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Genetic population structure and range colonisation of *Nezara viridula*

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Abstract

Genetic variation in *Nezara viridula* (L.) sampled from 11 locations (Slovenia, Italy, Greece, France, Madeira, Guadeloupe, Galapagos, California, Brazil, Japan, and Botswana) was studied by sequencing 16S and 28S rDNA, cyt b and COI gene fragments and randomly amplified polymorphic DNA (RAPD) analysis. Sequencing revealed 11 distinct haplotypes clustering into three main lineages. Lineage C was limited to a single specimen from Botswana and lineage B to Japan, while lineage A haplotypes were found in the remaining populations as well as in Japan. RAPD data were more variable but consistent with the structuring of mtDNA sequences. Sequence and RAPD results both support the African origin of *N. viridula*, followed by early dispersal to Asia and, more recently, by expansion to Europe and America. Japanese specimens with mtDNA lineage A haplotypes were revealed as Euro-Asian hybrids by RAPD analysis, suggesting a multiple colonisation of Japan. Invariant sequences of the 28S rDNA combined with other results do not support the hypothesis that cryptic (sibling) species exist within the populations investigated in this study.

Key words: cryptic species, dispersal, mitochondrial DNA, native area, RAPD.

Introduction

Nezara viridula (L.) is thought to have originated in the Afrotropic realm (Hokkanen, 1986; Jones, 1988), but it now occurs worldwide between latitudes 45° N and 45° S (Todd, 1989). It is still an active invading species and it has been suggested that its current spread into temperate regions is made possible by global warming (Musolin and Numata, 2003). This study was set to examine molecular genetic structure and differentiation of allopatric populations of *N. viridula* in order to decipher the native range of the species and broad-scale dispersal pattern, as well as to check whether molecular data support the hypothesis that the taxon *N. viridula* comprises a complex of cryptic species. The results of the study have been published in Kavar *et al*. (2006).

Materials and methods

N. viridula was sampled from 11 locations [Slovenia, Italy, Greece, France, Madeira (Portugal), Guadeloupe (Lesser Antilles), Galapagos (Ecuador), California, Brazil, Japan and Botswana] and stored in 96% ethanol. DNA was extracted from dissected thoracic muscles.

The fragments of the nuclear 28S rDNA, and mitochondrial 16S rDNA, COI and cyt b genes were amplified using previously published primers (Muraji and Tachikawa, 2000; Muraji *et al*., 2000a; 2000b) and sequenced on ABI PRISM 310 DNA Sequencer (Applied Biosystems). Sequences were aligned using ClustalW. Distance matrix based on the Kimura's two-parameter method was analysed by neighbour-joining (NJ) algorithm. Sequences of two other pentatomid bugs, *Piezodorus lituratus* (F.) and *Rhaphigaster nebulosa* (Poda) were used as out-groups.

Random amplified polymorphic DNA (RAPD) PCR amplifications were performed with 12 primers (Operon Technologies). A unique RAPD profile was generated for each individual (n = 68) by scoring 94 (3-16 per primer, 200-800 bp) reproducible polymorphic bands. Distance matrix based on Nei and Li's similarity coefficient was analysed by principal components analysis (PCA) and NJ algorithm.

Results and discussion

All *N. viridula* 28S rDNA sequences were identical, but differed from those of out-group species. The three mtDNA gene fragments generated a single 1201 bp sequence data set. 11 haplotypes were identified, clustering into three main lineages (figure 1a): lineage C was limited to a single specimen from Botswana and lineage B to Japan, while the specimens from lineage A were found in all of the remaining populations as well as in Japan. RAPD data were more variable, but consistent with structuring of mtDNA sequences (figure 1b). Both sets of data supported the African origin of *N. viridula*, followed by early dispersal out of Africa and, more recently, by expansion to Europe and America. Assuming 2.3% sequence divergence per million years (Brower, 1994), the observed values suggest that the African and non-African gene pools have separated in mid-Pliocene (3.7-4 Ma), and European and Far Eastern lineages in mid-Pleistocene (1.2 Ma). Both splits can be explained by vicariance due to changes in climate and vegetation. Contemporary populations inhabiting Eurasia most likely originated from postglacial expansion of refugial populations in Central Africa, in the Indian subcontinent and in SE Asia. Colonisation of Europe may have been facilitated by the Neolithic spread of agriculture from the Levant. Considering the similarities and distribution of lineage A haplotypes, two distinct routes appear to have led to the colonisation of America. One leading from the E Mediterranean (Greece, Italy) to Central

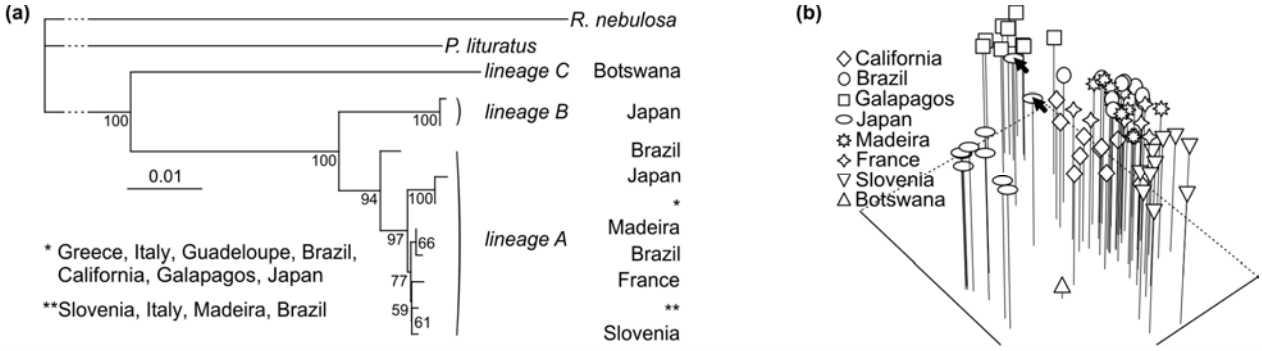

Figure 1. *N. viridula* molecular genetic analysis. (a) Neighbour-joining tree showing relationship among composite mtDNA haplotypes (16S rDNA, COI, cyt b). Sequences of *P. lituratus* and *R. nebulosa* were used as out-groups. Bootstrap values are given at the nodes. (b) Principal components plot based on RAPD data from eight allopatric samples. Arrows point to the two Japanese specimens of inferred hybrid origin.

America and from there on to the Eastern USA and the Western coastal areas of S America (and possibly also to California); and another, which originated in the W Mediterranean (Iberian peninsula) and led to the E coastal areas of S America. Two Japanese specimens with mtDNA lineage A haplotypes were revealed as Euro-Asian hybrids by RAPD analysis, which is strongly suggestive of a recent secondary introduction ("cryptic invasion") of a European/American haplotype into the islands.

Genetic distances among the non-African populations were in the range found at the intraspecific level in insects (e.g. Brown *et al*., 1994). Therefore we cannot, on the basis of genetic differentiation, consider these populations as distinct species. The specimen from Botswana, on the other hand, was characterised by three specific RAPD fragments and numerous nucleotide substitutions. Since its sequence differed from other haplotypes by 8.5 to 9.3%, which is more typical of interspecific comparisons in insects (Funk, 1999), the specimen may represent a distinct species.

Conclusion

This study lends further support to the previously proposed African origin of *N. viridula* and presents evidence of pre-Pleistocenic dispersal into Asia and postglacial (re)colonization of Eurasian and American continents. However, detailed inferences about the historical range, dispersal pattern and existence of cryptic species will require application of high-resolution molecular markers in conjunction with more comprehensive sampling of populations.

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References

BROWER A. V. Z., 1994.- Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution.- *Proceedings of the National Academy of Sciences*, 91: 6491-6495.

BROWN J. M., PELLMYR O., THOMPSON J. N., HARRISON R. G., 1994.- Phylogeny of Greya (Lepidoptera: Prodoxidae), based on nucleotide sequence variation in mitochondrial cytochrome oxidase I and II: congruence with morphological data.- *Molecular Biology and Evolution*, 11: 128-141.

FUNK D. J., 1999.- Molecular systematics of cytochrome oxidase I and 16S from Neochlamisus leaf beetles and the importance of sampling.- *Molecular Biology and Evolution*, 16: 67-82.

HOKKANEN H., 1986.- Polymorphism, parasites and the native area of *Nezara viridula* (Hemiptera, Pentatomidae).- *Annales Entomologici Fennici*, 52: 28-31.

JONES W. A., 1988.- World review of the parasitoids of the southern green stink bug *Nezara viridula* (L.) (Heteroptera: Pentatomidae).- *Annals of the Entomological Society of America*, 81: 262-273.

KAVAR T., PAVLOVČIČ P., SUŠNIK S., MEGLIČ V., VIRANT-DOBERLET M., 2006.- Genetic differentiation of geographically separated populations of the southern green stink bug *Nezara viridula* (Hemiptera: Pentatomidae).- *Bulletin of Entomological Research*, 96: 117-128.

MURAJI M., TACHIKAWA S., 2000.- Phylogenetic analysis of water striders (Hemiptera: Gerroidea) based on partial sequences of mitochondrial and nuclear ribosomal RNA genes.- *Entomological Science*, 3: 615-626.

MURAJI M., KAWASAKI K., SHIMIZU T., 2000a.- Phylogenetic utility of nucleotide sequences of mitochondrial 16S ribosomal RNA and cytochrome b genes in anthocorid bugs (Heteroptera: Anthocoridae).- *Applied Entomology and Zoology*, 35: 293-300.

MURAJI M., KAWASAKI K., SHIMIZU T., 2000b.- Nucleotide sequence variation and phylogenetic utility of the mitochondrial COI fragment in anthocorid bugs (Hemiptera: Anthocoridae).- *Applied Entomology and Zoology*, 35: 301-307.

MUSOLIN D. L., NUMATA H., 2003.- Timing of diapause induction and it's life-history consequences in *Nezara viridula*: is it costly to expand the distribution range.- *Ecological Entomology*, 28: 694-703.

TODD J. W., 1989.- Ecology and behavior of *Nezara viridula*.- *Annual Review of Entomology*, 34: 273-292.

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