GENETIC DISTANCE IN THE GENUS EPHIPPIGER (ORTHOPTERA, TETTIGONIOIDEA) – A RECONNAISSANCE

by

L. OUDMAN, W. LANDMAN & M. DUIJM

ABSTRACT

Oudman, L., W. Landman & M. Duijm, 1989. Genetic distance in the genus *Ephippiger* (Orthoptera, Tettigonioidea). – a reconnaissance. – Tijdschrift voor Entomologie 132: 177-181, figs 1-2, tabs 1-3. [ISSN 0040-7496]. Published 1 December 1989.

Genetic distances were determined by means of enzyme electrophoresis for a number of *Ephippiger* (sub)species, mainly from southern France and northern Italy. For each (sub)species and form one 'typical' location was selected. The results are summarized in a dendrogram. The three groups distinguished by Duijm & Oudman (1983) on the base of copulatory behaviour and morphological characters are confirmed. Nei's genetic distances between *E. ephippiger*, *E. cruciger* and *E. cunii* appeared to be low for genuine species. A comparison with *Uromenus rugosicollis* is included.

Keywords. - Ephippiger, enzyme electrophoresis, genetic distance, dendrogram.

L. Oudman, Department of Genetics, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands; W. Landman, Noorder Dierenpark/Zoo, Emmen, The Netherlands; M. Duijm, Department of Zoology, University of Groningen; present adress: Onnerweg 41, 9751 VB Haren, The Netherlands.

INTRODUCTION

In France, the northern part of Spain and NW Italy a number of Ephippiger (sub)species occur (e.g. Chopard 1951; Harz 1969). For this region Duijm & Oudman (1983) recognised three groups of (sub)spieces, viz. 1 - Ephippiger provincialis (Yersin, 1854), 2 - E. terrestris (Yersin, 1854) with the three (sub)species E.t. terrestris, E.t. bormansi (Brunner von Wattenwyl, 1882) and E. t. caprai Nadig, 1980, 3 - E. ephippiger (Fieber, 1853) (the subspieces E. e. vitium (Serville, 1831)¹) and E. e. vicheti Harz, 1966) E. cunii (Bolivar, 1877) and E. cruciger (Fieber, 1853). Between these groups no mating is possible. Within group 2 no mating barriers were found, whereas mating between species of group 3 is possible (Hartley & Warne 1984), but not in all cases (Duijm & Oudman 1983). Identification of single specimens and even populations belonging to group 3 often meets with considerable difficulties owing to the large variability of the morphological characters. The present study is an attempt to elucidate the relations between these Ephippiger taxa by the investigation of enzyme polymorphism. For comparison the Ephippigerid Uromenus rugosicollis (Serville, 1839) is used.

MATERIALS AND METHODS

The insects were collected during field trips in August and September of 1979, 1980, 1981, 1982 and 1983. Generally we succeeded in collecting a sufficient number (c 20) from a restricted area of a few acres. This area had to be small to limit ourselves to one population (or part of it) and so to avoid the mixing of different populations. The animals were killed, measured, photographed and frozen in solid carbon dioxide (-79°C).

For this "reconnaissance" it appeared desirable to omit the intra- (sub)specifical variation in order to get a clearer picture. For each taxon we therefore selected one locality that we considered sufficiently typical. In this selection we used – if possible – the type locality or our nearest collecting site and otherwise a locality that was in good concordance with the morphological description and/or in the neighbourhood of the centre of distribution²). The

¹) According to Kruseman (1988) the correct name for this subspecies is *E. ephippiger diurnus* Dufour, 1841.

²) Later work (Landman et al. 1989) showed that the population used in this study as representative for *E. t. terrestris* (Col de Castillon, No. 2), though in many respects very close to the nominate form cannot be regarded as entirely "pure".

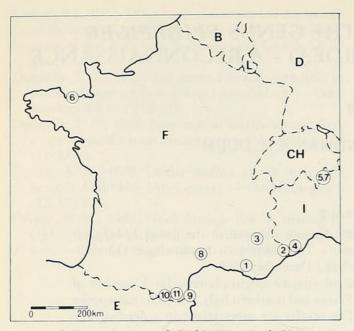


Fig. 1. Collection sites of *Ephippiger* and *Uromenus* species in SW Europe. Numbers denote the sites, see table 1.

sites selected are summarised in table 1 and fig. 1.

For preparation of the samples, electrophoretic techniques and the preparation of the horizontal polyacrylamide gels we refer to Van Dijk & Van Delden (1981).

The following loci were analysed: Alcohol dehydrogenase (Adh), Tetrazolium oxidase (To), two Phosphoglucomutases (Pgm-2 and Pgm-3), Malic enzyme (Me), Fructosediphosphate aldolase (Ald), Esterase-2 (Est-2), Hexokinase-3 (Hk-3), Fumarate hydratase (Fum), Xanthine dehydrogenase (Xdh), Isocitrate dehydrogenase (Idh), Glucose oxidase-3 (Gluo-3), α -Glycerophosphate dehydrogenase (α -Gpdh). For polymorphic loci Mendelian inheritance was assumed on base of enzyme band patterns and checked by testing genotype frequencies for every location for Hardy-Weinberg equilibrium.

Between populations Nei's genetic distances were calculated (Nei 1975). From the matrix of

No.	(Sub)species	Location	Country/dept.	Alt. m.	Date
1.	E. provincialis	Plan d'Aups	F 84	680	24-viii-80
2.	E.t. terrestris	Col de Castillon	F 06	700	31-viii-81
3.	E.t. terr. f minor	Col de Maure	F 04	1350	24-viii-81
4.	E.t. caprai	Cle. Scravaion	I Liguria	820	3-ix-81
5.	E.t. bormansi	Naggio (L. di Como)	I Lombardia	800	5/6-ix-81
6.	E.e. diurnus	Cap Fréhel	F 22	70	9-ix-83
7.	E.e. vicheti	Naggio (L. di Como)	I Lombardia	750	5/6-ix-81
8.	E. cruciger	Gignac	F 34	60	3-vii-82
9.	E. cunii	Cerbère	F 66	10	9-vii-82
10.	E. cunii f. jugicola	Val d'Eyne	F 66	1600 -	22-viii-82
11.	Uromenus rugosicollis	Canigou	F 66	840	31-viii-79

Table 1. Sites of collection of the selected populations of Ephippiger and Uromenus species.

Table 2. Allele frequencies of the polymorphic loci of Ephippiger and Uromenus species.

(sub)species		n	Pgm-2	Pgm-2		i dinusi	Pgm-3	oldigeo	nung a ba	na na
	and the second		12	14	16	20	26	29	32	35
1	E. provincialis	16	0	0	0	1.00	0	.14	.86	0
2	E.t. terrestris	18	.50	.50	0	0	.27	.67	0	.07
3	E.t.t.f. minor	13	.23	.77	0	0	0	0	.69	.31
4	E.t. caprai	23	.28	.65	.07	0	0	.96	.04	0
5	E.t. bormansi	18	.69	.31	0	0	0	0	1.00	0
6	E.e. diurnus	20	0	0	0	1.00	.70	.30	0	0
7	E.e. vicheti	21	0	0	0	1.00	1.00	0	0	0
8	E. cruciger	26	0	0	0	1.00	.35	.23	.31	.11
9	E. cunii	20	0	0	0	1.00	.20	.80	0	0
10	E. cunii f. jugicola	24	0	0	0	1.00	0	1.00	0	0
11	Uromenus rugosicollis	15	ing the second	-	-	-	.13	.53	.33	0

genetic distances a dendrogram was constructed following the UPGMA method (Sneath and Sokal 1973).

Samples of collected (sub)spieces from all localities, including tips of abdomens used for electrophoresis, will be deposited in the Entomological collection of the Institute for Taxonomical Zoology (Zoological Museum) in Amsterdam.

RESULTS

Thirteen loci were investigated of which eight were monomorphic. Five loci showed polymorphism: Est-2, Pgm-2, Pgm-3, To and Adh, with respectively 4, 4, 4, 3 and 2 alleles. The allozyme frequencies are given in table 2. To is fixed in most populations. Adh is only polymorphic in *E. t. caprai*. Pgm-2 is only variable in *E. terrestris*. The most variable enzymes are Pgm-3 and Est-2.

The genetic distances are shown in table 3 and the dendrogram, calculated from these distances, in fig. 2.

Based on a preliminary investigation (Landman, 1981) we determined the genetic distance between *Uromenus rugosicollis* and a number of *Ephippiger* (sub)spieces (18 populations, 9 loci, 18 alleles) at 0.3473. An indication of this distance is added to the dendrogram.

DISCUSSION

The genetic distance (0.35) between the closely related genera *Uromenus* and *Ephippiger* appears to be very low in view of the range for genera mentioned in reviews (e. g. Thorpe 1982, Menken & Ulenberg 1987). The distance between genera generally is 1, in the mean 1.30, and minimally 0.62.

The distance found by us between *E. provincialis* and the other *Ephippiger* species (0.20) as well as

the distance between our groups 2 and 3 (0.16) are very low for congeneric species. According to Thorpe (1982) only in 3% of the cases studied distances below 0.16 are found for congeneric species.

The distances between the subspecies of *E. ter*restris (0.015 - 0.11) are within the range generally found between subspecies: 0.02 - 0.22 (Menken & Ulenberg 1987) except one: the distance (0.015)between *E.t. caprai* and *E.t. terrestris* from Col de Castillon. This very small distance is one of the indications that the population of Castillon is not quite representative for the nominate form of *E. t. terrestris*.

The distances between the species within our group 3 are strikingly small (0.03); they lie in the range for subspecies. This is in accordance with the results of Hartley and Warne (1984).

The dendrogram of fig. 2 offers a picture that is mainly in accordance with current taxonomical opinion. It also conforms to our grouping (Duijm & Oudman 1983) based on morphological data and on the existence of mating barriers. Within group 3, however, *E. e. vicheti* occupies a rather separate position ¹). The relations between *E. e. diurnus*, *E. cruciger* and *E. cunii* as well as those within the *terrestris*-group will be dealt with in later publications.

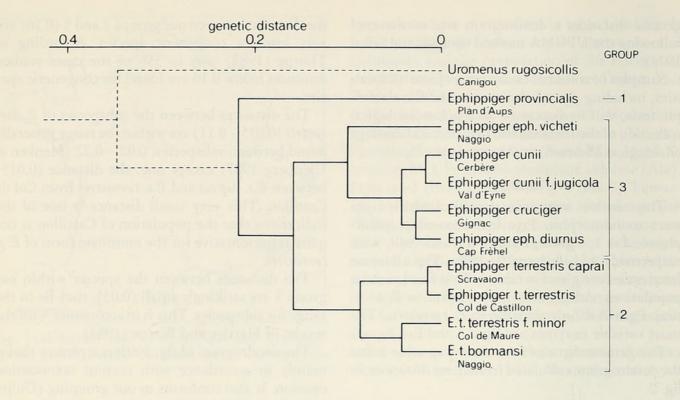
ACKNOWLEDGEMENTS

We would like to thank the Uyttenboogaart-Eliasen Stichting in Amsterdam for financial support. We are particularly indebted to Marten Zijlstra and Klaas Vrieling who helped us with the

 Nadig (1987,p.331) raises this taxon to species-level: E. vicheti Harz, 1966.

1	Tal	10	2	continued	1
(Tat	ne	2	continued)

Est-2					То		Adh				
15	17	20 -	23		40	63	- 65	67	18	22 -	
.78-	.22	0	0		0	1.00	0	0	1.00	0	Dist.
.08	.67	.25	0		0	0	0	1.00	1.00	0	
.42	.23	.35	0		0	0	. 0	1.00	1.00	0	
.10	.57	.33	. 0		0	. 0	0	1.00	.80	.20	
0	.24	.76	0		0	0	0	1.00	1.00	0	
0	1.00	0	0		0	0	0	1.00	1.00	0	
0	0	.08	.92		0	0	0	1.00	1.00	0	
.04	.50	.46	0		.06	0	0	.94	1.00	0	
.25	.67	.08	0		0	0	0	1.00	1.00	0	
.08	.33	.54	.04		0	0	0	1.00	>1.00	0	
0	.02	.90	.08		0	0	1.00	0	1.00	0	



computing work. We thank Prof. W. van Delden for providing laboratory facilities and for valuable discussions and continued interest. Our thanks are due to H. van Dijk for his instruction in electrophoretic techniques. We are very grateful to Dr. A. Nadig (Chur), who generously supplied us with information about his collecting sites. We thank A. Decae, G. J. van Klinken, H. Hidding and G. Overkamp, who assisted in collecting the insects. We are grateful to Dr. M. G. Ritchie for critically reading the manuscript and for correcting the English. Furthermore we thank Mrs. J. Poelstra for typing the manuscript and D. Visser for making the drawings. Fig. 2. Dendrogram of Ephippigeridae based on the genetic distances of table 3. An estimation of the distance between *Uromenus* and the *Ephippiger* (sub)species is added on the base of preliminary work (Landman, not published).

Group	(sub)species		1	2	.3	4	5	6	7	8	9	10
1	1	E. provincialis	-	.2522	.1840	.2668	.2167	.1971	.2337	.1408	.1631	.1890
2	2	E.t. terrestris		-	.0680	.0147	.0899	.0858	.1687	.0846	.0687	.0815
	3	E.t.t. f. minor			-	.0792	.0409	.1649	.1956	.1016	.1439	.1514
	4	E.t. caprai				-	.1115	.1232	.2152	.1073	.0761	.0726
	5	E.t. bormansi					-	.1908	.2192	.1059	.1771	.1598
3	6	E.e. diurnus						-	.0832	.0288	.0276	.0717
	7	E.e. vicheti							-	.0766	.1109	.1309
	8	E. cruciger								-	.0287	.0363
	9	E. cunii									-	.0179
	10	E. cunii f. jugicola	2									- 66

Table 3. Genetic distances based on ailozyme frequencies of thirteen loci of Ephippiger species.

References

- Chopard, L., 1951. Orthopteroides. Faune de France 56: 1-359.
- Van Dijk, H. & W. van Delden, 1981. Genetic variability in *Plantago* species in relation to their ecology. I. Genetic analysis of the allozyme variation in *P. major* subspecies. – Theoretical and Applied Genetics 60: 285-290.
- Duijm, M. & L. Oudman, 1983. Interspecific mating in *Ephippiger* (Orthoptera, Tettigonioidea). – Tijdschrift voor Entomologie 126: 97-108.
- Ferguson, A., 1980. Biochemical Systematics and Evolution. – Blackie, London.
- Hartley, J. C. & A. C. Warne, 1984. Taxonomy of the Ephippiger ephippiger complex (ephippiger, cruciger and cunii) with special reference to the mechanics of copulation. - Eos 60: 43-54.
- Harz, K., 1969. Die Orthopteren Europas, 1. Series Entomologica 5: i-xx, 1-749.
- Kruseman, G., 1988. Matériaux pour la faunistique des Orthoptères de France, Fascicule I, Les Ensifères. – Verslagen en Technische gegevens 51. Instituut voor Taxonomische Zoölogie (Zoölogisch Museum), Universiteit van Amsterdam.
- Landman, W. L., 1981. Genetische afstand en verwantschap in de Ephippigeridae. – Internal report Department of Genetics, University of Groningen. [unpublished].

- Landman, W. L., L. Oudman & M. Duijm, 1989. Allozymic and morphological variation in *Ephippiger terrestris* (Yersin) 1854. – Tijdschrift voor Entomologie 132: 183-198.
- Menken, S. B. J. & S. A. Ulenberg, 1987. Biochemical Characters in Agricultural Entomology. – Agricultural Zoology Reviews 2: 305-360.
- Nadig, A., 1987. Saltatoria (Insecta) der Süd und Südostabdachung der Alpen zwischen der Provence im W, dem pannonischen Raum im NE und Istrien im SE. I Tettigoniidae. – Revue suisse de Zoologie 94: 257-356.
- Nei, M., 1975. Molecular population genetics and evolution. - North Holland Publ. Co., Amsterdam.
- Sneath, P. H. A. & R. R. Sokal, 1973. Numerical Taxonomy. – Freeman, San Francisco.
- Thorpe, J. P., 1982. The Molecular Clock Hypothesis: Biochemical Evolution, Genetic Differentiation and Systematics. – Annual Review of Ecology and Systematics 13: 139-168.

Received: 4 January 1989 Revised version accepted: 29 May 1989



Oudman, L, Landman, W, and Duijm, M. 1989. "Genetic distance in the genus Ephippiger (Orthoptera, Tettigonioidea) - a reconnaissance." *Tijdschrift voor entomologie* 132, 177–181.

View This Item Online: <u>https://www.biodiversitylibrary.org/item/89703</u> Permalink: <u>https://www.biodiversitylibrary.org/partpdf/66449</u>

Holding Institution Harvard University, Museum of Comparative Zoology, Ernst Mayr Library

Sponsored by Harvard University, Museum of Comparative Zoology, Ernst Mayr Library

Copyright & Reuse Copyright Status: In copyright. Digitized with the permission of the rights holder. License: <u>http://creativecommons.org/licenses/by-nc-sa/3.0/</u> Rights: <u>https://biodiversitylibrary.org/permissions</u>

This document was created from content at the **Biodiversity Heritage Library**, the world's largest open access digital library for biodiversity literature and archives. Visit BHL at https://www.biodiversitylibrary.org.