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Mating behaviour and barriers to hybridization in the cave beetle of the Speonomus delarouzeei complex (Coleoptera, Catopidae, Bathysciinae)

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SUMMARY

The complex *Speonomus delarouzeei* combines 6 species which were previously synonymized. Using behavioural data, based on 12 populations, the author assesses the validity of 4 species and points out the occurrence of 2 species as yet undescribed. Constant and important differences during the mating appear between these different species. They concern the number of behavioural steps, the duration, the number of clappings of antennae, the abdominal male movements and the rubbing of female abdomen. The results of crossing experiments between different species indicate a prezygotic reproductive isolation with atypical matings and no sperm deposit. Between the populations of *S. delarouzeei* s. str., having the same mating pattern some small differences observed do not represent barriers to hybridization and they may represent a speciation event at a very early stage.

Numerous populations of Bathysciinae beetles exist in the hypogean biotope of French Catalonia and north of Spanish Catalonia. These belong to one species complex, *Speonomus (Parvospeonomus) delarouzeei* according to Bellès (1974) and Escola and Bellès (1984). They live in 2 hypogean habitats: caves (or deep underground habitat) and the « milieu souterrain superficiel » (M.S.S.) (or shallow underground habitat) lying under the last soil layer (Juberthie et al., 1981). Both are characterized by a total darkness, a very high moisture level and temperature maxima and minima which are very attenuated compared to those of the overlying epigean biotope. The studied populations about 60 km N-S, 100 km E-W and between 300 and 2200 m in elevation above the sea level.

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The complex *Speonomus delarouzeei*, in fact, combines 6 species or subspecies which were synonymized because, on one hand, they exhibit very few morphological differences and, on another hand, some intermediary forms exist. The taxa involved are *S. delarouzeei delarouzeei* Fairm. 1860, *S. brucki* Fairm. 1863, *S. delarouzeei catalonicus* Jeann. 1910, *S. faurai faurai* Jeann. 1910, *S. guimjuani* Zaraquiey 1919 and *S. faurai esponellai* Zaraquiey 1940.

Ecophysiological data (Delay, 1978), alloenzyme studies of some populations (Delay et al., 1980, 1985; Sbordoni, 1982; Juberthie et al., 1984) as well as preliminary hybridization experiments (Cobolli Sbordoni et al., 1983; Sbordoni in litt.), suggested that *S. delarouzeei* was a group of several sibling species.

In cave animals geographical isolation and gene flow are now studied either by biochemical analysis (Avise and Selander 1972; Peters et al., 1975; Cockley et al., 1977; Giuseffi et al., 1978; Sbordoni et al., 1980, 1981; Cesaroni et al., 1981; Crouau-Roy, 1983, 1986; Caccone, 1985) or less frequently by experimental hybridization (Wilkens, 1971, 1976; Sbordoni, 1982; Peck, 1983).

The purpose of this study was to estimate the behavioural differentiation between different populations or species and to measure their degree of reproductive isolation by the study of their sexual behaviour and their hybrid crossings. Some preliminary studies have pointed out constant and sometimes important differences in the mating behavioural pattern, between several populations (Juberthie-Jupeau and Cazals, 1984, 1985a) and the lack of hybridization between 2 populations (Juberthie-Jupeau and Cazals, 1985b) that led us to assess the validity of 4 taxa: *S. delarouzeei* s. str., *S. brucki*, *S. catalonicus* and *S. guimjuani*.

MATERIALS AND METHODS

Data are based on 12 populations; 4 were gathered in or near the type localities of 4 of the taxa involved: Resurgence de l'Empereur Cave for *S. delarouzeei*, La Mine Cave for *S. brucki*, Rialb Cave for *S. catalonicus*, Bora Major Cave for *S. guimjuani*, and 8 populations were collected in new stations: Valmanya Cave, Crouanques Cave, Oms Cave, Montbolo Cave, Beget M.S.S., Banat M.S.S., Col d'Ares France M.S.S. and Col d'Ares Spain M.S.S. (Fig. 1).

For the study of sexual behaviour animals were kept in the laboratory cave at Moulis at 11,5°C, males and females being set apart. For observations one male and one female were put together in a plaster box (« nid Janet ») having 6 x 4 x 2.5 cm

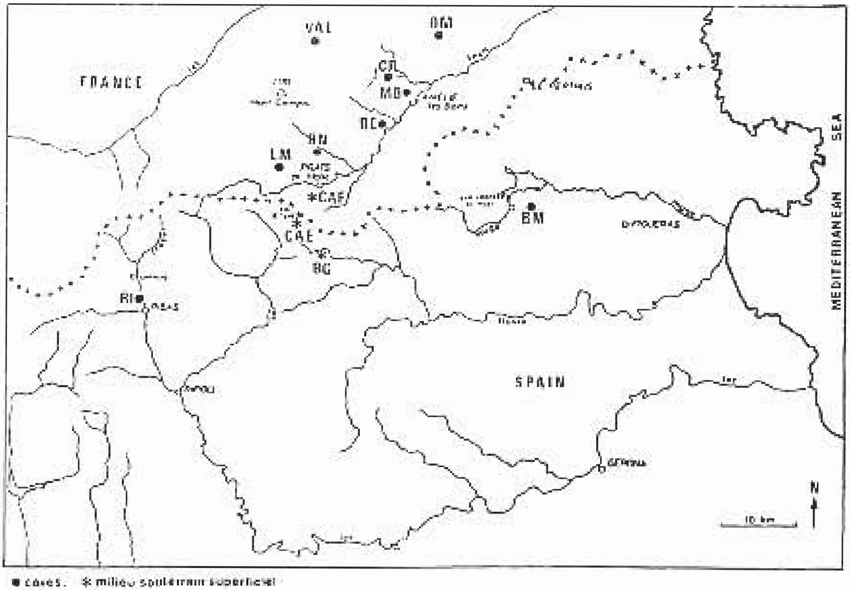

Fig. 1 - Geographic distribution of locality populations. BG = Beget; BM = Bora Major; BN = Banat; CAE = Col d'Ares Spain; CAF = Col d'Ares France; CR = Crouanques; LM = La Mine; MB = Montbolo; OM = Oms; RE = Résurgence de l'Empereur; RI = Rialb; VAL = Valmanya.

in its internal dimensions, the inner walls and bottom of which were coated with clay; it was shut with a glass lid. The number of matings is indicated for each population.

In crossing experiments only virgin females were used; one female of a population was placed with a male of another population; they were observed 1½ hour and if attempts to copulate occurred both were kept together. The number of interpopulational crossing studied and the number of pairs used for controls are indicated for each crossing.

MATING BEHAVIOUR

The mating, in which the male assumes an active role, shows several common aspects in the 12 populations.

The precopulatory orientation of male which wants to copulate is a few mm behind and facing posterior end of the female's body. He follows her when she walks and stops when she stops. After a non specific time, he mounts the female and copulates in almost upright position. However, male behaviour, during copulation, exhibits constant and sometimes important differences between the different populations. Two main manners were observed, based on the number of behavioural steps: 3 in 4 populations, and 1 in the other populations.

Mating involving 3 behavioural steps

This mode was observed in *S. delarouzeei* s. str. living in Resurgence de l'Empereur Cave and in 3 populations: Montbolo, Oms and Beget (fig. 2).

— The first step is the premating; the male mounts the female for a short time, about 1 min. It inserts its aedeagus, however no sperm is deposited as a histological control has shown.

— The second step is the intermediary rest, which lasts from 50 to 90 sec, the male keeping quiet behind the female.

— The third step is the mating itself; it lasts between 5 and 6 min, during which sperm deposit takes place.

Some variations appear in the duration of the behavioural steps within each population (tab. 1). However some significant differences were observed between 2 populations in the duration of one step (tab. 3).

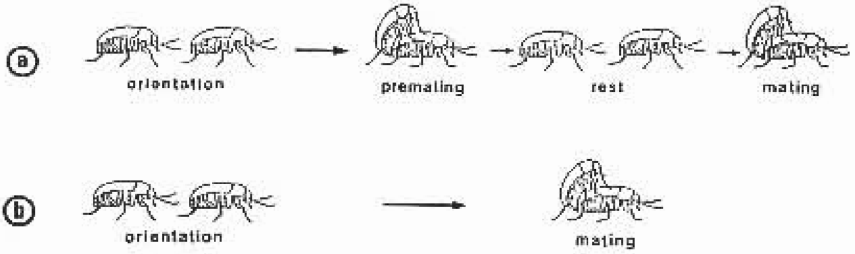

Fig. 2 - a. Mating involging 3 behavioural steps; b. Mating involving 1 behavioural step.

Table 1 - Mean and standard error ( ) of the duration (in seconds) of the 3 behavioural steps in 4 populations having the Resurgence de l'Empereur mating pattern. N = number of observed matings.

Populations	Premating		Rest		Mating itself	
	N	Duration	N	Duration	N	Duration
Resurgence de l'Empereur	44	72.18 (4.18)	36	50.94 (2.04)	40	328.50 (11.30)
Montbolo	13	71.69 (5.61)	17	90.00 (6.92)	13	350.05 (16.77)
Oms	24	84.88 (5.64)	24	75.42 (3.31)	28	352.25 (14.76)
Beget	17	38.24 (2.87)	17	50.71 (1.04)	20	297.15 (12.84)

Table 2 - Mean and standard error ( ) of some characteristics of 4 populations having the Resurgence de l'Empereur mating pattern. N = number of observed matings.

Populations	Lateral abdominal movements		Antennal taps premating		Antennal taps mating	
	N	Number	N	Number	N	Number
Resurgence de l'Empereur	21	14.71 (1.62)	24	6.38 (1.22)	46	37.96 (1.66)
Montbolo	16	14.06 (1.70)	15	0.20 (0.11)	13	3.42 (1.36)
Oms	25	20.92 (1.54)	24	1,71 (0.61)	29	13.72 (2.00)
Beget	14	6.57 (0.64)	16	4.75 (1.14)	20	47.30 (2.64)

In the course of the premating and mating itself the male performs antennal and abdominal movements (tab. 2). The first are antennal clappings which consist of tapping on the female's elytra by the antennae of the male. These taps are few in number in the premating step (between a mean of 0.2 among Montbolo males and 6.3 among Resurgence de l'Empereur males) and more numerous during the mating itself, between a mean of 3.4 in the Montbolo and 47.8 in the Beget populations.

Abdominal movements, which are lateral shakings, appear almost only in the premating step; their mean number ranges from 6.5 in the Beget population to 20 in the Oms populations.

Therefore, there are interpopulational differences both in the time spent in copula and in the number of movements per-

Table 3 - Comparison by Student's test between the main characteristics of 4 populations having the Resurgence de l'Empereur mating pattern. I, premating duration; II, rest duration; III, mating duration; IV, lateral abdominal movements; V, antennal taps during premating; VI, antennal taps during mating itself; ( ), degrees of freedom; +, significantly different at the 0.05 level.

Populations		Resurgence de l'Empereur	Montbolo	Oms
Montbolo	I	(55) 0.003—		
	II	(51) 49.27 +		
	III	(57) 1.155—		
	IV	(35) 0.075—		
	V	(37) 15.84 +		
	VI	(63) 127.0 +		
Oms	I	(66) 3.262—	(35) 2.281—	
	II	(58) 44.43 +	(39) 4.335 +	
	III	(56) 1.686—	(45) 0.009—	
	IV	(44) 7.625 +	(39) 8.421 +	
	V	(46) 11.73 +	(37) 3.747—	
	VI	(73) 72.10 +	(46) 14.45 +	
Beget	I	(59) 23.63 +	(28) 32.32 +	(39) 42.58 +
	II	(51) 0.006—	(32) 30.55 +	(39) 35.00 +
	III	(58) 2.903—	(37) 6.354 +	(46) 7.169 +
	IV	(33) 15.58 +	(28) 15.26 +	(37) 45.35 +
	V	(38) 0.851—	(29) 14.73 +	(38) 6.516 +
	VI	(64) 7.805 +	(37) 211.4 +	(47) 106.2 +

formed by the male, some of which are significant at the level of 0.05 (tab. 3). The number of significantly different characteristics is maximum between the pairs of the most separated populations (Beget and Montbolo, Beget and Oms), nevertheless we think these 4 populations belong to the same species *S. delarouzeei* s. str. This statement is strenghtened by the results of Juberthie et al. (1984) which demonstrate that these populations are biochemically closely related (alloenzyme studies).

Mating involving one behavioural step

This mode was observed in all 8 other populations. The male mounts the females and copulates (fig. 1). However its behaviour is so different in some populations that 5 patterns were discriminated.

La Mine population pattern.

La Mine, Valmanya, Col d'Ares France and Col d'Ares Spain populations exhibit this pattern. The mean of mating duration in each population varies from 192 sec (La Mine) to 226 sec (Col d'Ares Spain) (tab. 4). The pattern of mating is very similar in these 4 populations. It may be divised in 4 phases:

Table 4 - Mean and standard error ( ) of some characteristics of 4 populations of La Mine mating pattern. N = number of observed matings.

Populations	Mating		Abdominal movements		Antennal taps	
	N	Duration (sec.)	N	Number	N	Number
La Mine	52	192.31 (3.76)	25	73.04 (2.98)	27	60.44 (2.61)
Valmanya	21	226.29 (4.69)	20	90.00 (3.75)	20	39.65 (3.41)
Col d'Ares France	27	221.85 (3.55)	22	94.77 (4.60)	23	68.26 (2.87)
Col d'Ares Spain	20	225.90 (5.08)	14	92.07 (6.09)	15	72.53 (3.32)

Table 5 - Comparison by Student's test between the main characteristics of 4 populations having the La Mine mating pattern. I, duration of mating (sec); II, number of abdominal movements; III, antennal taps; ( ), degrees of freedom; +, significantly different at the 0.05 level.

Populations		La Mine	Col d'Ares	France Valmanya
Valmanya	I	(71) 26.20+		
	II	(43) 12.84+		
	III	(45) 24.29+		
Col d'Ares France	I	(75) 15.72+	(46) 0.59 —	
	II	(45) 16.42+	(40) 0.63 —	
	III	(48) 4.06+	(41) 41.81 +	
Col d'Ares Spain	I	(70) 24.07+	(39) 0.003—	(45 0.45—
	II	(37) 11.96+	(32) 0.11 —	(34) 0.14—
	III	(40) 7.93+	(33) 45.52 +	(36) 0.92—

— 1st phase, with strong antero-posterior abdominal movements in the male;

— 2nd phase, with tapping of the antennae on the female elytra;

— 3rd phase with strong antero-posterior abdominal movements and tapping of antennae alternately;

— 4th phase without antennal tapping and abdominal movements which are infrequent in few animals in the La Mine population and more numerous and occurring in all the males in Col d'Ares Spain.

The number of abdominal movements and antennal taps varies somewhat in each population. In spite of some interpopulational significant differences at the level of 0.05 (tab. 5), I believe these 4 populations belong to *S. brucki*.

Banat population pattern.

In the Banat (M.S.S.) population the mating is long and variable, in mean 472 sec ($N = 46$; $\sigma = 221$); it is twice as long as in the La Mine population pattern. The males performed antero-posterior abdominal movements which are, in a first phase very strong and few, in mean 28 ($N = 10$; $\sigma = 6.6$), and in a second phase very weak and numerous, in mean 171 ($N = 10$; $\sigma = 95$). This population must be separated from the others and belongs to *S. emiliae* recently described.

Rialb population pattern.

The mating which lasts in mean 252 sec ($N = 29$; $\sigma = 49$) is characterized by numerous and weak antero-posterior abdominal movements, the mean of which is 168 ($N = 21$; $\sigma = 48$); they are performed in several periods separated by a complete motionlessness. 70% of the males do not any antennal taps to the female, while the others only touch once. This pattern is characteristic of a species what was formerly known as *S. d. catalonicus*. This taxon must now be elevated to species rank *S. catalonicus*.

Bora Major population pattern.

The mating time is 348 sec in mean ($N = 26$; $\sigma = 61$). During all this time the males do not display any abdominal movements and perform only a few antennal taps, 8.3 in mean. The population that exhibits this pattern should be called *S. guimjuani*.

Crouanques population pattern.

The duration of mating varies in a wide range; some matings are brief, 318 sec in mean ($N = 23$; $\sigma = 122$), others are long, 782 sec in mean ($N = 21$; $\sigma = 99$) without demonstrating a structured sequence although the times are distributed in a bimodal fashion. Throughout the mating the male abdomen stands motionless while the antennae display taps to the female elytra. Brief and long matings have the same clapping frequency; thus, the number of taps is about 56 in the brief and 114 in the long ones.

The second legs do not lean on the substratum as they do in the other populations, but are bent towards the female's body and, from time to time, they rub against her abdomen. It is the only population in which the leg rubbing was observed. The mating behaviour indicates that this population belongs to *S. charlottae* recently described.

In short, 6 different patterns in the mating behaviour were pointed out in the 12 studied populations of *Speonomus*.

SEXUAL ISOLATION

In order to better understand the significance of the results of the behavioural studies in relation to species limits in these beetles, some interpopulational breedings were attempted between 2 populations with different mating patterns and between 2 populations with the same pattern. For this purpose reared virgin females were used because a preliminary experiment had shown that spermatozoa sometimes remain active in the spermatheca one and a half years after insemination.

Interbreeding between 2 populations with different patterns.

The Resurgence de l'Empereur population (RE) with a 3 behavioural steps pattern was crossed with the La Mine population (LM) with a 1 behavioural step pattern. For crossings 45 pairs females RE x males LM and 69 pairs females LM x males RE were studied and for controls 27 pairs of females RE x males RE and 28 pairs females LM x males LM were used.

In crossings females RE x males LM, in 34 pairs (75%), 65 unsuccessful attempts to copulate and 59 successful but atypical matings were observed. The time spent in copula is short: 34 matings last less than 1 min, 3 between 1 and 2 min, 9 between 2 and 3 min and 13 a little more than 3 min. In these crossings, the males LM have a 1 behavioural step mating, so that, it is the mating which is really observed.

In crossings females LM x males RE, in 42 pairs (60%), 55 unsuccessful attempts to copulate and 62 successful but atypical matings occurred. The time spent in copula is extremely brief: 49 matings lasted less than 1 min, 11 between 1 and 2 min, 2 between 2 and 3 min; the most correspond certainly to the premating.

In a general manner, the female frequently decamps when the male takes the precopulatory orientation, so that relatively few attempts to copulate were observed. When mating do occur, they are briefer than control matings and atypical concerning the male movements. Also the female is always walking or running to escape.

The fecundity as used here is the mean of number of eggs layed by a female during a month. In crossings females RE x males LM the fecundity was 0.70 (controls: 3.38), and in crossings females LM x males RE it was 0.47 (controls: 2.62). Thus, the fecundity was about a fifth of that of the controls.

In these experimental crossings, 386 eggs were obtained; all failed to develop. In controls the rate of egg hatching is about 87%. A histological study was performed for 13 females (9 females RE and 4 females LM), several months after the beginning of their crossing and for which atypical copulations were observed. No sperm was observed in the spermatheca, neither in female RE not in female LM which explain the non - success of these interbreedings. In controls the spermathecae were full of sperm.

Similar results were obtained in another trial of interbreedings concerning the La Mine population (LM) and the Banat population (BA). In spite of the fact that males of both mate in one behavioural step, they exhibit different patterns. For crossings 15 pairs of females LM x males BA and 15 pairs of females BA x males LM were used and for controls 28 pairs of females LM x males LM and 20 pairs of females BA x males BA were studied. These crossings are unsuccessful so that the 2 populations should be considered different species.

Interbreedings between 2 populations of the same pattern

The interbreedings between the Resurgence de l'Empereur and Montbolo populations which have the same mating pattern with only some small differences are a success of the F1 generation. These trials must go on for the F2 generation but it is a long work, as each generation requires one year to develop.

DISCUSSION

Both the comparative mating behaviour studies between 12 populations, which discriminated 6 different patterns, and the few interpopulational interbreeding experiments demonstrate that *S. delarouzeei* is a complex of 6 sibling species as previous studies allow to suspect (Juberthie-Jupeau and Cazals, 1984, 1985 a, b). Taking their origins into account there are 6 species: *S delarouzeei* s. stricto from Resurgence de l'Empereur Cave, *S. brucki* from La Mine Cave, *S. catalonicus* from Rialb Cave, *S. guimjuani* from Bora Major Cave and 2 new species, one of Crouanques and one of Banat.

Results of behavioural observations and cross-breeding experiments agree with allozymic evidence (Delay et al., 1980; Juberthie et al., 1984) obtained for 12 loci, in the same 12 populations herein studied. Specially the dendrogram of Delay (in Juberthie et al., 1984) emphasized similar clusters as the behavioural methods, that is to say: a first cluster grouping the 4 populations having 3 behavioural steps (Resurgence de l'Empereur pattern corresponding to *S. delarouzeei* sensu stricto), a second cluster grouping 4 populations of the La Mine pattern and corresponding to *S. brucki*, and 4 populations more or less separated. Concerning the genetic distances (using Nei's method) among different groups, they are comparable with those reported for different species (Delay et al., 1980; Sbordoni, 1982).

The recent results of Caccone (1985) in several populations of *S. delarouzeei* sensu Bellès, corroborate this point of view. That author studied the gene flow by Slatkin's method (1985) between 8 populations among which I have observed the mating behaviour in 5 populations: Resurgence de l'Empereur, Montbolo, La Mine, Valmanya and Crouanques. Caccone reported that the 8 populations belong to 3 clusters. Concerning my 5 populations, they are distributed among these 3 clusters. The Resurgence de l'Empereur and Montbolo populations belong to the same group and so do the La Mine and Valmanya populations; for these 5 populations the 3 groups belong in fact to 3 different species for which behavioural data allow a good discrimination.

The results from the hybridization studies between *S. delarouzeei* and *S. brucki* indicate that reproductive isolation is prezygotic and is a behavioural isolation with atypical matings and no sperm deposit.

Between the populations of *S. delarouzeei* s. str. having the same mating pattern, the small differences observed in the different populations do not now represent barriers to hybridization. Following an increase of these divergences in one or several populations these barriers may appear, leading to several species. Therefore, the observed divergences reported here, may represent a speciation event, at a very early stage.

Between the studied species of *Speonomus* we observed the completion of reproductive isolation that is not always the case in subterranean beetles. According to Peck (1983) in several species of the genus *Ptomaphagus* which are cavernicolous beetles of Alabama and Kentucky, behavioural mating barriers seem not to be present in 14 reciprocal crosses between 11 populations. Hybridization occurs but the tested populations show various levels of genetic barriers to interpopulational hybridization. In populations that are morphologically distinguishable, this barrier is measured by a reduced fecundity and a lowered success at producing fertile F1, and F2 larvae and adults.

We may conclude that the *Speonomus* studied, which live only in the hypogean habitat, have isolated populations in spite of the fact they are geographically near. Several phenomena play a part in this partitioning. Geological and pedological barriers exist, and the thermal requirements of the beetles are so narrow that the populations are restricted to quite small ranges.

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REFERENCES

AVISE, J. C. and R. K. SELANDER, 1972. Evolutionary genetics of cave-dwelling fishes of the genus *Astyanax*. Evolution 26: 1-19.

BELLES, X. 1974. Revision de los *Speonomus* del grupo *delarouzeei* (Fairmaire, 1860). Com. VII Congr. Int. Est. Piren. (Resumen p. 48) Seu d'Urgell.

CACCONE, A. 1985. Gene flow in cave Arthropods: a qualitative and quantitative approach. Evolution 30 (6): 1223-1235.

CESARONI, D., G. ALLEGRUCCI, A. CACCONE, M. COBOLLI SBORDONI, E. DE MATTHAEIS, M. DI RAO and V. SBORDONI. 1981. Genetic variability and divergence between populations and species of *Nesticus* cave spiders. Genetica 56: 81-92.

COBOLLI SBORDONI, M., B. DELAY, E. DE MATTHAEIS and V. SBORDONI. 1983. Divergenza genetica e barriere al flusso genico tra popolazioni cavernicole di *Speonomus delarouzeei* (Coleoptera, Catopidae). Atti XII Congr. Naz. Ital. Entom., Roma 1980, 2: 247-252.

COCKLEY, D.E., J.L. GOOCH and D.P. WESTON. 1977. Genetic diversity in cave dwelling crickets (*Ceuthophilus gracilipes*). Evolution 31: 313-318.

CROUAU-ROY, B., 1983. Variabilité génétique entre les populations de *Speonomus zophosinus*. Biochem. System and Ecol. 11: 55-61.

CROUAU-ROY, B. 1988. Genetic divergence between populations of two closely related troglobitic beetle species (*Speonomus*: Bathysciinae. Coleoptera). Genetica 66: 97-103.

DELAY, B. 1978. Milieu souterrain et écophysiologie de la reproduction et du développement des Coléoptères Bathysciinae hypogés. Mém. Biospéol. 5: 1-349.

DELAY, B., V. SBORDONI, M. COBOLLI SBORDONI and E. DE MATTHAEIS. 1980. Divergences génétiques entre les populations de *Speonomus delarouzeei* du Massif du Canigou (Col. Bathysciinae). Mém. Biospéol. 7: 235-247.

DELAY, B., L. JUBERTHIE-JUPEAU and C. JUBERTHIE. 1985. Sibling species and barriers to hybridization in cave - beetles. 3rd Int. Cong. Syst. Evol. Biol. Brighton, p. 45.

ESCOLA, O. and X. BELLES. 1984. Distribucio geographica de los *Speonomus* del subgenere *Parvospeonomus* (Col. Catopidae Bathysciinae) al versant meridional del Pyreneus. III Sessio Entomol. ICHN-SCL, Mataro, 19-28.

GIUSEFFI, S., T. C. KANE and W. F. DUGGLEBY. 1978. Genetic variability in the Kentucky cave beetle *Neaphaenops tellkampfii* (Coleoptera: Carabidae). Evolution 32 (3): 679-681.

JUBERTHIE, C., B. DELAY and M. BOUILLON. 1981. Sur l'existence d'un milieu souterrain superficiel en zone calcaire. Mém Biospéol., 8: 77-93.

JUBERTHIE, C., B. DELAY, L. JUBERTHIE-JUPEAU, J. DURAND, O. ESCOLA, X. BELLES and M. BLAS. 1984. Le complexe *Speonomus delarouzeei*: un modèle d'approches associées pour l'étude de la spéciation. Mém.. Biospéol., 11: 141-164.

JUBERTHIE-JUPEAU, L. and M. CAZALS. 1984. Accouplement et comportement sexuel chez un Bathysciinae souterrain. *Speonomus delarouzeei*. Fairm. Behavioral Process 9: 147-155.

JUBERTHIE-JUPEAU, L. and M. CAZALS. 1985 a. Différences éthologiques dans l'accouplement de quatre populations de Coléoptères Catopidae (*Speonomus delarouzeei* Fairm.) en relation avec la spéciation. C. R. Acad. Sc. Paris 300, série III: 559-562.

JUBERTHIE-JUPEAU, L. and M. CAZALS. 1985 b. Isolement reproducteur entre deux populations ayant valeur d'espèces dans le complexe *Speonomus delarouzeei*. C. R. Acad. Sc. Paris 301, série III: 393-398.

PECK, S. B. 1983. Experimental hybridizations between populations of cavernicolous *Ptomaphagus* beetles (Coleoptera: Leiodidae: Cholevinae). Can. Ent. 115 (5): 445-452.

PETERS, N., A. SCHOLL and H. WILKENS. 1975. Der Micos-Fisch, Höhlenfisch in statu nascendi oder Bastard. Ein Beitrag zur Evolution der Höhlentier. Z. zool. Syst. Evolut.-forsch. 13: 110-124.

SBORDONI, V. 1982. Advances in speciation of cave animals, p. 219-240. In C. Barrigozzi (ed.), Mechanisms of speciation, A.R.Liss, New York.

SBORDONI, V., G. ALLEGRUCCI, A. CACCONE, D. CESARONI, M. COBOLLI SBORDONI and E. DE MATTHAEIS. 1980. A preliminary report of the genetic variability in troglobitic Bathysciinae: *Leptodirus hohenwarti* and two *Orostygia* species (Coleoptera, Catopidae). Fragm. Entomol. 15: 327-336.

SBORDONI, V., G. ALLEGRUCCI, A. CACCONE, D. CESARONI, M. COBOLLI SBORDONI and E. DE MATTHAEIS. 1981. Genetic variability and divergence in cave populations of *Troglophilus cavicola* and *T. andreinii* (Orthoptera, Rhaphidophoridae). Evolution 35 (2): 226-233.

SLATKIN, M. 1985. Rare alleles as indicator of gene flow. Evolution 39: 53-65.

WILKENS, H. 1971. Genetic interpretation of regressive evolutionary process: studies on hybrid eyes of two *Astyanax* cave populations (Characidae. Pisces). Evolution 25: 530-544.

WILKENS, H. 1976. Genotypic and phenotypic variability in cave animals. Studies on a phylogenetically young cave population of *Astyanax mexicanus* (Filippi) (Characidae, Pisces). Ann. Spéléol. 31: 137-148.