrophoresis to study allozyme variation in 6 populations from 3 islands. Twelve presumptive loci, including 9 polymorphic ones, were analysed. The results show low heterozygosity values, with the lowest genetic diversity on the youngest island. F-statistics (mean F_{ST} = 0.431) indicate a very large amount of genetic differentiation between populations. Hierarchical analysis indicates little inter-island gene flow but also considerable genetic variation between populations occurring on the same island. These results strongly suggest a metapopulation structure with recurrent extinctions and recolonisations of populations within each island. Recent field observations support these findings.

Key words: Chrysomelidae, Galápagos, evolution, population genetics, genetic differentiation, phytophagy, metapopulation structure, gene flow, genetic drift.

INTRODUCTION

The Galápagos Archipelago is situated in the Pacific Ocean about 1000 km from the South American coast, straddling the Equator. It is a group of 13 large islands, 6 smaller ones and 107 islets and rocks, with a total land area of about 8000 square kilometres. The islands are volcanic in origin and several volcanoes in the west of the archipelago are still active (*e.g.* recent volcanic activity on Cerro Azul, Isla Isabela in September-October 1998).

The archipelago is home to many radiated groups of endemic species, the most famous examples being the Darwin finches, the giant tortoises, the mockingbirds, the *Opuntia* cacti, the *Scalesia* trees and the lava lizards. Although the invertebrate communities also have a considerable portion of endemics and include striking examples of radiated groups (COPPOIS, 1984; PECK, 1996; FINSTON & PECK, 1997), until now evolutionary research on these islands has mainly focused on vertebrate species (*e.g.* GRANT, 1981; FRITTS, 1984; SNELL *et al.*, 1984; STERN & GRANT, 1996; RASSMANN *et al.*, 1997). Since 1982, entomologists from the Royal

Belgian Institute of Natural Sciences have been conducting systematic and ecological work on insects and spiders of Galápagos (*e.g.* BAERT & MAELFAIT, 1986a, 1986b; DESENDER *et al.*, 1989, 1990) collecting material during expeditions in 1982, 1986, 1988 and 1991. In spring 1996, another expedition to three of the major Galápagos islands was organised in order to collect material in liquid nitrogen for studying population genetic aspects of several model spider and insect species, now also including phytophagous beetles. The aim of this study is to obtain information about genetic variability within and genetic exchange between the islands for several invertebrates, and to gain insight into the genetic structure of their populations.

In this paper we present the first results of these studies on a specialised herbivorous leaf beetle from Galápagos: *Nesaecrepida darwini* (Mutchler, 1925). This species probably occurs on all islands and is monophagous on saltbush (*Cryptocarpus pyriformis*), a plant that often is very abundant along the coastline in the littoral zone. Although the plant can cover vast areas of land, the beetles are only found on isolated patches, constituting geographically well-defined populations of hundreds to several thousands of individuals (pers. obs.). Large areas of host plant are left unoccupied by the beetles. It is unknown why these animals do not occur as continuous populations over larger areas.

Until now population genetic aspects of only one group of Galápagos insects have been published: FINSTON & PECK (1995, 1997) provided data on population structure and gene flow in the Galápagos beetles of the genus *Stomion,* a species swarm (13 species) of flightless beetles that are generalised litter feeders. In this study we focus on a genus with only one described endemic species that is able to fly, is present on all major islands and is a highly specialised feeder (monophagous). Population genetic results on other beetle species will be given in a future contribution (DESENDER & VERDYCK, unpublished data).

MATERIAL AND METHODS

During our 1996 expedition to Galápagos we collected at least 40 individuals from six populations of *Nesaecrepida darwini* on three islands (Isla San Cristóbal [estimated age 3 million years], Isla Santa Cruz [estimated age between 0.7 and 1.5 million years], and Volcan Sierra Negra on Isla Isabela [estimated age less than 0.7 million years]). For electrophoresis the abdomen of each individual was removed and homogenised in 35 µl of distilled water. After a pilot study on some 30 enzyme loci, a selection of 12 presumptive loci, showing clearly interpretable banding patterns, was used for analysis. The allozyme loci studied were arginine phosphokinase (*APK*, E.C. 2.7.3.3, 2 loci), aspartate aminotransferase (*AAT,* E.C. 2.6.1.1, 2 loci), isocitrate dehydrogenase (*IDH*, E.C. 1.1.1.42, 2 loci), malic enzyme (*ME*, E.C. 1.1.1.40), malate dehydrogenase (*MDH*, E.C. 1.1.1.37, 2 loci), peptidase-A (dipeptide substrate: leucyl glycine, *PEP-A*, E.C. 3.4.-.-), peptidase-D (dipeptide substrate: phenylalanine proline, *PEP-D*, E.C. 3.4.-.-), 6-phosphogluconate dehydrogenase (*6PGDH*, E.C.1.1.1.44). Staining recipes were as in HEBERT & BEATON (1989). Alleles were designated alphabetically according to decreasing mobility, the slowest allele being A. Nine loci (*APK1, AAT1, AAT2, IDH1, IDH2, ME, MDH1*, *PEP-A* and *PEP-D*) were polymorphic (five loci at the 95% level).

After interpretation, data were further analysed using BIOSYS-1 (SWOFFORD & SELANDER, 1989) and GENEPOP (RAYMOND & ROUSSET, 1995). Four genetic variability measures (mean number of alleles per locus, percentage of loci polymorphic, direct count heterozygosity and

heterozygosity values expected under Hardy-Weinberg conditions) were calculated at twelve loci in all populations (see Table 1). We tested for deviations of Hardy Weinberg equilibrium using exact probabilities (SWOFFORD & SELANDER, 1989), correcting for errors resulting from multiple tests by means of the tablewide sequential Bonferroni prosedure (HOLM, 1979; RICE 1989).

TABLE 1

Allele frequencies in all populations of Nesaecrepida darwini

		Bahia Tortuga (Isla Santa Cruz)	*CDRS* (Isla Santa Cruz)	*Caleta Sapho* (Isla San Cristóbal)	*La Loberia* (Isla San Cristóbal)	*El Estero* (Isla Isabela)	*Villamil* (Isla Isabela)
APK1	N	45	50	43	43	45	42
	A	0.000	0.000	0.012	0.000	0.000	0.000
	B	1.000	1.000	0.988	1.000	1.000	1.000
APK2	N	45	50	43	43	45	42
	A	1.000	1.000	1.000	1.000	1.000	1.000
AAT1	N	45	50	43	43	46	43
	A	0.267	0.580	0.651	0.115	0.537	0.035
	B	0.733	0.420	0.349	0.885	0.463	0.965
AAT2	n	45	50	43	43	46	43
	A	0.978	0.990	1.000	1.000	1.000	1.000
	B	0.022	0.010	0.000	0.000	0.000	0.000
IDH1	n	44	49	43	43	45	43
	A	0.114	0.347	0.000	0.000	0.000	0.000
	B	0.886	0.653	1.000	1.000	1.000	1.000
IDH2	n	45	50	43	43	46	43
	A	0.000	0.000	0.000	0.012	0.000	0.000
	B	1.000	1.000	0.988	0.988	1.000	1.000
	C	0.000	0.000	0.012	0.000	0.000	0.000
ME	n	45	50	43	43	46	43
	A	0.000	0.070	0.000	0.000	0.000	0.000
	B	1.000	0.930	0.977	1.000	1.000	1.000
	C	0.000	0.000	0.023	0.000	0.000	0.000
MDH1	n	45	50	43	42	46	43
	A	1.000	0.990	0.605	0.571	1.000	1.000
	B	0.000	0.000	0.081	0.012	0.000	0.000
	C	0.000	0.010	0.314	0.417	0.000	0.000
MDH2	n	45	50	43	43	46	43
	A	1.000	1.000	1.000	1.000	1.000	1.000
PEPA	n	45	40	43	38	41	43
	A	0.011	0.000	0.047	0.000	1.000	0.721
	B	0.989	1.000	0.953	1.000	0.000	0.279
PEPD	n	45	38	43	36	39	43
	A	0.000	0.000	0.012	0.000	0.000	0.000
	B	1.000	1.000	0.988	0.986	1.000	1.000
	C	0.000	0.000	0.000	0.014	0.000	0.000
6PGDH	n	45	49	43	43	46	43
	A	1.000	1.000	1.000	1.000	1.000	1.000

Rogers' Genetic distances were calculated between all populations and both UPGMA and distance Wagner dendrograms were constructed.

Population structure was analysed using Wright's F-statistics (WRIGHT, 1978) (F_{ST} measures the amount of differentiation between subpopulations relative to the limiting amount under complete fixation). A hierarchical analysis of population differentiation was performed at two levels: localities within islands, and between islands.

RESULTS

Allele frequencies for all populations studied are shown in Table 1. The different genetic variability measures for all populations are shown in Table 2. Only the locus *AAT1* in the population of Caleta Sapho (one out of more than 20 tests only) shows significant deviation from Hardy-Weinberg equilibrium, due to a heterozygote excess.

TABLE 2

Genetic variability measures for all populations (standard errors in parentheses)

Population	*Mean Sample Size per Locus*	*Mean no. of alleles per locus*	*% of loci polymorphic*	*Mean Heterozygosity*	
				Direct count	*HdyWbg expected*
Bahia Tortuga (Santa Cruz)	44.9 (0.1)	1.3 (0.1)	33.3	0.054 (0.033)	0.055 (0.035)
CDRS (Santa Cruz)	48.0 (1.2)	1.4 (0.1)	41.7	0.082 (0.045)	0.093 (0.053)
La Loberia (San Cristobal)	41.6 (0.7)	1.4 (0.2)	33.3	0.063 (0.042)	0.064 (0.044)
Caleta Sapho (San Cristobal)	43.0 (0.0)	1.7 (0.2)	58.3	0.079 (0.050)	0.100 (0.054)
Villamil (Isabela)	42.8 (0.1)	1.2 (0.1)	16.7	0.045 (0.039)	0.040 (0.034)
El Estero (Isabela)	44.3 (0.7)	1.1 (0.1)	8.3	0.028 (0.028)	0.042 (0.042)

Rogers' genetic distances between all populations are presented in Table 3. Distances between populations vary from 0.054 to 0.156, distances between populations of one island generally being smaller than distances between populations of different islands. The UPGMA and distance Wagner dendrograms are shown in Figs 1 and 2. In both dendrograms, three main groups can be distinguished, each corresponding to the populations of one island. Isla Isabela is separated first, whereas Santa Cruz and San Cristóbal are clustered more closely. These last two islands are considerably older than Isla Isabela.

Table 4 provides a summary of the F-statistics at all loci (FIT is the overall inbreeding coefficient of an individual, which includes a contribution due to actual nonrandom mating within subpopulations [FIS] and another contribution due to the subdivision itself [F_{ST}]). F_{ST} values vary between 0.010 and 0.795. The mean F_{ST} value over all loci is 0.431. Variance components and F-statistics combined across loci for the hierarchical analysis are shown in Table 5. About half of the total variance is attributable to variance between islands. $F_{ISLAND\text{-}TOTAL}$ is somewhat smaller than $F_{LOCALITY\text{-}ISLAND}$, hence genetic differentiation is considerably important also between populations within an island.

TABLE 3

Matrix of Rogers (1972) genetic distances between populations

Population	*Bahia Tortuga*	*CDRS*	*Villamil*	*El Estero*	*La Loberia*
Bahia Tortuga (Santa Cruz)	-				
CDRS (Santa Cruz)	0.054	-			
Villamil (Isabela)	0.090	0.142	-		
El Estero (Isabela)	0.116	0.123	0.065	-	
La Loberia (San Cristobal)	0.062	0.111	0.104	0.156	-
Caleta Sapho (San Cristobal)	0.081	0.077	0.143	0.124	0.061

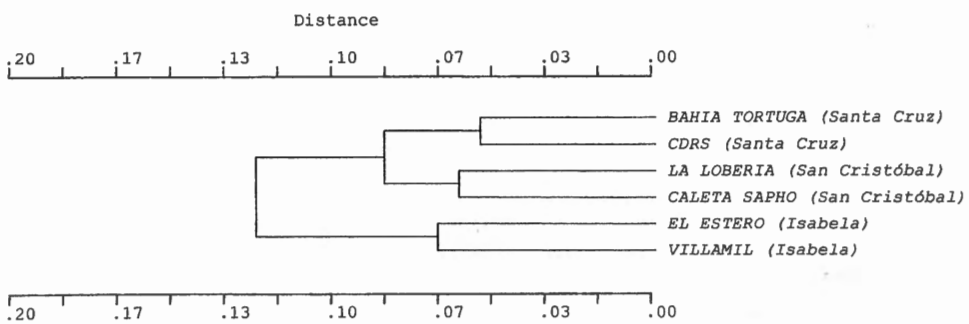

Fig. 1. – UPGMA dendrogram based on Rogers' genetic distance

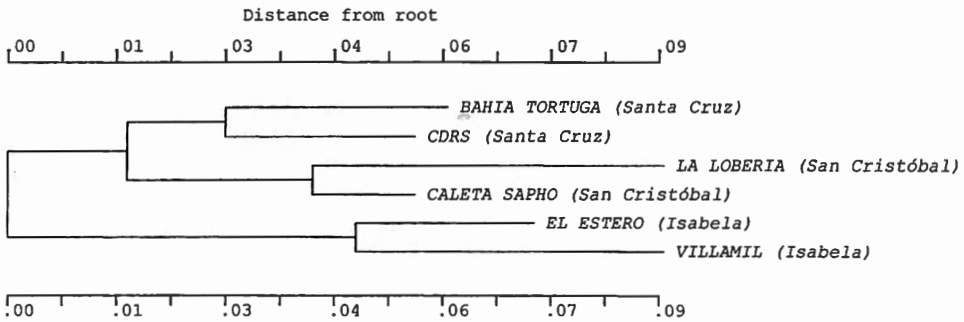

Fig. 2. – Distance Wagner dendrogram based on Roger's genetic distance

TABLE 4

Summary of F-statistics at all loci

Locus	*F(IS)*	*F(IT)*	*F(ST)*
APK1	-0.012	-0.002	0.010
AAT1	0.256	0.444	0.244
AAT2	-0.019	-0.005	0.013
IDH1	-0.033	0.205	0.230
IDH2	-0.012	-0.003	0.009
ME	-0.062	-0.013	0.046
MDH1	-0.050	0.246	0.281
PEPA	-0.041	0.787	0.795
PEPD	-0.013	-0.003	0.010
Mean	0.096	0.469	0.413

TABLE 5

Variance components and F-statistics combined across loci

Comparison		*Variance*	F_{xy}
X	*Y*	*Component*	
Locality	Island	0.25405	0.244
Locality	Total	0.53930	0.407
Island	Total	0.28525	0.215

DISCUSSION

Heterozygosity values (Table 2) in all populations are relatively low compared to those found in studies on continental Chrysomelidae (see overview in VERDYCK, in press) and other beetles (see overview in HSIAO, 1989). Observed heterozygosity is also considerably higher in the flightless Galápagos species of the genus *Stomion* (average 0.085) (FINSTON & PECK, 1997).

Populations from Isla Isabela (Villamil and El Estero), the youngest island investigated in our study, show the lowest genetic diversity for all measures. This can be explained if the origin of these populations is to be found in a more recent colonisation of this island by a limited number of individuals (founder effects), originating from nearby older islands (*e.g.* Floreana, Santa Cruz).

The observed clustering pattern of both dendrograms may be explained by the fact that populations of the two oldest islands (Santa Cruz and San Cristóbal) probably have had some genetic exchange in their recent history, whereas Isabela is genetically much more separated.

The mean F_{ST} value of 0.431 is one of the highest ever recorded between populations of a chrysomelid species (an overview of genetic population structuring in chrysomelids at different micro- and macrogeographical scales is provided by KNOLL *et al.* (1996)). VERDYCK *et al.* (1998) studied continental populations of the alticine beetle *Phyllotreta tetrastigma*. This species has (apart from belonging to the same subfamily of beetles) several other characteristics in common with *N. darwini*. It is a monophagous species, capable of flying, with a discontinuous distribution (because its host plant *Cardamine amara* is restricted to wet woodlands), which can sometimes be very locally abundant. However *P. tetrastigma* shows remarkably little genetic differentiation over a relatively large geographic scale (Western Europe). For the only other Galápagos insects studied, FINSTON & PECK (1995) found a mean F_{ST} of 0.30 across taxa in the flightless *Stomion* beetles.

The results obtained for *N. darwini* are very remarkable and in contrast with those found for other good flying and even for flightless species. The high amount of genetic differentiation between the islands indicates that, although the species is macropterous, inter-island gene flow is not sufficient to counteract effects of differentiation between islands. Moreover, we observed a relatively high differentiation between populations from the same island, sometimes even at relatively small distances. The main reason for this might be a metapopulation structure (patchy distribution) in combination with a pattern of recurrent extinctions and recolonisations giving rise to many founder events. Recent field observations in spring 1998 showed that the population on Villamil had probably become extinct whereas the populations on Santa Cruz (CDRS and Bahia Tortuga) still existed (the other populations were not visited in 1998). This confirms the hypothesis of a metapopulation model for *N. darwini*. A more general concluding hypothesis is that the population genetics and thus the evolution of this species in Galápagos have been profoundly shaped by genetic drift during recurrent founder events within the archipelago. This hypothesis will be further tested in the future, by means of genetic data on additional populations and by using other biochemical markers, such as mtDNA-data.

ACKNOWLEDGEMENTS

The authors want to thank the Belgian DWTC (former Ministry of Education), the Leopold III foundation and the Fund for Scientific Research – Flanders for their financial support. Excellent cooperation and field logistic support were provided during our 1996-expedition by the Charles Darwin Research Station (CDRS, Santa Cruz, Galápagos), C. Blanton, Director and her staff, and the Galápagos National Park Service (A. Izurieta, Superintendent, Dept. Forestry, Ministry of Agriculture, Ecuador). TAME airline kindly issued reduced travel tickets. Also sincere thanks to Dr. L. Baert, Dr. J.P. Maelfait, Prof. Dr. S. Peck and Dr. L. Roque for their help with the fieldwork. P. Verdyck is a Postdoctoral Fellow of the Fund for Scientific Research – Flanders (Belgium) (F.W.O.).

REFERENCES

BAERT, L. & J.P. MAELFAIT (1986a) – Spider communities of the Galápagos Islands (Ecuador). *Actas X. Congreso Internacional Aracnologica Jaca, Espana*, **I**: 193-188.

BAERT, L. & J.P. MAELFAIT (1986b) – A contribution to the knowledge of the spider fauna of Galápagos (Ecuador). *Bull. Inst. R.. Sc. Nat. Belg. : Entomologie*, **56**: 93-123.

COPPOIS, G. (1984) – Distribution of bulimulid land snails on the northern slope of Santra Cruz Island, Galápagos. *Biol. J. Linn. Soc.*, **21**: 217-227.

DESENDER, K., L. BAERT & J.P. MAELFAIT (1989) – Contribution to the knowledge of Carabid beetles of Galápagos (Ecuador). *Bull. Inst. R.. Sc. Nat. Belg. : Entomologie*, **58**: 55-64.

DESENDER, K., L. BAERT & J.P. MAELFAIT (1990) – Evolutionary Ecology of Carabids in the Galápagos Archipelago. In *: The role of Ground Beetles in Ecological and Environmental Studies.* STORK, N.E. (Ed.): 13-20.

FINSTON, T.L. & S.B. PECK (1995) – Population structure and gene flow in *Stomion* flightless beetles of the Galápagos Islands, *Heredity,* **75**: 390-397.

FINSTON, T.L. & S.B. PECK (1997) – Genetic differentiation and speciation in *Stomion* (Coleoptera: Tenebrionidae): flightless beetles of the Galápagos Islands, Ecuador, *Biol. J. Linn. Soc.*, **61**: 183-200.

FRITTS, T.H. (1984) – Evolutionary divergence of giant tortoises in Galápagos. *Biol. J. Linn. Soc.*, **21**: 165-176.

GRANT, P.R. (1981) – Speciation and the adaptive radiation of Darwin's finches. *Am. Sci.*, **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).

HOLM, S. (1979) – A simple sequentially rejective multiple test procedure. *Scand. J. Stat.*, **6**: 65-70.

HSIAO, T. (1989) – Estimation of genetic variability amongst Coleoptera. In *: Electrophoretic Studies on Agricultural Pests.* LOXDALE, H.D. & J. DEN HOLLANDER (Eds.). Clarendon Press, Oxford: 143-180.

KNOLL, S., M. ROWELL-RAHIER, P. MARDULYN & J.M. PASTEELS (1996) – Spatial genetic structure of leaf beetle species with special emphasis on Alpine populations. In: *Chrysomelidae Biology, vol. 1: The classification, phylogeny and genetics.* JOLIVET, P.H.A. & M.L. COX (Eds.). SPB Academic Publishing, Amsterdam, The Netherlands: 379-388.

PECK, S.B. (1996) – Origin and development of an insect fauna on a remote archipelago: The Galápagos Islands, Ecuador. In: *The origin and evolution of Pacific Island biotas, New Guinea*

to Eastern Polynesia : patterns and processes. A. KEAST & S.E. MILLER (Eds.). SPB Academic Publishing, Amsterdam, The Netherlands : 91-122.

RASSMANN, K., D. TAUTZ, F. TRILLMICH & C. GLIDDON (1997) – The microevolution of the Galápagos marine iguana *Amblyrhynchus cristatus* assessed by nuclear and mitochondrial genetic analyses. *Mol. Ecol.*, **6** : 437-452.

RAYMOND, M. & F. ROUSSET (1995) – GENEPOP (version 1.2) : population genetics software for exact tests and ecumenicism. *J. Heredity*, **86** : 248-249.

RICE, W.R. (1989) – Analyzing tables of statistical tests. *Evolution,* **43** : 23-30.

SNELL, H.I., H.M. SNELL & C.R. TRACY (1984) – Variation among populations of Galápagos land iguanas (*Conolophus*) : contrasts of phylogeny and ecology. *Biol. J. Linn. Soc.*, **21** : 185-207.

STERN, D.L. & P.R. GRANT (1996) – A phylogenetic reanalysis of allozyme variation among populations of Galápagos finches. *Zool. J. Linn. Soc.,* **118** : 119-134.

SWOFFORD, D.L. & R.B. SELANDER (1989) – *BIOSYS-1 : a computer program for the analysis of allelic variation in population genetics and biochemical systematics.* Illinois Natural History Survey (43 pp).

VERDYCK, P. (in press) – Genetic diversity and host plant use in the phytophagous Chrysomelidae : an evaluation of the niche width variation hypothesis. *Proceedings of the Fourth International Symposium on the Chrysomelidae, XX International Congress of Entomology, Firenze 1996.*

VERDYCK, P., H. DE WOLF, K. DESENDER, J. HULSELMANS & P. GROOTAERT (1998.) – Genetic comparison of two colour morphs of *Phyllotreta tetrastigma* (Coleoptera : Alticinae). *Belg. J. Zool.*, **128** : 119-134.

WRIGHT, S. (1978) – Evolution and the genetics of populations, vol. **4** Variability within and among natural populations. University of Chicago Press, Chicago (580 pp).