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MORPHOLOGICAL AND GENETIC DIFFERENTIATION OF *GAMMARUS STUPENDUS* PINKSTER, 1983 IN THE MASSIF DE LA SAINTE BAUME, FRANCE

by

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ABSTRACT

Morphologically distinct populations of the *Gammarus pulex* group which should be attributed to at least three species applying morphological criteria postulated by Karaman & Pinkster (1977) have been reported in the Massif de la Sainte Baume (southern France). These populations proved to be completely interfertile and thus were considered conspecific. This group of populations, in which three major forms A, B and C were distinguished, has been described as *Gammarus stupendus* Pinkster, 1983.

Systematic sampling was carried out to determine the distribution of the various forms of *G. stupendus* in the Massif de la Sainte Baume and adjacent areas, and the relation between morphological and genetic differentiation has been investigated. Genetic differentiation has been studied in four polymorphic gene loci: *Gpi*, *Got-1*, *Mdh-1*, and *Mdh-2*.

As the populations used in hybridization experiments by Pinkster (1983) proved to be genetically very similar, additional crossbreeding experiments have been carried out with two genetically more distinct populations. Moreover, experiments were run to test the stability of diagnostic characters in each form A, B and C of *G. stupendus*.

No clear correlation could be found between any of the factors morphology, genetic differentiation, and geographic distribution pattern. In the crossbreeding experiments genetically more distinct populations appeared to be incompletely interfertile. Thus, the various *G. stupendus* populations, exhibiting different degrees of interfertility which may ultimately result in reproductive isolation, may indicate different stages in the process of speciation.

RÉSUMÉ

Des populations de Gammares du groupe *pulex* morphologiquement distinctes qui devraient, en appliquant les critères formulées par Karaman & Pinkster (1977), être attribuées au moins à trois espèces différentes, ont été rapportées du Massif de la Sainte Baume (Midi de la France). Ces populations, dont trois formes principales A, B et C ont été distinguées et qui ont prouvées d'être tout à fait interfertiles, étaient donc considérées conspécifiques et ont été décrites comme *Gammarus stupendus* Pinkster, 1983.

Un échantillonage systématique a été effectué afin de déterminer la répartition des formes différentes dans le Massif de la Sainte Baume et les régions avoissinantes, et la relation avec la différenciation génétique. La variation génétique de quatre loci polymorphiques a été étudiée: *Gpi*, *Got-1*, *Mdh-1* et *Mdh-2*.

Étant donné que les populations utilisées par Pinkster (1983) dans des essais d'hybridation étaient très semblables du point de vue génétique, d'autres essais d'hybridation ont été effectués avec des populations génétiquement plus différentes. En plus, des essais ont été effectués pour tester la stabilité des caractères diagnostiques de chacune des formes A, B et C de *G. stupendus*.

Une corrélation nette entre ni la différenciation morphologique et génétique d'une part, ni la différenciation morphologique ou génétique et la répartition géographique d'autre part, n'a pu être démontrée. Dans les essais d'hybridation, des populations génétiquement plus différentes ne se sont prouvées pas tout à fait interfertiles. Donc, les populations différentes de *G. stupendus*, en montrant plusieurs niveaux d'interfertilité dont pourrait ultérieurement résulter une isolation reproductive, devraient vraisemblablement être considérées comme des stades différents dans le processus de spéciation.

INTRODUCTION

Pinkster (1983) investigated morphologically different populations of the *Gammarus pulex* group in the Massif de la Sainte Baume and the adjacent karst areas in southern France. The material he collected could not be identified satisfactorily with the aid of the key by Karaman & Pinkster (1977). Some populations could be identified as *G. fossarum* Koch, 1835, but most of the populations could not be attributed to any of the known taxa. Applying a set of so-called stable characters (Pinkster, 1983) at least three species should have been distinguished, but even then uncertainty remained because of the enormous variability.

Although not one population seemed to be identical to any other, Pinkster (1983) was able to recognize three major forms A, B and C. These populations all have certain characters in common with *G. fossarum*, but differ from it (and often from each other) in two or more characters which should be discriminative according to Karaman & Pinkster (1977). Two of these forms resemble the French populations of *G. ibericus* Margalef, 1951 (later described as *G. orinos* Pinkster & Scholl, 1984) in more than one respect. However, in crossbreeding experiments the forms A, B and C proved to be interfertile, whereas crosses between any of these populations and standard populations of "true" *G. fossarum* and *G. orinos* failed to give any offspring (Pinkster, 1983). Therefore it was decided that populations of forms A, B and C belonged to a new species, which was described as *G. stupendus* Pinkster, 1983.

The results of the crosses between the forms A, B and C and the morphology of the offspring did raise some doubt about the taxonomical value of certain morphological characters as proposed by Karaman & Pinkster (1977): even those which have so far been considered stable in some species were too variable in others and could not be used for discrimination.

The primary aim of the present study was to determine if morphological differentiation in *G. stupendus* (i.e. the forms A, B and C) coincides with genetic differentiation. Second goal was to determine if there is any relation between either morphological or genetic variation and the geographical distribution pattern.

Genetic variation was investigated by starch-gel enzyme electrophoresis. Sampling for electrophoretical analysis was preceded by systematic sampling to map in more detail the actual distribution of the morphological forms A, B and C of *G. stupendus*. Thus, sampling carried out in 1980-1981 and earlier (Pinkster, 1983) was completed and differences in temporal and spatial distribution patterns of these forms were checked.

Laboratory experiments were carried out to verify whether the morphological differentiation is constant, i.e. not subject to seasonal and/or environmental fluctuations. During electrophoretical analysis it was noticed that populations used in crossbreeding experiments by Pinkster (1983) were genetically rather similar to one another. Moreover, "true" *G. fossarum* used in his experiments came from northern France and the Alps. In my opinion local *G. fossarum* must also be used in these crosses. After the first electrophoretic results it was decided to rerun a series of crossbreeding experiments with (a) two genetically more distinct populations of different morphological forms, (b) these populations and a local *G. fossarum* population, and (c) a local *G. fossarum* population and a *G. fossarum* population from northern France.

STUDY AREA

As the variability of *G. stupendus* should be seen in the context of a highly structured environment, the study area will be discussed in more detail. The Massif de la Sainte Baume and the adjacent limestone areas (fig. 1) include four main drainage systems: the Arc, the Argens, the Huveaune and the Gapeau. The upper reaches of the largest system, the Argens, can be subdivided in four smaller tributary systems: the Eau Salée, the Cauron, the Caramy and the Isolle. Superficial drainages of these systems are well separated. However, as some springs in the upper reaches are very close to one another (in some cases less than 100 m) subsidiary drainage into subterranean conduits (a phenomenon common in karst areas) through the watershed can not be excluded. Thus, an effective geographic barrier may be absent.

The study area is situated in the Mediterranean region and climatical factors as rainfall and temperature can be of decisive importance to local hydrology. Rainfall is in the order of 700-1000 mm a year with maxima at the end of the winter, in spring and in autumn (Kiener & Ollier, 1970; Giudicelli et al., 1980). Incidently, rainfall fails in autumn. The area is subject to a well-defined "dry season", ranging roughly from June to September. According to

Bagnouls & Gaussen (1953) the "dry season" is defined as the period in which the monthly means of rainfall (in mm) is equal to or less than twice the temperature (in degrees centrigrade). The xerothermic index as defined by these authors is 60 for Marseille (for comparison: the xerothermic index for Lyon is 0; for Marakéch, Morocco, it is 178).

The upper reaches of the rivers Gapeau and Argens depend for their water supply directly on subterranean groundwater basins and their discharge is quite independent on local rainfall. The discharge of other tributaries, however, is directly related to local rainfall (Kiener & Ollier, 1970; Giudicelli et al., 1980; pers. obs.). The Latay for instance is in the dry season reduced to a series of stagnant pools, which often are not interconnected anymore, and under extreme conditions as prevailing in the summers of 1985 and 1986 some tributaries desiccate almost entirely (e.g. Cauron, Isolle, upper reaches of Huveaune and Eau Salée). This is even known to happen incidently to the Gapeau system as a whole (Kiener & Ollier, 1970). This situation can last for several months (up to seven and perhaps more; pers. obs. 1985-1986).

The frequent desiccation of riverbeds in the dry season, resulting in the formation of isolated river sections and pools subsisting for at least several months, may provide a temporal genetic barrier. It is obvious however, that when complete desiccation occurs, gammarids may be absent for quite some time.

MATERIAL AND METHODS

Sampling and collection sites

In November 1984 intensive sampling was carried out in the study area to determine the distribution of the various morphological forms. In total at 48 localities *Gammarus* were found (table I, fig. 1). In many localities however, no gammarids occurred at all. At every locality samples of approximately 100 animals have been taken. The animals were killed on the spot with 4% formaldehyde and later on transferred to and stored in 70% ethanol. From the 48 localites, 21 were selected for additional morphological and electrophoretic studies. Sampling for this purpose has been carried out in November 1985. For comparison two populations from the adjacent Massif des Maures were sampled as well.

Animals sampled for electrophoretic experiments were anaesthetized with Sandoz MS 222 (1 : 1000), identified in the field and immediately transferred into a liquid nitrogen container (-170°C). In addition to each frozen sample, an ethanol control sample was taken for morphological study. As all sampling was done in the winter period, in July 1985 additional samples have been taken at six localities to check eventual seasonal changes in morphology.

Material collected by Pinkster (1983) and by J. Berner (unpubl.) was reexamined. As far as these localities do not correspond with my sampling stations they are indicated as stations a-i in table I and fig. 1.

In November 1984 *G. stupendus* forms A, B and C of representative stations have been collected (stations 1, 32 and 43) to test the stability of morphological characters under uniform laboratory conditions. Part of the animals collected were frozen to compare allele frequencies in November 1984 and November 1985.

In November 1985 and July 1986 population samples of *G. stupendus* form B and C, and of *G. fossarum* were collected at stations 43, 1, and 13, respectively, for additional crossbreeding experiments. "True" *G. fossarum* for these experiments were collected in August 1986 in the river Slack in Ambleteuse (dépt. Pas-de-Calais, northern France).

Laboratory experiments

After being transported in plastic bags with habitat water under oxygen, the animals of each population were kept in storage basins of 40 × 50 × 30 cm. Rough stones were provided for shelter and air was blown constantly through the water. The animals were fed with *Stellaria media* Vill., a common terrestrial weed.

To test the stability of morphological characters under uniform laboratory conditions 25 males and 25 females of each population A, B and C of *G. stupendus* were kept in special units. Each unit consisted of two boxes, an outer box (25 × 25 × 10 cm) and an inner box (24.5 × 24.5 × 7 cm). The bottom of the inner box was replaced by a wire netting (mesh 750 μm). The adults, unable to pass the wire netting, were kept in the inner box. Juveniles sank through the meshes and concentrated on the bottom of the outer box, so that they could not be eaten by the adults. The experiment was done at 15°C and a light/dark period of 16/8 hours. Once a month the morphology of 10 animals of each of the forms A, B and C was examined. The same procedure was applied to the F1 and F2 when raised to maturity.

The methods used for the crossbreeding experiments were identical to those described by Pinkster (1983). The B population he used (collected at station 29) was genetically very similar to his C population (station 1; tables I and II). Therefore, in the present study the station 29 population has been replaced by the B population from station 43. Contrary to Pinkster (1983) an A population was not involved. The first series of crossbreeding

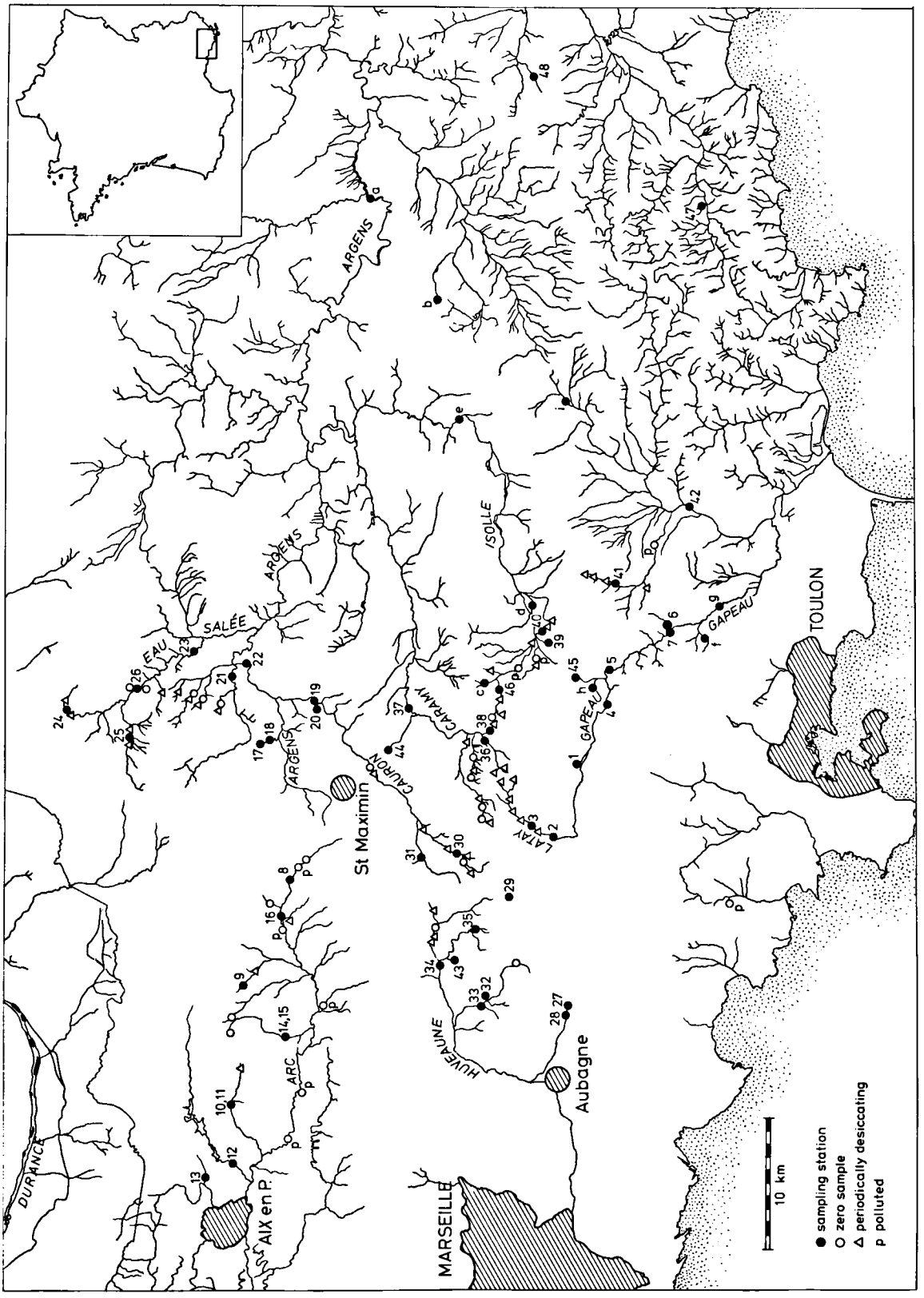

Fig. 1. Study area and collection sites. Stations numbered 1-48 have been sampled for the present study; those numbered a-i have been sampled by J. Berner (unpublished).

experiments was run in the period November 1985-March 1986; the second series in the period August 1986-January 1987.

Electrophoresis

Electrophoresis and staining procedures generally followed Siegismund et al. (1985). Of 20 enzyme systems assayed, four polymorphic enzymes gave good results: glucose phosphate isomerase (GPI, E.C. No. 5.3.1.11), glutamic oxaloacetic transaminase (GOT-1, E.C. No. 2.6.1.1), and malate dehydrogenase (MDH-1 and MDH-2, E.C. No. 1.1.1.37). The genetic interpretation of the variation is inferential.

The allele frequencies have been used for calculating a matrix of Nei's indices of normalized genetic identity, *I* (Nei, 1972). Based upon these figures, an UPGMA dendrogram (Sneath & Sokal, 1973) has been constructed.

RESULTS

Distribution of the major morphological forms (Table I, figs. 1 and 2)

The Arc system

In the Arc system *G. stupendus* was not found. *G. pulex gallicus* S. Karaman, 1935 dominated, and only at three localities (stations 8, 12 and 13) *G. fossarum* occurred. As a whole, the Arc system is rather polluted (fig. 1) and it is probably due to this pollution that *G. fossarum* populations regressed and progressively have been replaced by populations of *G. p. gallicus*, a species inhabiting generally middle and lower reaches, having a higher tolerance to pollution.

Upper reaches of the Argens system

In the upper reaches of the Argens system (Argens, Eau Salée and Cauron) *G. stupendus* forms A and B, and *G. fossarum* were found. Populations of *G. stupendus* form A showed only to a small extent the diagnostic characters of this form as given by Pinkster, 1983 (in particular the setation of P5-P7) and can therefore very easily be confused with *G. fossarum* (both species occur sympatrically at stations 17, 18, 19, 20, 21 and 22). The morphology of these populations was stable and distinct (individuals showing transitional characters toward other forms of *G. stupendus* have not been found). Thus, at localities where *G. stupendus* forms A and B occur sympatrically (stations 19, 20, 21 and 22) they could be separated without difficulties.

The Caramy subsystem

In the Caramy *G. stupendus* form B dominated. Although individuals showing transitional characters attributable to both *G. stupendus* forms A and C (as described by Pinkster, 1983) were found, no discrete different forms could be distinguished.

The Isolle subsystem

In the Isolle all three forms A, B and C of *G. stupendus* were encountered. Morphological characters of *G. stupendus* form A in the population of station 39 were stable, but in both A and C populations from station 40 intermediate forms showing typical form B characters (especially the setation of P5-P7) were frequently found. Material collected by J. Berner at the localities c, d and e (see table I and fig. 1) contained predominantly *G. stupendus* form A identical to the population from station 39.

The Huveaune system

The lower reaches of the Huveaune (stations 34, 35 and 43) are populated by *G. stupendus* form B. The morphology of these populations was rather variable. Individuals supposed to belong (according to Pinkster, 1983) to *G. stupendus* form A were occasionally found; this applies particularly to the populations of stations 34 and 35. However, as all intermediate stages between form B and form A were found, this variability can be seen as intra-populational variation. The same applies to the *G. stupendus* form A populations in the upper reaches of the Huveaune (stations 27, 28, 32 and 33). Thus, the *G. stupendus* form A populations of the upper reaches and the form B populations of the lower reaches showed an overlap in morphology.

Older material from the lower Huveaune reaches (stations 35 and 43) showed a form A morphology and might indicate intermittent temporal changes in morphology over longer periods as well as shifts in the distribution pattern.

TABLE I

Sampling localities and morphological forms: distribution over the various drainage systems (cf. fig. 1 and footnote).

drainage (sub)system	station	morphology 1970-1971*	1980-1981**	1984	1985
Arc	8	—	—	*f*	—
	16	—	—	*p*	—
	9	—	—	*p*	—
	14/15	—	*p*	*p*	—
	10/11	—	*p*	*p*	—
	12	—	f,*p*	—	—
	13	—	*f*	*f*	*f*
Eau Salée	24	—	B,*f*	*f*	*f*
	26	—	A	B,*f*	—
	25	—	*f*	*p*	p,*f*
	23	—	*f*	B	—
Argens	17/18	—	A	A,*f*	A,*f*
	21	A	A	A,*f*,B	—
	22	—	—	A,B,*f*	f,B,A
	a	B	—	—	—
	b	A	—	—	—
Cauron	19/20	—	—	B,A,*f*	A,B,*f*
	31	—	—	B,*p*	—
	30	—	B	B,*p*	B
Caramy	44	—	—	B	B
	37	—	—	B	B
	36	—	—	B	B
	38	—	B	B,A	B
Isolle	46	B,A	B	O	B
	39	—	—	A,*p*,*f*	—
	40	—	—	C,A	C,A
	c	B	—	—	—
	d	B, A	—	—	—
	e	A,B	—	—	—
Huveaune	35	A	B	B	—
	34	B	B	B	B
	43	A	—	B	B
	32	—	—	A	A
	33	—	—	A,*p*	—
	27/28	—	A	A	A
	29	B	B	B	B
Gapeau	3	—	—	C	C
	2	—	—	C	C
	1	C	C	C	C
	4	C	—	C	—
	45	A	—	C,A	C,A
	5	C	—	C	—
	6/7	A,C	—	C,A	C,A
	41	B	—	A	O
	42	A	—	A	—
	f	A	—	—	—
	g	A	—	—	—
	h	A	—	—	—
	i	A	—	—	—
Maravenne	47	—	*f*	—	B
Préconil	48	—	B	—	B

Compared to the other sampling sites the Fontaine de St. Pilon (station 29) represents a particular case. This isolated fountain, situated just under the edge of the Sainte Baume mountain crest, apparently does not belong to any stream system. However, in periods of rainfall water from the fountain basin seeps down through the woodlands along the mountain slope, most probably toward the upper reaches of the Huveaune system. The very small population of this locality ("type-locality" of *G. stupendus* form B in Pinkster, 1983) generally showed a form B morphology, although intergrades with more or less pronounced characters of *G. stupendus* form A were also found. It should be emphasized, however, that some diagnostic characters (e.g. setosity of P5-P7) provided by Pinkster (1983) revealed to be sexually dimorphic in many populations of *G. stupendus* forms A and B, and their growth appears to be allometric (i.e. animals showing a form B morphology in the younger life stages may end with a form A morphology). For these reasons, it was difficult to attribute a certain number of individuals to one form or another.

The Gapeau system

In the Gapeau system *G. stupendus* form C dominated, although *G. stupendus* form A was also found. The *G. stupendus* form C populations showed little variation in morphology. *G. stupendus* form A populations were more variable compared to one another. The populations at stations 6 and 45 exhibited a stable morphology, and resembled *G. stupendus* form A populations from the Argens system (stations 17, 18, 19, 20, 21 and 22). In populations of stations 41 and 42 the setosity of P5-P7 was more variable, and in this respect these populations resembled the *G. stupendus* form A populations in the upper reaches of the Huveaune (stations 27, 28, 32 and 33). Older material collected at localities f, g, h and i was identical to form A populations from stations 6 and 45.

f = *G. fossarum*; *p* = *G. pulex gallicus*; A = *G. stupendus* form A; B = *G. stupendus* form B; C = *G. stupendus* form C; bold type = form predominant (figures in order of decreasing abundance); O = station desiccated, * = leg. J. Berner; ** = leg. Pinkster (1983).

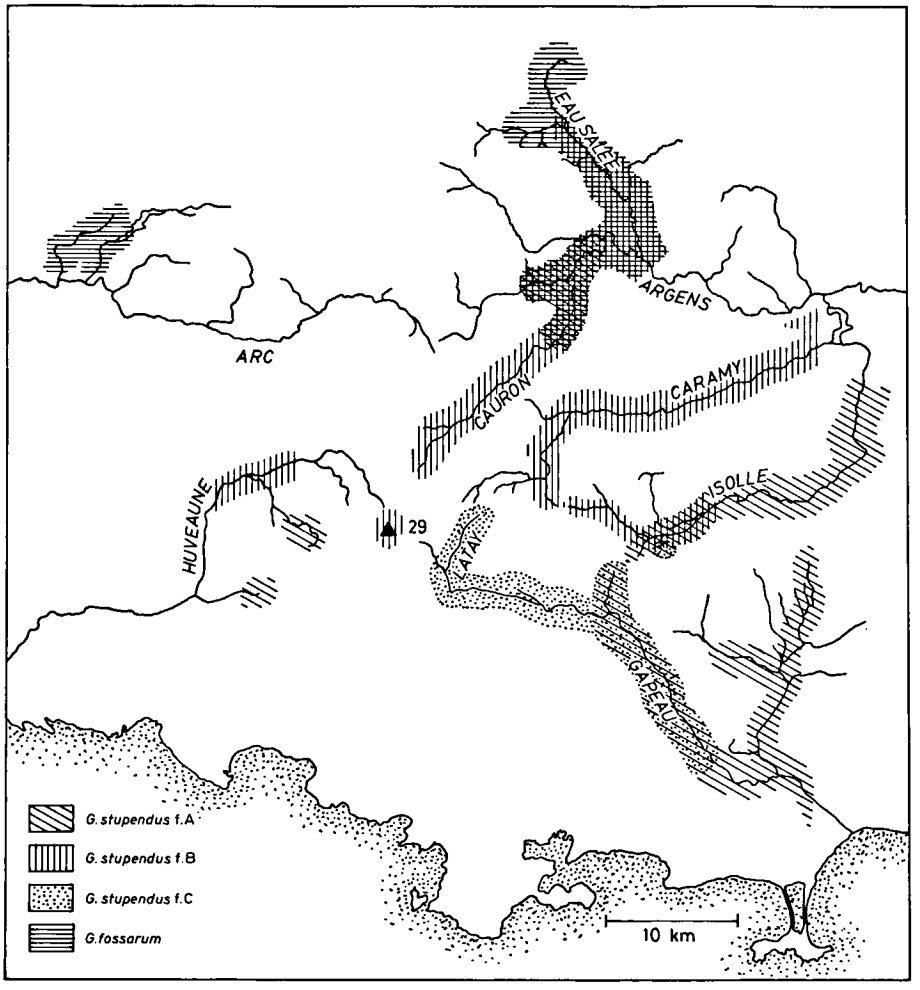

Fig. 2. Distribution of the major morphological forms A, B and C of *G. stupendus*, and *G. fossarum*.

The *G. stupendus* form C populations from the Gapeau system were identical to the former population from the type-locality of *G. stupendus*, a spring in the Vallat de Fontanieu (Pinkster, 1983). This location is nowadays canalized and polluted (fig. 1: approx. 10 km N.W. of Toulon), and gammarids were not found anymore.

Although *G. stupendus* form C was largely restricted to the Gapeau system whereas the tributaries to the Argens were predominantly populated by *G. stupendus* form B populations, there does not seem to be a clear correlation between the different morphological forms of *G. stupendus* and the drainage systems studied (fig. 2). Notably the occurrence of *G. stupendus* form A populations across all these systems suggests the absence of any correlation.

Seasonal changes in morphology

To check seasonal variation in morphology, the following stations were sampled: 1 (Gapeau resurgence, *G. stupendus* form C), 17 (Argens resurgence, predominantly *G. stupendus* form A), 27 and 32 (sources of the Huveaune, *G. stupendus* form A), 43 (spring in one of the lower reaches of the Huveaune, *G. stupendus* form B) and 29 (Fontaine de St. Pilon, *G. stupendus* form B). Samples collected in July 1985 at stations 1,

TABLE II

Distribution of allelic frequencies at four polymorphic enzyme loci. For legends of morphological forms and station numbers see table I; a = fastest moving allele; b-g = slower moving alleles; Sa = silent allele; N = sample size; h = heterozygosity per locus (direct count); H = average heterozygosity (direct count) over the four loci studied; (*) = significant departure from Hardy-Weinberg equilibrium ($P<0.05$).

station		13	24	25	17	17	22	22	22	19	19	19	30	44	37	38	46	40	40	34	43	32	27	29	3	1	45	45	6	6	47	48
morph.		*f*	*f*	*f*	*f*	A	*f*	A	B	*f*	A	B	B	B	B	B	B	A	C	B	B	A	A	B	C	C	A	C	C	A	B	B
Gpi	a	1	1	0.97	1	-	0.72	-	-	0.94	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	b	-	-	-	-	-	0.26	-	-	0.06	-	-	-	-	-	0.11	0.01	-	0.07	-	0.01	0.1	-	0.03	0.05	0.03	0.66	-	0.03	0.05	-	0.12
	c	-	-	0.03	-	0.01	0.02	-	0.04	-	0.01	0.09	0.01	-	0.17	0.76	0.89	-	0.84	0.09	0.31	0.82	0.81	0.96	0.92	0.94	0.26	0.89	0.92	0.9	0.95	0.81
	d	-	-	-	-	0.04	-	-	0.75	-	0.02	0.78	0.65	0.51	0.44	0.08	0.08	0.23	0.08	0.67	0.52	0.08	-	-	0.03	0.03	0.08	0.11	-	-	0.05	0.05
	e	-	-	-	-	0.9	-	0.5	-	-	0.91	-	0.22	0.49	0.01	0.05	0.01	0.69	0.01	0.02	-	-	-	0.01	-	-	-	-	0.05	0.05	-	0.02
	f	-	-	-	-	0.04	-	0.5	0.19	-	0.06	0.13	0.12	-	0.37	-	0.01	0.05	-	0.22	0.16	-	0.17	-	-	-	-	-	-	-	-	-
	g	-	-	-	-	0.01	-	-	0.02	-	-	-	-	-	0.01	-	-	0.03	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-
	N	56	30	19	6	71	85	4	28	8	59	39	39	47(*)	39	31	87	39	42	29	44(*)	33	26	42	74	74	67	18	20	11	20	30
	h	0	0	0.05	0	0.18	0.33	1	0.43	0.12	0.17	0.38	0.44	0.72	0.67	0.4	0.22	0.44	0.29	0.45	0.45	0.3	0.3	0.07	0.12	0.12	0.44	0.22	0.15	0.18	0.1	0.2
Got-1	a	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	0.01	0.04	0.23	-	-	-	-	-	-	-	-	-	-	-	-	-
	b	-	-	-	-	-	-	-	0.84	-	-	0.9	0.97	-	0.73	0.87	0.63	-	-	0.9	0.62	0.06	0.4	0.31	0.86	-	1	-	-	0.81	0.65	0.83
	c	0.99	0.96	0.94	1	-	0.99	-	-	1	-	0.07	-	-	0.26	0.01	0.17	-	-	-	0.08	-	-	-	0.03	-	-	-	0.02	0.19	0.1	0.05
	d	-	-	-	-	0.11	-	0.5	0.12	-	-	0.03	0.03	1	0.01	0.07	0.18	0.96	0.73	0.1	0.3	0.94	0.6	0.69	0.11	0.9	-	1	0.92	-	0.2	0.07
	e	0.01	0.04	0.06	-	0.76	0.01	0.5	-	-	0.94	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.02	-	-	0.05
	f	-	-	-	-	0.13	-	-	-	-	0.06	-	-	-	-	0.05	0.01	-	0.04	-	-	-	-	-	-	0.1	-	-	0.04	-	0.05	-
	N	55	28	16	7	63	76	3	28(*)	7	58	30	40	65	37	49	75(*)	39	42	30	42	33	25(*)	43	73	74	67	20	27	13	20	30
	h	0.18	0.07	0.12	0	0.4	0.01	0	0.1	0	0.12	0.2	0.05	0	0.27	0.22	0.28	0.03	0.36	0.13	0.4	0.12	0.32	0.35	0.23	0.2	0	0	0.15	0.4	0.4	0.23
Mdh-1	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.73
	b	0.03	1	1	1	0.97	0.8	1	1	1	1	1	1	0.28	0.52	1	0.99	0.05	0.98	1	1	1	1	1	1	1	0.84	0.97	1	1	0.7	0.27
	c	0.97	-	-	-	0.03	0.2	-	-	-	-	-	-	0.72	0.48	-	0.01	0.91	0.02	-	-	-	-	-	-	-	-	0.03	-	-	0.3	-
	d	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.16	-	-	-	-	-
	e	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	N	55	30	16	6	63	77	5	28	7	59	37	30	56	29	50	87	39	42	30	35	32	26	43	84	53	67	20	27	13	20	30
	h	0.5	0	0	0	0.06	0.3	0	0	0	0	0	0	0.37	0.34	0	0.01	0.13	0.05	0	0	0	0	0	0	0	0.22	0.05	0	0	0.3	0.27
Mdh-2	Sa	-	-	-	-	-	-	-	-	-	-	-	-	-	0.09	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	a	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	0.02	0.08	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-
	b	-	-	-	-	0.1	0.01	-	-	-	0.02	-	-	0.01	0.16	0.63	0.08	-	-	-	-	-	-	-	0.83	0.01	-	0.03	0.02	-	-	0.05
	c	1	0.99	1	1	0.98	0.98	1	1	1	0.98	1	1	0.99	0.75	0.35	0.79	0.97	0.98	1	1	1	0.98	1	0.37	0.98	0.99	0.97	0.98	1	0.8	0.75
	d	-	0.01	-	-	-	0.01	-	-	-	-	-	-	-	-	-	0.04	0.03	-	-	-	-	0.02	-	-	0.01	0.01	-	-	-	0.2	-
	e	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.01	-	-	-	-	-	-	-	-	-	-	-	-	0.2
	N	55	30	16	6	52	68	5	28	7	59	37	30	56	28	50	87	39	42	30	45	32	26	33	84	53	67	20	27	13	10	20
	h	0	0.03	0	0	0.04	0.03	0	0	0	0.02	0	0	0.02	0.43	0.5	0.37	0.05	0.05	0	0	0	0.04	0	0.43	0.04	0.02	0.05	0.04	0	0.4	0.5
	H	0.02	0.03	0.04	0	0.17	0.168	0.33	0.13	0.03	0.77	0.15	0.12	0.28	0.43	0.29	0.22	0.16	0.18	0.15	0.22	0.1	0.17	0.1	0.2	0.09	0.17	0.08	0.08	0.14	0.3	0.3

17, 29 and 43 showed no differences compared to those collected in November 1984 and 1985; samples from stations 27 and 32 showed some minor differences in the setation of P5-P7.

Stability of morphological characters under uniform laboratory conditions

The animals for this experiment were collected at stations 1 (*G. stupendus* form C), 32 (*G. stupendus* form A) and 43 (*G. stupendus* form B). The experiments were run from November 1984 to August 1985. The morphology of all three forms proved to be stable. Neither the animals collected, nor their progeny in F1 and F2 did show any deviation with regard to the initial morphology. However, the setosity of P5-P7 of males in form A and B populations diminished as they grew older. This phenomenon is much less expressed in females, especially those of form B.

Genetic variation

Variation in allelic distribution pattern

All enzymes, MDH-2 excepted, moved anodally. Major differences in allele distribution occurred at the *Gpi* and *Got-1* loci (table II). The allele distribution in *G. fossarum* at these loci differed almost completely from all the other populations studied; thus, these loci can be considered genetic markers for *G. fossarum* and *G. stupendus*.

Morphological forms of *G. stupendus* occurring sympatrically showed different allele distribution patterns (table II: stations 6, 19, 22, 40 and 45). Moreover, it can be seen that intra-areal populations do not necessarily exhibit a more similar allele distribution pattern than inter-areal populations (as reported by Gooch & Hetrick, 1979), and only part of the discontinuities in the distribution of allele frequencies coincides with differences in morphology.

Variation among morphological forms

Values of I (Nei's index of normalized genetic identity; Nei, 1972) range from 1, indicating identical allele frequencies, to 0.12, meaning that the complements of alleles only slightly overlap (table III). As in these indices identical monomorphic loci are not included in the averages (which increases the discriminatory power), these figures are not directly comparable with the ranges of indices characteristic of specific or subspecific differentiation (Ayala, 1975; Thorpe, 1982).

If morphological differentiation in the various forms of *G. stupendus* (viz. forms A, B and C) is paralleled by genetic differentiation, intra-form values of I should be higher than inter-form values. Following Gooch & Hetrick (1979) mean I and standard deviation of the genetic indices of each morphological form A, B and C of *G. stupendus*, and *G. fossarum* were calculated. These figures and the range lowest-highest value, I_{min}-I_{max} (to visualize the heterogeneity of I), are given in table IV. *G. fossarum*, showing the highest mean intra-form value of I, seems to represent a well-defined, genetically coherent group of populations in the area under study.

Differences in mean intra-form values of I in the three forms A, B and C of *G. stupendus* (table IV) are due on the one hand to the higher level of genetic differentiation among the populations showing a form A morphology (resulting in low mean intra-form I values), and on the other hand to the effect of high I values of neighbouring form B and C populations. Although minimum-maximum ranges of I in form B and C populations are still large (table IV), mean intra-form values of I can still be high as a consequence of a greater number of single high I values of intra-areal comparisons. However, as mentioned above, genetically different populations may occur sympatrically (tables I and II), and intra-area I values do not necessarily have to be higher.

Compared to *G. fossarum*, the mean intra-form value of I in form A of *G. stupendus* was low, with a wide range of I_{min}-I_{max} (table IV). Moreover, the mean inter-form A-C value of I is higher than the intra-form A value (table IV). Using the Mann-Whitney U-test, intra-I values of form A do not differ significantly at the 5% level from inter-A-B values ($P = 0.71$) and inter-

TABLE III

Matrix of Nei's indices of normalized genetic identity (*I*), based upon allele frequencies at four polymorphic enzyme loci. Stations in the same order as in table II, legends of morphological forms as in table I.

station	13	24	25	17	17	22	22	22	19	19	19	30	44	37	38	46	40	40	34	43	32	27	29	3	1	45	45	6	6	47	48
morph.	*f*	*f*	*f*	*f*	A	*f*	A	B	*f*	A	B	B	B	B	B	B	A	C	B	B	A	A	B	C	C	A	C	C	A	B	B
13 *f*	-																														
24 *f*	0.76	-																													
25 *f*	0.76	1	-																												
17 *f*	0.76	1	1	-																											
17 A	0.29	0.54	0.56	0.53	-																										
22 *f*	0.82	0.97	0.97	0.97	0.54	-																									
22 A	0.30	0.59	0.6	0.53	0.96	0.53	-																								
22 B	0.28	0.55	0.56	0.55	0.6	0.55	0.68	-																							
19 *f*	0.76	1	1	1	0.54	0.98	0.59	0.56	-																						
19 A	0.27	0.53	0.54	0.52	0.99	0.51	0.88	0.57	0.52	-																					
19 B	0.3	0.56	0.57	0.56	0.59	0.56	0.65	1	0.57	0.56	-																				
30 B	0.28	0.54	0.55	0.54	0.64	0.54	0.68	0.99	0.55	0.62	0.99	-																			
44 B	0.49	0.36	0.37	0.36	0.57	0.43	0.66	0.56	0.37	0.5	0.53	0.53	-																		
37 B	0.43	0.44	0.45	0.44	0.51	0.49	0.64	0.89	0.45	0.47	0.88	0.87	0.59	-																	
38 B	0.12	0.4	0.42	0.4	0.45	0.4	0.48	0.7	0.41	0.43	0.72	0.72	0.26	0.7	-																
46 B	0.3	0.58	0.59	0.58	0.58	0.58	0.64	0.79	0.59	0.55	0.8	078	0.44	0.75	0.89	-															
40 A	0.52	0.28	0.29	0.28	0.54	0.37	0.6	0.41	0.29	0.48	0.37	0.41	0.96	0.52	0.17	0.34	-														
40 C	0.29	0.55	0.56	0.54	0.62	0.55	0.75	0.66	0.55	0.57	0.64	0.61	0.65	0.63	0.66	0.87	0.55	-													
34 B	0.28	0.55	0.56	0.55	0.6	0.55	0.69	1	0.56	0.58	1	0.99	0.54	0.9	0.73	0.81	0.4	0.66	-												
43 B	0.33	0.62	0.63	0.61	0.65	0.62	0.76	0.97	0.62	0.66	0.96	0.95	0.62	0.88	0.76	0.89	0.47	0.81	0.97	-											
32 A	0.27	0.52	0.54	0.52	0.59	0.53	0.74	0.65	0.53	0.54	0.63	0.6	0.67	0.63	0.66	0.86	0.56	0.99	0.65	0.81	-										
27 A	0.29	0.56	0.57	0.56	0.62	0.56	0.77	0.76	0.56	0.58	0.74	0.73	0.6	0.74	0.78	0.94	0.5	0.96	0.77	0.89	0.96	-									
29 B	0.28	0.54	0.55	0.53	0.6	0.54	0.72	0.7	0.54	0.56	0.69	0.67	0.6	0.68	0.76	0.93	0.5	0.97	0.71	0.85	0.98	0.99	-								
3 C	0.12	0.4	0.41	0.4	0.42	0.39	0.46	0.67	0.4	0.41	0.68	0.68	0.25	0.68	0.99	0.9	0.16	0.69	0.7	0.74	0.69	0.8	0.79	-							
1 C	0.27	0.52	0.54	0.52	0.59	0.52	0.73	0.62	0.53	0.54	0.6	0.57	0.65	0.6	0.66	0.85	0.55	0.99	0.62	0.79	1	0.96	0.98	0.69	-						
45 A	0.29	0.51	0.52	0.51	0.55	0.57	0.59	0.84	0.53	0.53	0.84	0.86	0.4	0.77	0.77	0.82	0.32	0.64	0.86	0.85	0.64	0.76	0.72	0.74	0.61	-					
45 C	0.27	0.51	0.52	0.51	0.58	0.51	0.73	0.62	0.51	0.53	0.6	0.58	0.69	0.62	0.64	0.83	0.58	0.98	0.63	0.79	1	0.95	0.97	0.67	1	0.59	-				
6 C	0.27	0.52	0.54	0.52	0.6	0.52	0.74	0.61	0.53	0.55	0.59	0.57	0.65	0.61	0.68	0.85	0.55	0.98	0.61	0.78	0.99	0.95	0.97	0.71	0.99	0.6	0.99	-			
6 A	0.33	0.59	0.6	0.58	0.58	0.6	0.62	0.79	0.59	0.56	0.81	0.81	0.39	0.75	0.86	0.98	0.31	0.81	0.82	0.89	0.79	0.91	0.89	0.88	0.79	0.86	0.76	0.78	-		
47 B	0.38	0.49	0.51	0.49	0.51	0.52	0.57	0.72	0.5	0.48	0.74	0.72	0.5	0.78	0.83	0.96	0.44	0.83	0.75	0.83	0.82	0.91	0.91	0.85	0.82	0.78	0.81	0.81	0.95	-	
48 B	0.25	0.33	0.34	0.33	0.36	0.36	0.39	0.61	0.34	0.34	0.63	0.63	0.32	0.65	0.7	0.79	0.28	0.6	0.64	0.67	0.6	0.71	0.7	0.72	0.59	0.72	0.57	0.59	0.81	0.81	-

TABLE IV

Mean intra- and inter-morphological form values of the genetic identity (*I*) and standard deviation, based upon four polymorphic enzyme loci. Intra-form values in bold face; range I_{min}-I_{max} in italics.

	G. stupendus form A	*G. stupendus* form B	*G. stupendus* form C	*G. fossarum*
G. stupendus form A	**0.65 ± 0.18** *(0.31-0.99)*			
G. stupendus form B	0.65 ± 0.18 *(0.17-0.99)*	**0.76 ± 0.16** *(0.28-1)*		
G. stupendus form C	0.69 ± 0.19 *(0.16-1)*	0.7 ± 0.14 *(0.25-0.99)*	**0.87 ± 0.16** *(0.67-1)*	
G. fossarum	0.49 ± 0.17 *(0.27-0.6)*	0.47 ± 0.11 *(0.12-0.63)*	0.46 ± 0.11 *(0.12-0.56)*	**0.92 ± 0.1** *(0.76-1)*

A-C ($P = 0.40$) values of *I*. Thus, the morphological form A is not paralleled by a specific genetic differentiation.

The mean intra-form value of *I* in *G. stupendus* form B is higher than in form A, but still lower than in *G. fossarum* and single *I* values are very heterogeneous. Intra-form values of *I* in form B are not significantly different from the inter-B-C values at the 1% level ($P = 0.0174$), but differ significantly from the inter-A-B values ($P = 0.0006$). This means that part of the form B populations shows a uniform genetic differentiation.

The mean intra-form value of *I* in *G.*

TABLE V

Mean intra- and inter-zone values of the genetic identity (I) and standard deviation of four zones of high genetic identity, based upon four polymorphic enzyme loci. Intra-zone values in bold face; range I_{min}-I_{max} in italics.

zone	Huveaune-Gapeau	Huveaune-Cauron-Argens	*G. stupendus* form A Argens	*G. fossarum* Argens
Huveaune-Gapeau	**0.98 ± 0.02** *(0.95-1)*			
Huveaune-Cauron-Argens	0.68 ± 0.09 *(0.56-0.89)*	**0.98 ± 0.02** *(0.95-1)*		
G. stupendus form A Argens	0.63 ± 0.08 *(0.53-0.77)*	0.63 ± 0.05 *(0.56-0.76)*	**0.99 ± 0.01** *(0.99-1)*	
G. fossarum Argens	0.54 ± 0.18 *(0.5-0.57)*	0.57 ± 0.03 *(0.54-0.65)*	0.55 ± 0.03 *(0.51-0.6)*	**0.99 ± 0.01** *(0.99-1)*

stupendus form C is relatively high. Intra-form values differ significantly from inter-A-C ($P = 0.009$) and inter-B-C values ($P = 0.001$) at the 1% level, which means that most of the form C populations show a uniform genetic differentiation. It should be kept in mind, however, that different morphological forms may also be subject to similar genetic differentiation (see table II).

Mean inter-form values of the three forms A, B and C of *G. stupendus*, and *G. fossarum* (viz. I inter-A-*G. f.*, B-*G. f.* and C-*G. f.*) do not differ significantly at the 5% level ($P = 0.29$, 0.12 and 0.013, respectively), which means that on the average all three forms A, B and C of *G. stupendus* differ genetically to about the same extent from *G. fossarum*.

Genetic variation among areas

To examine in more detail the distribution of populations having a high degree of genetic identity, these populations were pooled and grouped into "zones of high identity" (fig. 3), and mean I values of these zones were calculated (table V). Since genetically different forms of *G. stupendus*, and *G. fossarum* occurred sympatrically, these zones may overlap partly or even entirely (fig. 3). The various zones and the morphological forms they include are listed below.

Huveaune-Gapeau: stations 27 and 32 (*G. stupendus* form A); station 29 (form B); stations 1, 6, 40 and 45 (form C).

Huveaune-Cauron-Argens: stations 19, 22, 30, 34 and 43 (all *G. stupendus* form B).

G. fossarum Argens: stations 19, 22, 24 and 25.

G. stupendus form A Argens: stations 17, 19 and 22.

It is striking that populations of different morphological forms occurring in the same stream system never belonged to the same zone of high identity (applies to all zones). To put it otherwise, populations of different morphological forms belonging to the same zone of high identity never occurred in the same stream system. It should be noticed as well that the extension of these zones may range across several stream divides. High genetic similarity across stream divides was also observed in populations of the neighbouring upper reaches of the Latay, Caramy (stations 3 and 38; $I_{3,38} = 0.99$) and Isolle (station 46; $I_{3,46} = 0.9$, $I_{38,46} = 0.89$). The same was observed in the Gapeau and Isolle (stations 45 and 40; $I_{45,40} = 0.98$).

When genetically different populations occur sympatrically, it is evident that discontinuities in allele distribution and thus lower I values will be found within the same stream system. However, dissimilarities in allele distribution occurred also in the same stream system among genetically less differentiated populations not susceptible to be attributed to different genetical or morphological forms (viz. stations 37 and 38: $I_{37,38} = 0.69$; stations 1 and 3: $I_{1,3} = 0.69$; station 40 population C and station 46: $I_{40,46} = 0.87$). Thus, I values among

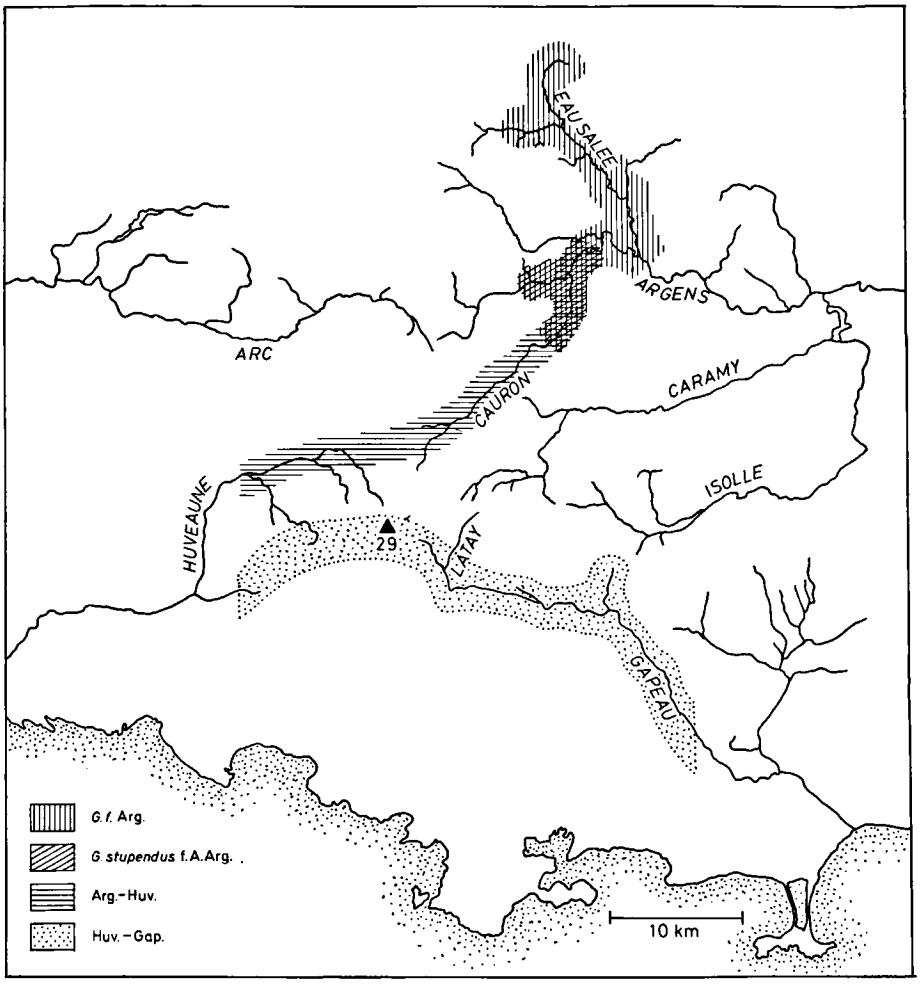

Fig. 3. Distribution of four zones of high genetic identity. The zones are composed of populations with high mutual genetic identity (*I*) levels, based upon four polymorphic enzyme loci. *G. f.* Arg. = *G. fossarum* Argens; *G. stupendus* f. A. Arg. = *G. stupendus* form A, Argens; Arg.-Huv. = Argens-Cauron-Huveaune; Huv.-Gap. = Huveaune-Gapeau.

populations across a stream divide may be higher than among populations in one and the same tributary.

The UPGMA dendrogram

The results discussed above are summarized in an UPGMA dendrogram (cf. Sneath & Sokal, 1973) (fig. 4). Two main clusters (*G. stupendus* and *G. fossarum*) can be distinguished. The *G. stupendus* cluster can be subdivided in smaller clusters. The smallest cluster consists of populations of stations 40 (form A) and 44. These populations are somewhat more closely related to *G. stupendus* than to *G. fossarum* and may have more affinities to populations that have not been investigated electrophoretically (e.g. stations 39, 41 and 42, localities a-i; table I, fig. 1). The same applies to the population of station 48, from the Massif des Maures. Other clusters cover the zones *G. stupendus* form A Argens, Huveaune-Cauron-Argens and Gapeau-Huveaune (table V, fig. 4).

A number of populations, that previously could not be attributed to a zone of high identity because of relatively low *I* values with respect to all other populations studied, are

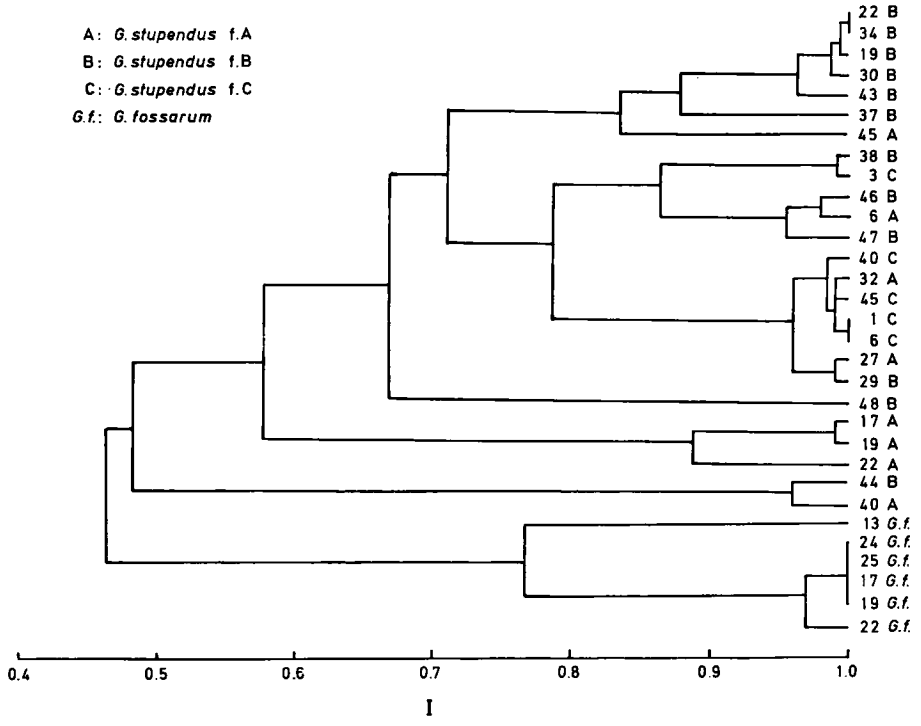

Fig. 4. UPGMA dendrogram based upon four polymorphic enzyme loci. I = Nei's index of normalized genetic identity; for legends of station numbers see table I and fig. 1.

included in the dendrogram. However, as the cophenetic coefficient is relatively low (0.864), their position is arbitrary. For this reason, and because of sampling errors due to small sample sizes, the dendrogram becomes less reliable close to its extremities. However, from this dendrogram it becomes clear that there is no correlation between morphological and genetic variation, and that there is no distinct relation between either morphological or genetic variation and the stream systems studied.

Comparison of allele frequencies in 1984 and 1985

Allele frequencies at the *Gpi* and *Got-1* locus in populations of stations 1, 32 and 43 are presented in table VI. P values indicate that the allele frequencies at these loci in November 1984 and November 1985 did not differ significantly in any of the three populations studied.

Cross-breeding experiments

Local *G. fossarum* and *G. fossarum* from northern France (f_l and f_n in tables VI and VII) proved to be completely interfertile in reciprocal crosses: the F2 (F1 × F1) could be obtained. In crosses between local *G. fossarum* and *G. stupendus* form C only one female gave offspring (cross C × f_l, table VIII). From crosses between local *G. fossarum* and *G. stupendus* form B several females with offspring could be obtained (f_l × B); in the reciprocal cross only one female gave offspring. F1 from these crosses (except for C × f_l) have been raised to maturity, but failed to give offspring. Experiments to hybridize *G. stupendus* form B and C were more successful. The most striking result from these experiments is that although several dozens of F1 juveniles of both crosses B × C and C × B have been raised to maturity, no F2 (F1 × F1) could be obtained.

TABLE VI

Allele frequencies at the *Gpi* and *Got-1* locus in populations of stations 32, 43 and 1 in November 1984 and November 1985; b = fastest moving allele; N = sample size; *P* = probability in Chi-square contingency test (* = probability in Fisher's Exact test).

	Gpi						*Got-1*					
	b	c	d	f	N	*P*	b	c	d	f	N	*P*
station 32 (1984)	0.02	0.88	0.1	—	48	0.043	0.04	—	0.96	—	48	0.5*
(1985)	0.1	0.82	0.08	—	33		0.06	—	0.94	—	33	
station 43 (1984)	—	0.18	0.61	0.21	31	0.53	0.62	0.03	0.35	—	16	0.6
(1985)	0.01	0.31	0.52	0.16	44		0.62	0.08	0.3	—	42	
station 1 (1984)	0.04	0.84	0.12	—	34	0.16	—	—	0.92	0.08	69	0.92
(1985)	0.03	0.94	0.03	—	74		—	—	0.9	0.1	74	

DISCUSSION AND CONCLUSIONS

The situation described in this study resembles in more than one respect the findings of Gooch & Hetrick (1979), who studied the genetic structure of *G. minus* in a North American karst area. These authors state that genetic similarity may be largely a neighbourhood effect quite independent of ecophenotypic differentiation (in this study: population 1 of form A is likely to have a higher *I* with population 2 of form C in the same area than with population 3 of form A in another area); they conclude that the forms are independently evolved phenotypes at most localities rather than genetically knit distinct "varieties", subspecies or species. In their opinion it is shown as well that convergent morphologies can evolve without an accompanying restructuring of the entire genome toward a greater similarity. However, based upon three loci, their statement might be somewhat premature. Nevertheless, the results of the present study (based upon four loci) appear to indicate the occurrence of morphological differentiation without restructuring the entire genome (e.g. populations of *G. stupendus* forms A, B and C, placed in the same cluster in fig. 4 and in the same zone of high identity in table V). These results indicate as well that convergent morphologies may evolve independently, or that genetic restructuring to some degree may occur without accompanying changes in morphology (e.g. *G. stupendus* form B populations in Cauron, Caramy, Isolle and Préconil; the various form A populations in Argens, Cauron, Huveaune, Isolle and Gapeau).

Otherwise, the results of the present study are more complex than the situation reported by Gooch & Hetrick (1979) and there is one essential difference: in the area under study genetically and morphologically different forms occurred sympatrically. Different morphological forms occurring (whether or not sympatrically) in the same stream system were all genetically different (e.g. *G. stupendus* forms A and B in the Argens) and sometimes populations within the same stream system attributed to the same morphological form differed also genetically (e.g. *G. stupendus* form B in the Caramy). In this context for instance, it is likely that *G. stupendus* form A from the Argens-Cauron represents such a genetically-knit distinct "variety" (as mentioned above) in which morphological changes have been accompanied by genetic differentiation, or vice versa. Occurring in sympatry with genetically different populations of *G. stupendus* form B (stations 19 and 22), these populations should probably be considered as a separate species. However, there is no certainty that the morphology is a direct expression of the changing genome. Therefore, based upon the four loci studied, it can be concluded that there is no direct relationship between morphological and genetical differentiation.

TABLE VII

First series of crossbreeding experiments: November 1985-March 1986; f_l = local *G. fossarum* population from station 13; f_n = "true" *G. fossarum* population from Ambleteuse (northern France); B = *G. stupendus* form B (station 43); C = *G. stupendus* form C (station 1).

cross ♂ × ♀	N ♂♂	N ♀♀	N ovig. ♀♀	N ♀♀ with offspring	F2 obtained
$f_l \times f_l$	30 f_l	36 f_l	16	8	+
B × B	37 B	40 B	13	3	+
C × C	34 C	34 C	28	14	+
$f_l \times f_n$	42 f_l	42 f_n	35	12	+
$f_n \times f_l$	48 f_n	50 f_l	28	4	+
f_l × B	35 f_l	22 B	10	2	—
B × f_l	62 B	59 f_l	9	1	—
f_l × C	50 f_l	50 C	25	—	—
C × f_l	52 C	52 f_l	7	—	—
B × C	77 B	77 C	13	2	—
C × B	78 B	65 B	10	1	—

TABLE VIII

Second series of crossbreeding experiments. For legends see table VII.

cross ♂ × ♀	N ♂♂	N ♀♀	N ovig. ♀♀	N ♀♀ with offspring	F2 obtained
$f_l \times f_l$	30 f_l	45 f_l	14	6	+
$f_n \times f_n$	32 f_n	32 f_n	15	10	+
B × B	98 B	128 B	117	20	+
C × C	78 C	106 C	78	7	+
$f_l \times f_n$	50 f_l	80 f_n	17	7	+
$f_n \times f_l$	100 f_n	100 f_l	34	9	+
f_l × B	100 f_l	108 B	37	3	—
B × f_l	94 B	126 f_l	38	—	—
f_l × C	100 f_l	117 C	28	—	—
C × f_l	54 C	73 f_l	12	1	—
B × C	50 B	68 C	28	14	—
C × B	100 C	100 B	43	15	—

A clear correlation between morphological variation and geographical distribution pattern could not be found (fig. 2). The genetical variation pattern is more coherent (fig. 3) and corresponds fairly well to the main clusters of the UPGMA dendrogram (fig. 4). The validity of the "zones" of high identity and the main clusters of the UPGMA dendrogram are confirmed by genetic differentiation at *Ldh* and *Pep* loci (Scheepmaker, in prep.). However, discontinuities in the allelic distribution do not coincide with apparent geographic barriers.

A number of populations had low *I*-levels with all other populations studied and therefore did not figure in any of the zones of high identity: the *G. stupendus* form A population from the Isolle (station 40) and the form B populations from Caramy (station 44) and Préconil (station 48, Massif des Maures). Possibly these populations are more closely related to the populations which have not been investigated electrophoretically (e.g. populations at stations 39, 41, 42 and at localities a-i; cf. table I and fig. 1).

Unfortunately *G. stupendus* form A from the Argens and the Cauron was not involved in the hybridization experiments. One of the main reasons for this was that this form always occurs sympatrically with *G. fossarum*, whereas it is very difficult to separate the two forms in the field. The taxonomic status of these populations will be treated in a future publication (Scheepmaker, in prep.).

Hybridization experiments show a close relationship between the forms considered in this study: all crosses gave offspring in at least one of the reciprocal combinations. However, crosses between *G. fossarum* and *G. stupendus* populations (stations 13, 43 and 1; $I_{13,43} = 0.33$, $I_{1,13} = 0.27$) were not very successful, and F2 could never be obtained (whether or not due to an artefact as a consequence of the limited number of F1 individuals that could be raised to maturity) and reproductive isolation between these species has most probably been achieved.

Results of the hybridization experiments improve stepwise when inter-populational crosses of *G. stupendus* are considered. Reciprocal crosses between form B and C (stations 1 and 43; $I_{1,43} = 0.79$) gave a numerous offspring, which could be raised to maturity. However, an F2 could not be obtained. Thus, unless these results are due to laboratory artefacts, reproductive isolation has probably been achieved. Finally, in hybridization

experiments carried out by Pinkster (1983), the offspring (F1) from all crosses between *G. stupendus* form A, B and C (collected at stations 1, 27 and 29; $I_{1,27} = 0.96$, $I_{1,29} = 0.98$, $I_{27,29} = 0.99$) were completely interfertile. The results of these experiments suggest several genome differences leading toward reproductive isolation among populations of *G. stupendus*.

According to Gooch & Hetrick (1979), high I values may be due to high gene flow, concordance of selective forces or, unless dealing with numerous loci, simple coincidence, whereas a low I, even based on a single locus, means incontrovertibly that the populations are isolated or experience different modes of selection. Unless coincidence, inter-areal populations (e.g. forms A and C, stations 27 and 1) separated by an obvious geographic barrier (over a long distance, across a stream divide) may have high I values and be completely interfertile, whereas other populations (e.g. those at stations 1 and 43), apparently separated by one and the same barrier do not seem to be completely interfertile anymore, thus advancing toward reproductive isolation.

On the one hand, the high I-level of stations 3 and 38 ($I = 0.99$) indicates that, unless coincidence, gene flow across stream divides is quite well possible. Although the upper reaches are very near to each other, they desiccate almost completely in the dry season. It is not known whether these reaches are recolonized during the "rainy season" or not; otherwise gene flow must occur by subterranean contact. Evidence is against a fast recolonization of dried-up water reaches: many localities where no gammarids were found in 1980, 1982 and 1984 (the "zero" samples in fig. 1) appeared to desiccate periodically in 1985.

On the other hand, populations in the same tributary showed discontinuities in the allelic distribution pattern. I values may be lower while no obvious genetic barrier is present (e.g. populations at stations 37 and 38, $I_{37,38} = 0.69$; at stations 1 and 3, $I_{1,3} = 0.69$). These discontinuities seem to be correlated with differences in average heterozygosity over the four loci studied. Average heterozygosity (H in table II) among populations in the same zone of high genetical identity is generally rather constant. Partial reproductive isolation might explain the maintenance of different allele frequencies in the same system where no obvious genetic barrier is present.

It can be seen from table I that changes (however slight) occur in the temporal distribution pattern of the morphological forms (e.g. stations 26, 23, 21, 46, 35, 45 and 47). These changes may represent simple shifts in relative abundance of the various forms due to changing environmental conditions favouring one form at the expense of another, or the coincidental replacement of one form by another when a tributary is recolonized, or both at the same time. Thus, the distribution of the various forms can be seen as a dynamic process, governed by intermittent environmental factors. For instance, many populations are repeatedly separated during longer periods of drought, breaking down migration routes and gene flow. As has been demonstrated by Goedmakers (1981) and Goedmakers & Pinkster (1981), gammarids are actively and sometimes massively migrating organisms. In northern France, migration maxima occur in summer; in winter the migration almost stops. If, in the area under study, migration maxima occur in late spring, summer or autumn, they will often coincide with periods of drought. Life cycles in lower reaches are possibly adapted to local environmental conditions, but in the upper reaches or in a source or cave environment, where temperature is more constant, temperature fluctuations may approximate conditions found in similar habitats in central and northern France.

Putting together all the data presented it can be concluded that the various forms of *G. stupendus*, showing different degrees of interfertility which may ultimately result in reproductive isolation, must probably be seen as different stages in the process of speciation.

In combination with isolation, genetic drift is often referred to as a mechanism to provoke discontinuities in the allele distribution pattern (Bulnheim, 1985; Bulnheim & Scholl, 1981a &

b, 1986; Siegismund et al., 1985). However, unless in the case of extreme bottlenecking, the effect of genetic drift is not sufficient to explain the differences observed. According to Ayala (1982), gene frequency changes will be governed primarily by random drift if $4\ Nx \ll 1$ (N = population size; x = rate of other deterministic processes). For example, if we assume a migration rate (m) of 2% (or two individuals for every 100) and there is no mutation or selection, gene frequencies will change toward the frequencies in the population from which the migrants came, even in a small population with only 25 individuals, because in such a case $4\ N\ m = 4 \times 25 \times 2\% = 2 > 1$. Computer simulation experiments by Bodmer & Cavalli Sforza (as discussed by Goodenough, 1984), with an hypothetical population of 25 individuals and two alleles with frequency 0.5, demonstrated that in the absence of other deterministic processes it may require 42 generations to get one of the alleles fixed. Similar experiments have been reported by Spiess (1977): 50 populations of 20 individuals and two alleles with frequency 0.5 came all to fixation by no later than the 94th generation and no earlier than the 6th. In the case of *G. stupendus* this represents respectively 17-24, and 1.5-55 years (Scheepmaker, unpublished data). The Fontaine de St. Pilon (station 29) for instance, inhabited by an isolated population of several dozens to several hundreds of individuals (depending on the period of the year) has still an average heterozygosity of 0.1 (table II), which is not exceptional compared to other populations.

However, the inter-area populations having high I-levels and being interfertile are all large and inhabit a stable environment (i.e. sources and upper reaches with a rather constant discharge). Thus, they are not subjected to important bottlenecking and less susceptible to the effects of genetic drift, whereas selective modes may be quite similar.

Otherwise, local conditions as substrate and temperature regime may be very variable (e.g. muddy and polluted sections in the lower reaches or muddy stagnant pools in desiccating riverbeds, in contrast to the upper reaches and springs with a constant discharge, stony substrate and constant temperature). Temperature in or in the neighbourhood of springs and caves will be rather constant (yearly fluctuations in the order of 1 or 2°C; Giudicelli et al., 1980; pers. obs.) whereas in other (lower) reaches temperature fluctuations will follow the air temperature throughout the year (differences of some 10 to 15°C between summer and winter).

According to Goodenough (1984), random drift and selection pressure allow a "splinter" population to acquire very different allelic frequencies from its parent Mendelian population within a very few generations (from about 7 in experiments performed by Powell & Richmond with *Drosophila paulistorum* as discussed by Goodenough, 1984). Thus, since in the area studied environmental conditions vary greatly it is very likely that genetic differentiation is due to genetic drift, bottlenecking and different selective forces acting together.

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