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Ecological and genetic studies on parapatric *Rhymogona silvatica* Roth. and *R. cervina* Verh. (Diplopoda: Craspedosomatidae) with special reference to hybrid populations in a zone of contact

by

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With 4 figures

ABSTRACT

Field studies did not detect differences in phenology, vertical distribution or habitat preference of *R. silvatica* and *R. cervina*. However, genetic differentiation of both taxa is revealed by a survey of allozymic variation based on starch gel electrophoresis. Enzyme electrophoretic data furthermore confirm hybridisation of *R. silvatica* and *R. cervina* in their zone of contact, as was previously suggested by morphological studies of male and female genitalia.

INTRODUCTION

Rhymogona is a small genus of the Diplopoda family Craspedosomatidae. It was described in 1896 by Cook and houses presently eight nominal species which are distinguished by subtle differences in morphology of genitalia, usually of males, but in some species of females as well. Several of these species are known from one or a few localities only. In fact the whole genus has a very restricted distribution, which extends

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north of the Swiss Alps to the Black Forest in the northeast and is limited from northwest to southwest by the Vosges, Côte d'Or and the Savoie.

The distribution of the genus *Rhymogona* was recently reviewed by PEDROLI-CHRISTEN (1990) with some comments on taxonomical problems. Field studies concentrated on two species, *R. silvatica* and *R. cervina,* which were discovered in the Swiss Jura. While analysing the distribution of these species, it became apparent, that they form zones of contact both in the Swiss Jura and in the alps. Morphological examination of specimens from these zones of contact suggested hybridisation of the two taxa. The contact zones are very small and range from a few hundred meters to about 5-10 km in width.

In this investigation we have analysed the phenology, vertical distribution and habitat preferences of *R. silvatica* and *R. cervina.* In addition we have attempted to substantiate the hypothesis of hybridisation of these taxa in their zones of contact by genetic studies involving a survey of allozymic variation based on starch gel electrophoresis.

MATERIAL AND METHODS

1. FIELD STUDIES

Investigation of phenology, vertical distribution and habitat preferences was conducted by observation and by pitfall (Barber) trapping. Occasional observations were made between 1976 and 1984, followed by a very intensive study in 1985 and 1986. In this latter period the population densities were estimated by a relative index (n ad/h) which gives the number of sexually mature individuals observed per hour. In order to more quantitatively compare the vertical distribution and habitat preferences of both taxa, we calculated a fraction of positive sampling sites. A sampling site was designated "positive" if specimens were found within one hour.

The type of vegetation was analysed for each observation site, using additional information from the vegetation maps of RICHARD (1962).

Pitfall trapping (Barber) was conducted as part of more general surveys of the soil fauna carried out by the following:

— PEDROLI-CHRISTEN (1981), sampling in habitats of a submediterranean oak forest *(Coronillo-Quercetum)* at Châtillons/NE, 560 m, and a European beech forest *(Carici-Fagetum)* at Voëns/NE, 730 m, between March 1977 and February 1978;
— BORCARD (1981), sampling in habitats of an ash forest *(Carici elongatae Fraxinetum)* at Staatswald/BE, 433 m, and a submediterranean oak forest *(Coronillo-Quercetum)* at La Coudre/NE, 610 m, between February and December 1979;
— DELARZE (1986), sampling in dry grassland with *Stipa capillata* at La Bâtiaz/VS, 550 m, and with *Bromus* and *Stipa eriocaulis* at Follaterres/VS, 550 m, between 1979 and 1982;
— BASSET (unpublished), sampling in a cave, Grotte de chemin de fer/NE, 610 m, in 1980 and 1981.

2. ENZYME ELECTROPHORESIS

2.1. Material

The material for enzyme electrophoretic studies was collected at 11 sampling sites in 1987. The selection of these sampling sites was based on the following considerations:

— Previous occurrence of relatively high population densities and sampling success;
— Four samples were taken at localities at which putative hybrid populations had occurred in previous years (populations 8-11 in Fig. 1);
— For both *R. silvatica* and *R. cervina* a population has been chosen close (some 5 km) to the zone of contact of both species (in *R. silvatica* this is population 2, at Mauborget/VD and in *R. cervina* population 4, at Le Prévoux/NE, see Fig. 1) as well as a population more distant from this zone (*R. silvatica:* population 1, at St. Georges/VD; *R. cervina:* population 5, at Pertuis/NE, see Fig. 1);

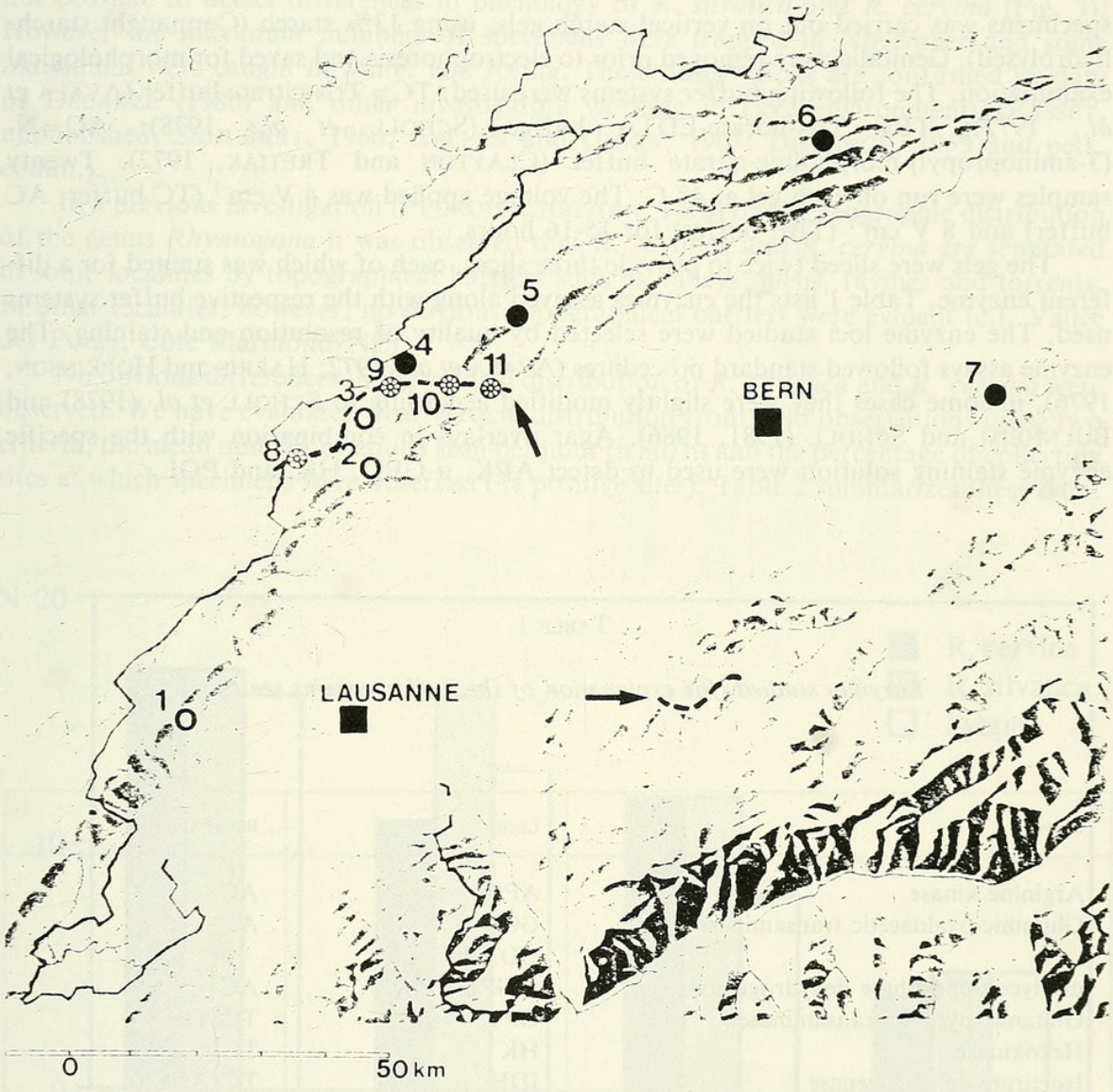

FIG. 1.

Sampling sites of *Rhymogona* populations used for electrophoretic studies. *R. silvatica* (O), 1: St. Georges/VD, 2: Mauborget/VD, 3: La Brévine/NE. *R. cervina* (●), 4: Le Prévoux/NE, 5: Pertuis/NE, 6: Schelten/BE, 7: Napf/BE. Putative hybrid populations (⊛), 8: La Côte-aux-Fées/NE, 9: La Chaux-du-Milieu/NE, 10: Mauvaise Combe/NE, 11: Peseux/NE. Dotted lines (arrows): Zones of contact of *R. silvatica* and *R. cervina*.
(The figure was designed using plate 3 of the Atlas of Switzerland, 1965, Ed. Imhof and H. Leuzinger, editors, reproducted by permission of the Federal Office of Topography, from February 3rd, 1989).

— Samples were also taken from three sites at which low numbers of individuals were caught but which are nevertheless of interest within the context of this investigation. These sites were at La Brévine/NE, located very close to the zone of contact (see Fig. 1), where a population of *R. silvatica* occurred and at Schelten/BE and Napf/BE, located 65 km northeast and 100 km east respectively from the zone of contact, where *R. cervina* was found (populations 3, 6 and 7 in Fig. 1).

2.2. Methods

The electrophoretic methods are essentially those of earlier studies (SCHOLL *et al.*, 1978; BULNHEIM and SCHOLL, 1981, 1986). Electrophoretic analysis of individual specimens was carried out on vertical starch gels, using 13% starch (Connaught starch-hydrolysed). Genitalia were removed prior to electrophoresis and saved for morphological examination. The following buffer systems were used: TC = Tris-citrate buffer (AYALA *et al.*, 1972); TBE = Tris-borate-EDTA buffer (SCHOLL *et al.*, 1978); AC = N-(3-aminopropyl)-morpholine-citrate buffer (CLAYTON and TRETIAK, 1972). Twenty samples were run on each gel at 4° C. The voltage applied was 4 V cm^{-1} (TC buffer, AC buffer) and 8 V cm^{-1} (TBE buffer) for 15-16 hours.

The gels were sliced twice to provide three slices, each of which was stained for a different enzyme. Table 1 lists the enzymes assayed along with the respective buffer systems used. The enzyme loci studied were selected by quality of resolution and staining. The enzyme assays followed standard procedures (AYALA *et al.*, 1972; HARRIS and HOPKINSON, 1976), in some cases they were slightly modified according to SCHOLL *et al.* (1978) and BULNHEIM and SCHOLL (1981, 1986). Agar overlays in combination with the specific enzyme staining solution were used to detect APK, α-GPD, HK, and PGI.

TABLE 1.

Enzymes studied (for explanation of the buffer systems see text)

Enzyme	Locus	Buffer system
Arginine kinase	APK	AC
Glutamic-oxaloacetic transaminase	GOT-1	AC
	GOT-2	
α-Glycerophosphate dehydrogenase	α-GPD	AC
Glutamic-pyruvic transaminase	GPT	TC/TBE*
Hexokinase	HK	TC
Isocitrate dehydrogenase	IDH	TC/AC*
Malate dehydrogenase	MDH-1	TC
	MDH-2	
Malic enzyme	MOD	TBE
6-Phosphogluconate dehydrogenase	6-PGD	AC/TC*
Phosphoglucose isomerase	PGI	TC

* = these buffer systems were used alternatively and gave identical results.

RESULTS AND DISCUSSION

1. Field studies

Adult specimens of *R. silvatica, R. cervina* and of the putative hybrid were only observed in the field in September and October, and no differences in phenology of the three forms could be detected (Fig. 2). Numbers appeared high in early September and decreased continuously until the end of October. No specimens were observed after the beginning of November.

In studies with pitfall traps (Borcard, 1981; Pedroli-Christen, 1981) again it was not possible to detect differences in phenology of *R. silvatica* and *R. cervina* (Fig. 3). However the maximum numbers of specimens were trapped in November and some individuals were caught in winter and spring. These observations are confirmed by data of Delarze (1986) and other investigators working in cave environments (Basset, unpublished, Schubart, 1960; Aellen and Gigon, 1963; Demange, 1959 and pers. comm.).

In a previous investigation (Pedroli-Christen, 1990) on the geographic distribution of the genus *Rhymogona* it was observed that *R. silvatica* and *R. cervina* are separated in some localities by topographical barriers such as valleys, glens, ravines and torrents. In other localities, however, no obvious topographical barriers were evident (c.f. Vallée des Ponts, Côte Marmoud/NE).

No obvious differences in the vertical distribution of *R. silvatica* and *R. cervina* were observed. We have evaluated their vertical distribution from field observations using two criteria, the mean number of adults seen per hour (n ad/h) and the percentage of collecting sites at which specimens were observed (% positive sites). Table 2 summarizes these data.

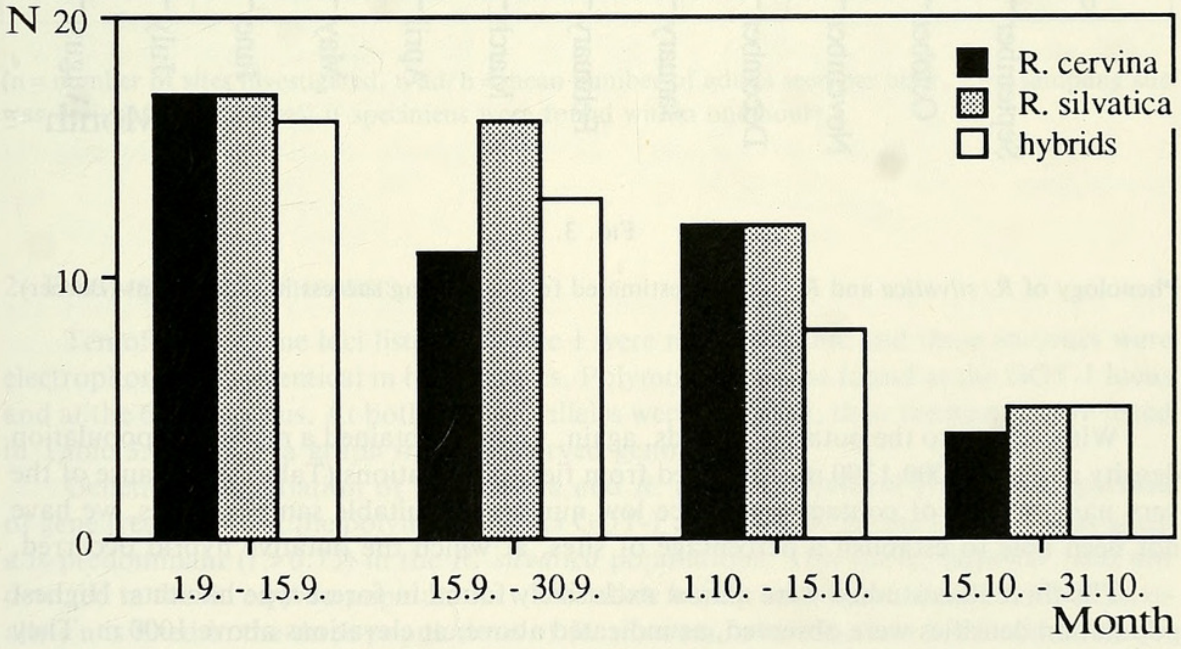

Fig. 2.

Phenology of *Rhymogona silvatica, R. cervina* and the putative hybrids, estimated from observation (adult specimens, pooled data from 1985 and 1986).

The mean number of adults seen per hour increases in both species from 6 specimens per hour at elevations below 750 m to about 14 specimens per hour above 1000 m. The fraction of positive sites increases in a similar fashion with altitude, it is low below 750 m, but much higher (more than 80%) at elevations above 1000 m.

In the alps both species were found in very low numbers above the tree line, at about 2000 m (*R. silvatica:* Rochers de Naye/VD, *R. cervina:* Kandersteg/BE, Arvenwald, 1900 m). At four sampling sites which are located between 430 m and 560 m they were occasionally observed in pitfall traps. Sampling success is very low at these low elevations, as is also evident from Table 2.

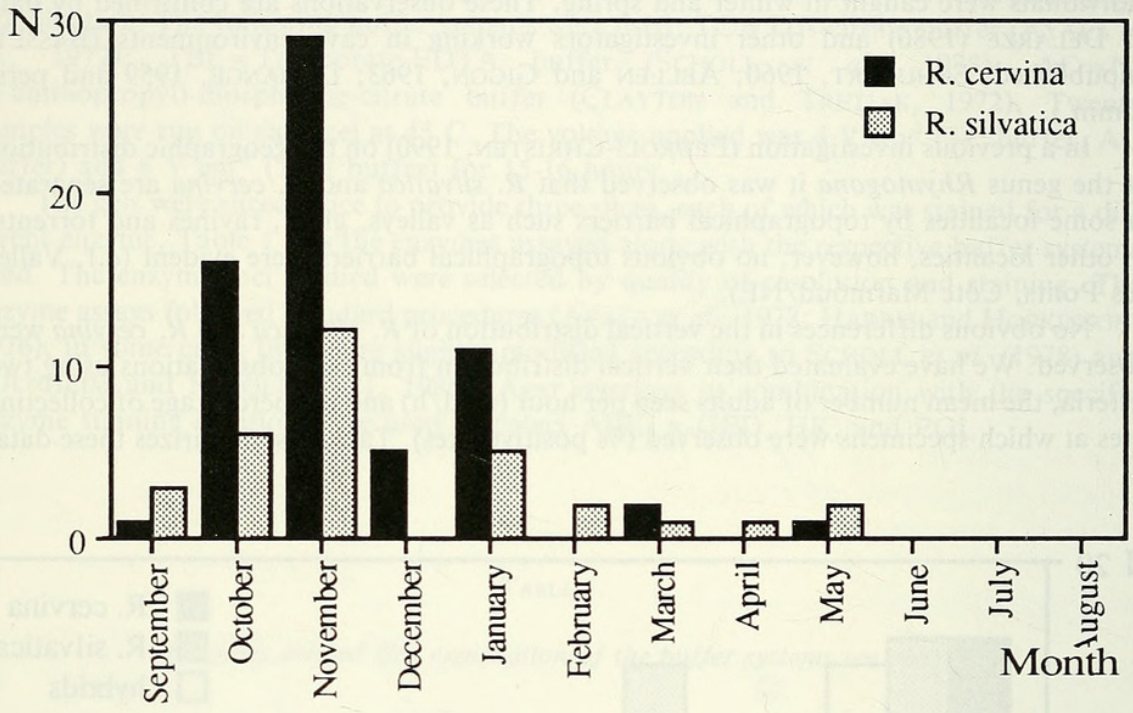

FIG. 3.

Phenology of *R. silvatica* and *R. cervina,* estimated from sampling success in pitfall traps (Barber).

With respect to the putative hybrids, again, we have obtained a maximum population density at about 1000-1300 m, as judged from field observations (Table 2). Because of the very narrow zone of contact and hence low number of suitable sampling sites, we have not been able to establish a percentage of sites, at which the putative hybrid occurred.

The three taxa studied were almost exclusively found in forest-type habitats. Highest population densities were observed, as indicated above, at elevations above 1000 m. They are associated with fir-beech forest *(Abieti-Fagetum)* of higher humidity with lush understory layer, dominated by *Adenostyles (Adenostylion)* or with vegetation of a more mosaic type, including fissured blocks covered with moss and *Vaccinium (Asplenio-Piceetum* or even *Aceri-Fagetum).* At elevations of about 2000 m the genus was found in

humid boulder fields with lush vegetation *(Adenostylion)*. Below 750 m there was a tendency to colonize mostly humid habitats such as fir-beech forest with *Petasites (Abieti-Fagetum),* ash forest *(Pruno-Fraxinetum)* and alder forest *(Carici elongatae-Alnetum).*

Captures of *R. cervina* in more submediterranean vegetation *(Coronillo-Quercetum)* as well as those of *R. silvatica* (two specimens) in dry grassland were unexpected.

TABLE 2.

Vertical distribution of Rhymogona silvatica, R. cervina *and the putative hybrid populations, estimated from observation at various elevations (pooled data from 1985 and 1986)*

	R. silvatica		*R. cervina*		putative hybrids	
altitude	n ad/h	n	n ad/h	n	n ad/h	n
< 750 m	6	2	6	1	4	1
750-1000 m	6,5	6	8,1	10	6,5	6
1000-1300 m	13,7	38	13,8	34	10,9	19
altitude	% positive sites*	n	% positives sites*	n		
< 750 m	22	9	33	3		
750-1000 m	60	10	67	15		
1000-1300 m	83	46	87	39		

(n = number of sites investigated, n ad/h = mean number of adults seen per hour, * = a sampling site was designated "positive" if specimens were found within one hour).

2. ENZYME ELECTROPHORESIS

Ten of the enzyme loci listed in Table 1 were monomorphic and these enzymes were electrophoretically identical in both species. Polymorphism was found at the GOT-1 locus and at the 6-PGD locus. At both loci two alleles were observed, their frequencies are listed in Table 3. Fig. 4 is a graph of the observed genotypes.

Genetic differentiation of *R. silvatica* and *R. cervina* is evident from a comparison of gene frequencies at the polymorphic loci GOT-1 and 6-PGD. At the GOT-1 locus allele **a** is predominant ($f > 0.75$) in the *R. silvatica* populations. This allele, however, was not detected in the *R. cervina* populations where allele **b** only was found. At the 6-PGD locus allele **a** is fixed in the three populations of *R. silvatica,* whereas allele **b** is fixed or nearly so in populations of *R. cervina* (Table 3, Fig. 4).

Even though there is some variation in gene frequencies at these loci in both *R. silvatica* and *R. cervina,* enzyme electrophoresis does allow for a biochemical identification of these taxa on the basis of GOT-1/6-PGD genotypes, as is illustrated in

TABLE 3.

Allele frequencies at polymorphic loci GOT-1 and 6-PGD in R. silvatica, R. cervina and putative hybrid populations in their zone of contact

Sampling site	N	GOT-1				6-PGD			
		b	a	H_o	H_e	b	a	H_o	H_e
R. silvatica									
St. Georges/VD	36	0.21	0.79	0.31	0.33	—	1.0		
Mauborget/VD	37	0.11	0.89	0.22	0.20	—	1.0		
La Brévine/NE	9	0.06	0.94	0.11	0.11	—	1.0		
R. cervina									
Le Prévoux/NE	31	1.0	—			0.98	0.02	0.05	0.04
Pertuis/NE	11	1.0	—			1.0	—		
Schelten/BE	4	(1.0)	—			(1.0)	—		
Napf/BE	3	(1.0)	—			(1.0)	—		
putative hybrid populations									
La Côte-aux-Fées/NE	12	0.96	0.04	0.08	0.08	0.67	0.33	0.67	0.44
La Chaux-du-Milieu/NE	16	0.66	0.34	0.31	0.45	0.53	0.47	0.56	0.50
La Mauvaise Combe/NE	12	0.50	0.50	0.33	0.50	0.63	0.37	0.58	0.47
Peseux NE	13	0.50	0.50	0.38	0.50	0.62	0.38	0.46	0.47

N = sample size, H_o = observed frequency of heterozygotes, H_e = expected frequency of heterozygotes.

Fig. 4 A. Samples from their zone of contact, however, show a very different picture, the majority of specimens are heterozygous either at the GOT-1 locus or at the 6-PGD locus or even at both loci (Fig. 4 B). In fact, the observed frequencies of heterozygotes (H_o) agreed fairly well with the expectation (H_e) if random mating is assumed (Table 3), and the observed frequencies of heterozygotes were not significantly different from Hardy-Weinberg frequencies. The electrophoretic data thus confirm the hypothesis of hybridisation of *R. silvatica* and *R. cervina* in their zone of contact, as was previously suggested (PEDROLI-CHRISTEN, 1990) based on morphological examination of male and female genitalia.

In order to evaluate gene flow estimates between *R. silvatica* and *R. cervina,* we have calculated parameters of G-statistics (NEI, 1975) from distributions of alleles at the two diagnostic loci GOT-1 and 6-PGD along a transect in the Swiss Jura. For this transect we have chosen the *R. silvatica* population at La Brévine and the *R. cervina* population at Le Prévoux, these populations are 10 km apart, and the putative hybrid-population at La

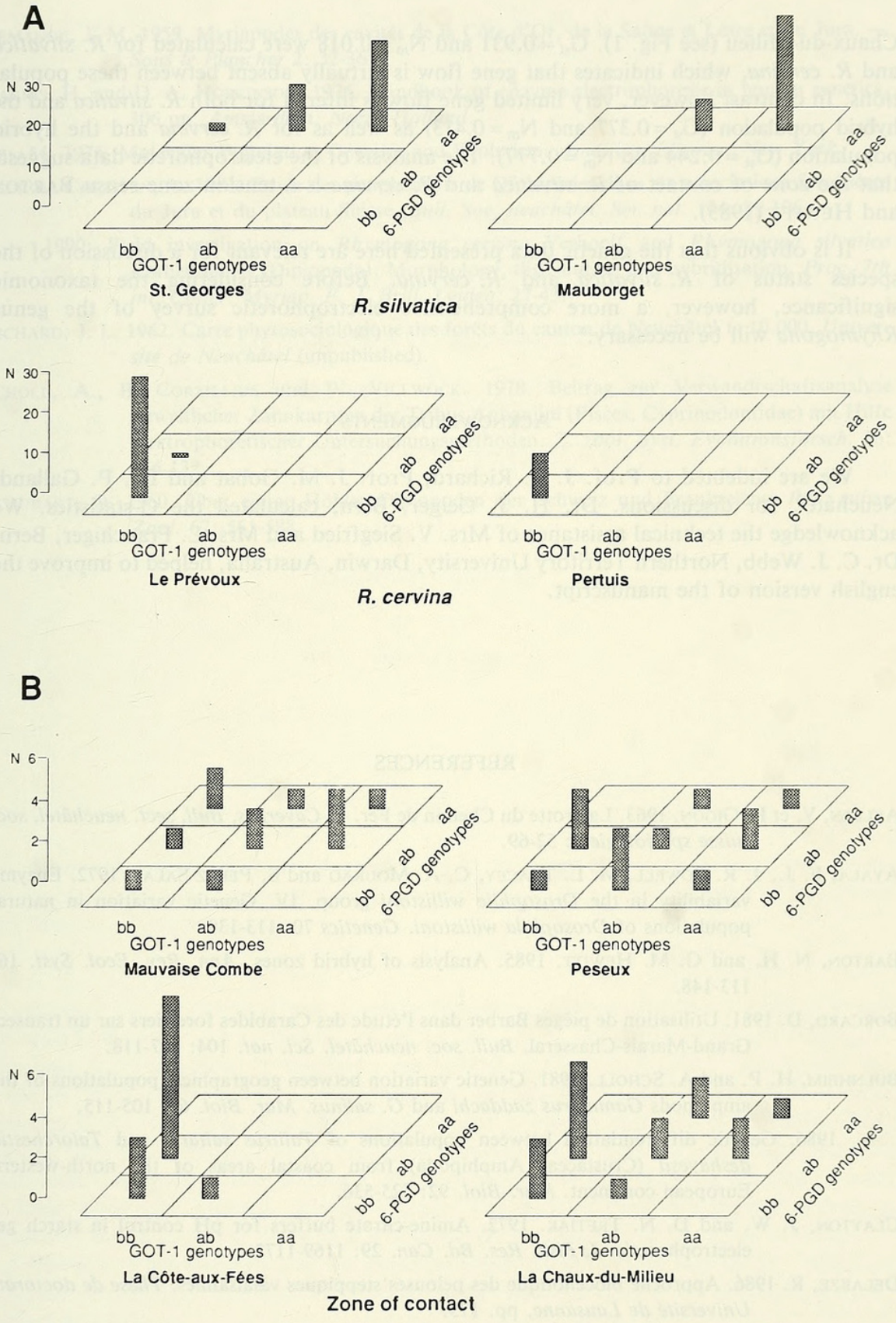

FIG. 4.

Graph of the enzyme genotypes found at the polymorphic loci GOT-1 and 6-PGD.
A: populations of *R. silvatica* and *R. cervina* outside the zone of contact.
B: populations within the zone of contact of *R. silvatica* and *R. cervina*.

Chaux-du-Milieu (see Fig. 1). $G_{st} = 0.931$ and $N_m = 0.018$ were calculated for *R. silvatica* and *R. cervina,* which indicates that gene flow is virtually absent between these populations. In contrast however, very limited gene flow is infered for both *R. silvatica* and the hybrid population ($G_{st} = 0.377$ and $N_m = 0.413$) as well as for *R. cervina* and the hybrid population ($G_{st} = 0.244$ and $N_m = 0.777$). The analysis of the electrophoretic data suggests that the zone of contact of *R. silvatica* and *R. cervina* is a tension zone sensu BARTON and HEWITT (1985).

It is obvious that the genetic data presented here are relevant for a discussion of the species status of *R. silvatica* and *R. cervina.* Before considering the taxonomic significance, however, a more comprehensive electrophoretic survey of the genus *Rhymogona* will be necessary.[1]

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