

Comparative evolution of P–M system and infection by the sigma virus in French and Spanish populations of *Drosophila melanogaster*

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Summary

In 1983, an extensive survey of populations of *D. melanogaster* was started in a southern French region (Languedoc) in two non-Mendelian systems: the P–M system of transposable elements and the hereditary Rhabdovirus sigma. Unexpectedly fast-evolving phenomena were observed and interesting correlations were noted, giving similar geographical pattern to the region in both systems. For these reasons, the analysis was continued and extended towards the north (Rhône Valley) and the south (Spain). In the P–M system, all the Languedoc populations evolved from 1983 to 1991 towards the Q type which is characteristic of the Rhône Valley populations. In contrast, M' strains are currently observed in the southernmost French populations and in all Spanish ones, so that there is a clear pattern in their geographical distribution.

The frequency of flies infected by the sigma virus dramatically increased from 1983 to 1988 in Languedoc; this increase was clearly correlated with some viral characteristics. But, in northern France, similar characteristics did not trigger any increase in the frequency of infected flies. The data presented here show that the distinctive features of Languedoc extend northwards through the Rhône Valley up to Lyon and disappears southwards before the Spanish border.

1. Introduction

Since 1983, an extensive analysis of the natural populations of *D. melanogaster* has been carried out in a southern French region (Languedoc) in two non-Mendelian systems: the status of infection by the sigma virus and the P–M system of transposable elements.

Most viruses are only known through extensive analysis of a few laboratory strains. Some characteristics of a Rhabdovirus, the sigma virus, make it possible to analyse its situation in the wild. In natural populations of *D. melanogaster*, throughout the world, a minority of individuals are infected by the sigma virus (for a review, see Fleuriet, 1988). The virus is not contagious from fly to fly but transmitted through male and female gametes (Brun & Plus, 1980; Emeny & Lewis, 1984). There is no integration into the fly chromosomes and the virus multiplies in the cytoplasm. An infected fly can be easily identified by the symptom of CO₂ sensitivity conferred by the virus upon its host. Because of this symptom, of vertical transmission and of the genetic knowledge we have of

both partners, it is possible to analyse the population genetics of the *Drosophila*–sigma system.

A few loci of the fly are known to give resistance to the virus (Gay, 1978). For the *ref(2)P* locus, a regular polymorphism has been found in populations for both permissive *ref(2)P^o* and restrictive *ref(2)P^p* alleles, with the *ref(2)P^p* allele in the minority. Two viral types, differing in their sensitivity to the *ref(2)P^p* allele coexist in the wild: Type I, which is very sensitive, and Type II, more resistant (Fleuriet, 1988).

In Languedoc, unexpectedly clear and fast-evolving changes were observed (Fleuriet *et al.* 1990; Fleuriet & Periquet, submitted). The most striking phenomenon was a dramatic increase in the frequency of infected flies from 1983 to 1988, while in northern and central France, it remained low (about 0.15) (Fleuriet, 1990). A few other characteristics of the system were observed to change, particularly viral adaptation to the *ref(2)P^p* allele. These modifications were considered to be the cause of the increase in the frequency of infected flies in Languedoc. In northern and central France, comparable variations in viral characteristics were also observed, without triggering any change in

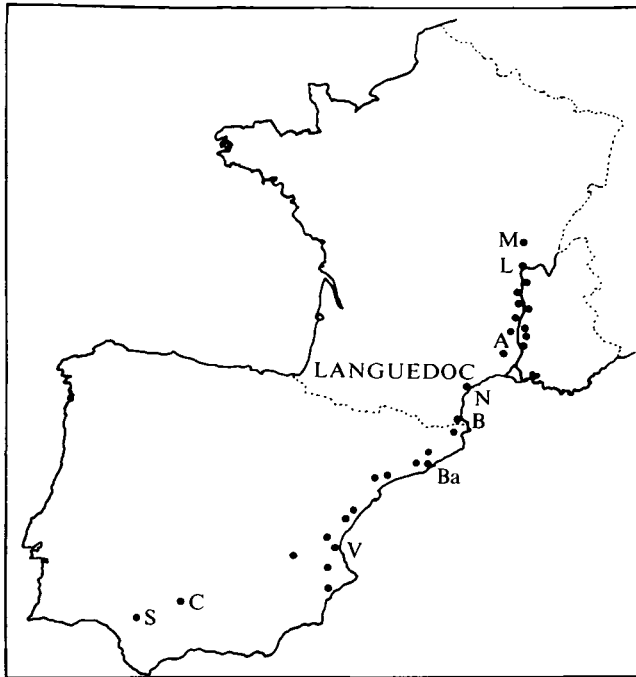


Fig. 1. Geographical location of the populations sampled. France: M, Mâcon (site of sampling – Biziati); L, Lyon (site of sampling – Ste Foy). Rhône Valley: nine populations sampled (from north to south Péage de Roussillon, St Désirat, Sarras, Tain, Loriol, St Gervais, Alba, Suze, St Alexandre). Languedoc: A, Alès; N, Narbonne; B, Banyuls. Spain: Ba, Barcelona (five populations sampled – Perelada, Artès, Sant Sadurni, Montbriol, Gandesa); V, Valencia (five populations sampled – Sant Mateu, Villafamès, Villar, Albaida, Monovar); C, Cordoba; S, Sevilla.

the frequency of infected flies (Fleuriot, 1990). The populations of Languedoc thus seemed to differ from those elsewhere in France, but the factors involved in this difference, physical or biological, or both, could not be identified. The question was then to determine how far from Languedoc this specificity would extend.

During the same period, *D. melanogaster* populations of Languedoc were also surveyed for their status in the P–M system of hybrid dysgenesis (Anxolabéhère *et al.* 1988*a*). In this system, the syndrome of hybrid dysgenesis, which induces gonadal sterility and high mutation levels, is known to be related to the activity of the transposable P element family (see review in Engels, 1989). It is manifested when active P elements are introduced into P susceptible individuals. Strains of *Drosophila* may be characterized on the basis of two properties related to the phenotypic effect of their P elements. Strains are specific in their ability to mobilize P elements that are in an unregulated state. This ability is referred to as ‘P activity potential’. Strains may also vary in their ability to regulate or suppress the activity of the autonomous P elements present in their genomes. This property is referred to as ‘P susceptibility’. It covers the joint action of all mechanisms affecting P element regulation including that of cytotyping (Engels, 1979). P strains have a high

level of P activity potential and no P susceptibility. Q strains have neither P activity potential nor P susceptibility. M’ strains, although containing some defective P elements, have no P activity potential but a significant level of P susceptibility.

Surveys of natural populations have shown that P strains predominate in the Americas and Central Africa, whereas M’ strains predominate in Eurasia and North Africa. In Eurasia, a gradient can be traced from Western Europe, where most strains are Q, to mid-Asian areas where M’ strains predominate (Anxolabéhère *et al.* 1988*b*). A similar gradient was also found in the Languedoc area, over a distance of 250 km, with weak P populations in the north, Q populations in the centre and M’ populations in the south (Anxolabéhère *et al.* 1988*a*) raising the question of the stability of this geographical distribution (Periquet *et al.* 1989).

Moreover, some interesting correlations were noted: a similar division of the Languedoc region into the same three zones was observed for the P–M system and the status of infection by the sigma virus (Anxolabéhère *et al.* 1988*a*). Taking into account all these data, it was therefore decided to analyse, in both systems, the evolution and characteristics of populations in neighbouring regions, in the Rhône Valley and in Spain (Barcelona and Valencia) (Fig. 1). The data collected on the sigma virus in the Languedoc region have been presented in another paper (Fleuriot & Periquet, submitted).

Materials and methods

(i) Collection of samples and culture conditions

Samples of adult flies were collected each autumn (Fig. 1). A detailed map of the Languedoc region is presented in Fleuriot *et al.* (1990). The characteristics of viral clones were measured immediately after fly collection. The frequency of infected flies was measured on GO flies (collected as adults in the wild). The valence and viral type were determined on G1 males. The P–M status was determined in the first three generations in the laboratory. In the laboratory, flies were maintained on axenic food (David, 1959), under natural light conditions.

(ii) P–M system: Determination of P–M status within a population

P potential activity was measured at 28.5 °C, according to the procedure of Periquet (1980). The percentage of dysgenic gonads (GD sterility) was determined in 50 F1 females obtained by crossing females from the Canton S reference strain with males from the tested strain (cross A). The P susceptibility level was measured at 28.5 °C by determining the percentage of dysgenic gonads in 50 F1 females

Table 1. Phenotypic characteristics of strains collected in the Languedoc-Roussillon area from 1983 to 1991, classified in three latitudinal zones

	North (Les Fumades-Gigean)			Centre (Mèze-Salses)			South (Tautavel-Cerbère)		
	No. pop.	P potential ^a	P susceptibility ^b	No. pop.	P potential ^a	P susceptibility ^b	No. pop.	P potential ^a	P susceptibility ^b
1983	14	5.8 ± 1.6	0.9 ± 0.2	12	0.8 ± 0.6	4.4 ± 2.2	9	1.9 ± 1.7	21.9 ± 8.4
1984	8	8.1 ± 3.0	0.4 ± 0.6	5	4.9 ± 3.4	1.1 ± 1.3	9	2.2 ± 1.3	11.4 ± 6.9
1985	5	5.0 ± 6.0	0.8 ± 1.2	13	1.1 ± 0.6	1.8 ± 1.4	8	1.4 ± 1.3	12.0 ± 5.1
1986	15	3.8 ± 1.5	0.7 ± 0.6	13	0.7 ± 0.8	1.5 ± 1.2	8	0.5 ± 0.6	8.9 ± 5.2
1987	11	5.4 ± 2.7	2.2 ± 1.9	11	1.7 ± 1.5	3.2 ± 2.1	8	0.4 ± 0.5	5.1 ± 2.6
1988	9	2.8 ± 1.5	0.4 ± 0.5	7	0.6 ± 0.7	1.4 ± 1.5	8	1.6 ± 1.6	5.6 ± 4.6
1989	5	1.0 ± 1.5	0.0 ± 0.0	6	0.2 ± 0.3	1.3 ± 1.2	8	0.0 ± 0.0	3.5 ± 3.9
1990	3	0.0 ± 0.0	0.0 ± 0.0	10	0.4 ± 0.7	0.2 ± 0.4	8	0.5 ± 0.6	0.5 ± 0.9
1991	6	0.3 ± 0.7	0.7 ± 0.9	6	0.0 ± 0.0	0.7 ± 0.8	8	0.1 ± 0.2	0.1 ± 0.2

^a P potential is the mean of P sterility potential of all strains tested in each zone and measured by per cent GD sterility in diagnostic cross A.

^b P susceptibility is the mean of P susceptibility level of all strains tested in each zone and measured by per cent GD sterility in diagnostic cross A*.

Each mean is given with its confidence interval at 5%.

obtained from a cross between males from the Harwich reference strain and females from the tested strain (cross A*).

(iii) *Sigma virus*

(a) *Frequency of infected flies.* The CO₂ test used to measure the frequency of infected flies is described in Plus (1954).

(b) *Valence and determination of the viral type.* Isofemale lines were isolated from each sample; only CO₂ sensitive lines were kept. They are assumed to carry one viral clone only. For each line, the valence of 5–10 males, i.e. the frequency of infected flies in their progeny, was determined by mating the males individually with *ref(2)P^o/ref(2)P^o* uninfected females. The frequency of CO₂ sensitive flies was then measured in their offspring. The average valence of males from a same line, characteristic of that line was obtained by pooling the values observed from each male.

The method used to determine the type (with reference to the *ref(2)P^p* allele) of a viral clone has been described in Fleuriet (1980). Taking as a reference the valence of a male when it is mated with a *ref(2)P^o/ref(2)P^o* female (see above), its valence with a *ref(2)P^p/ref(2)P^p* female is measured. If the latter value is zero (or close to zero) the male carries a viral type I; if it is only slightly lower than the reference value, it carries a viral type II.

(c) *Sensitivity of viral type II to the ref(2)P^p allele.* In the presence of the *ref(2)P^p* allele, viral clones can still be transmitted by males when they are of type II; they are not transmitted when they are of type I. But there is a wide range of sensitivities to the *ref(2)P^p* allele among type II clones. A parameter, designated as P/O

ratio in this paper, may be calculated to express the sensitivity of a clone:

$$P/O \text{ ratio} = \frac{\left(\begin{array}{l} \text{valence of males of a line mated} \\ \text{with } ref(2)P^p/ref(2)P^p \text{ females} \end{array} \right)}{\left(\begin{array}{l} \text{valence of the same males mated} \\ \text{with } ref(2)P^o/ref(2)P^o \text{ females} \end{array} \right)}$$

For a viral type I, this ratio is nearly zero. For a viral type II, it is usually lower than 1. When P/O = 1, it indicates that the *ref(2)P^p* allele has no effect upon the transmission of the viral clone examined. If P/O is greater than 1, the viral clone is better transmitted in the presence of the *ref(2)P^p* allele.

(d) *Genotypes at the ref(2)P locus.* Males are classified as *ref(2)P^o/ref(2)P^o*, *ref(2)P^o/ref(2)P^p* or *ref(2)P^p/ref(2)P^p* at the *ref(2)P* locus, depending on whether their adult progeny, after crossing with a female from a reference strain, is entirely CO₂ sensitive, half CO₂ sensitive or entirely CO₂ resistant (Fleuriet, 1976). This method allows determination of the genotypes of a few males only (about 40 for one measurement).

3. Results and discussion

(i) P-M system

Table 1 and Fig. 2 present the P-M status of strains collected in Languedoc from 1983 to 1991. From 1983 to 1986, both the P activity potential and the P susceptibility levels were significantly correlated with the rank of populations from north to south. Three zones were defined: a northern zone, with the highest P activity potential (weighted average: 5.4%) and the lowest P susceptibility (0.7%); a central zone with low

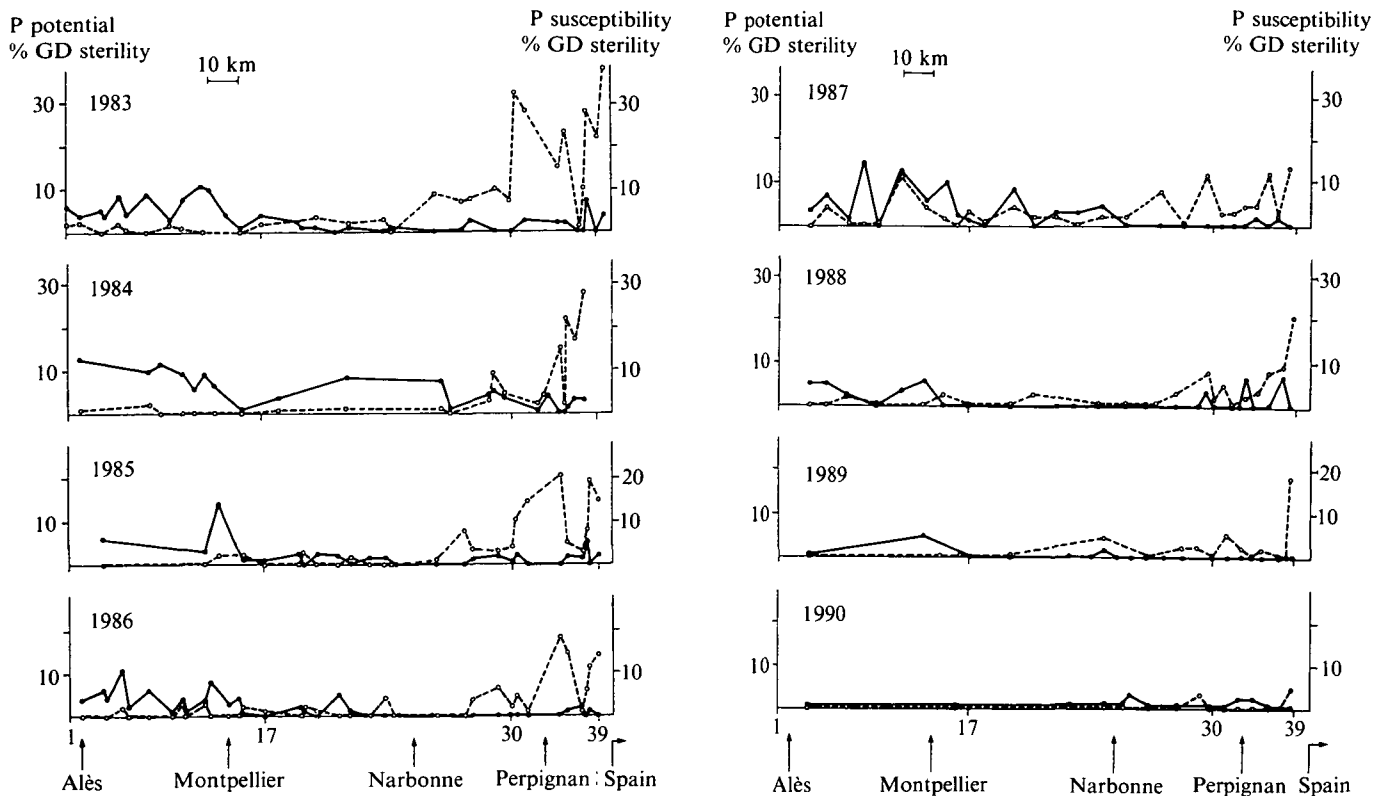


Fig. 2. Geographical and temporal distributions of strains collected in Languedoc according to their potential for the P-M system. The data collected in 1991 were similar to those of 1990 (see Table 1) and are not presented in this figure. Solid lines, P-potential activity; Dashed lines, P-susceptibility level.

Table 2. Phenotypic characteristics of strains (P-M system) in French and Spanish populations (measurements as in Table 1)

	Rhône Valley			Languedoc			Barcelona			Valencia		
	n	P pot.	P susc.	n	P pot.	P susc.	n	P pot.	P susc.	n	P pot.	p susc.
1988	7	0±0	0±0	24	1.8±1.3	2.4±2.2	—	—	—	—	—	—
1989	7	0±0	0±0	19	0.3±0.6	1.9±1.7	4	0.2±0.4	59.5±8.3	5	0.2±0.3	32±3.5
1990	5	0±0	0±0	21	0.4±0.4	0.3±0.4	4	0.5±0.8	57.0±7.5	5	0.4±0.4	31±7.0
1991	4	0±0	0±0	20	0.1±0.2	0.4±0.4	—	—	—	5	0.0±0.0	36±2.3

n, number of populations; P pot., P potential in % of induced GD sterility; P susc., P susceptibility in % of induced GD sterility.

values of both parameters (respectively 1.3 and 2.4%) and a southern zone, with low P activity potential (1.5%) and a moderate P susceptibility level (13.7%). During the five following years (1987–1991), significant differences were observed (Table 1 and Fig. 2). An overall trend towards low level of both parameters was recorded, with average values of 2.3, 0.7 and 0.6% respectively for the P activity potential in the three zones and 0.7, 1.5 and 3.7% for the P susceptibility level. During these 9 years then, we clearly witnessed the progressive elimination of the M' type in wild populations of this area and their replacement by the Q type. In 1991, only the southernmost French population, in Banyuls, was still of a weak M' type.

Table 2 and Fig. 3 present the P-M status of strains collected from 1988 to 1991 in the Rhône Valley and Spain (Barcelona and Valencia). For these years, there

was a marked difference between the French and Spanish populations. The populations of the Rhône Valley, like those of Languedoc, were of the Q type (with very low levels of P activity potential and P susceptibility), whereas the Spanish populations were clearly of the M' type (with intermediate level of P susceptibility, from 30 to 60% of induced GD sterility). No significant temporal trend was observed over the three years in Spain. This period might be too short for any differences to emerge, but, as will be seen below, the frequency of infected flies in Spain did not change either from 1989 to 1991. However, the data collected in Languedoc (Fleuriot & Periquet, submitted) showed that variations in this frequency were detectable there over one year only. This might be an indication that conditions prevailing in Spain are different from those in Languedoc, at least in the rate

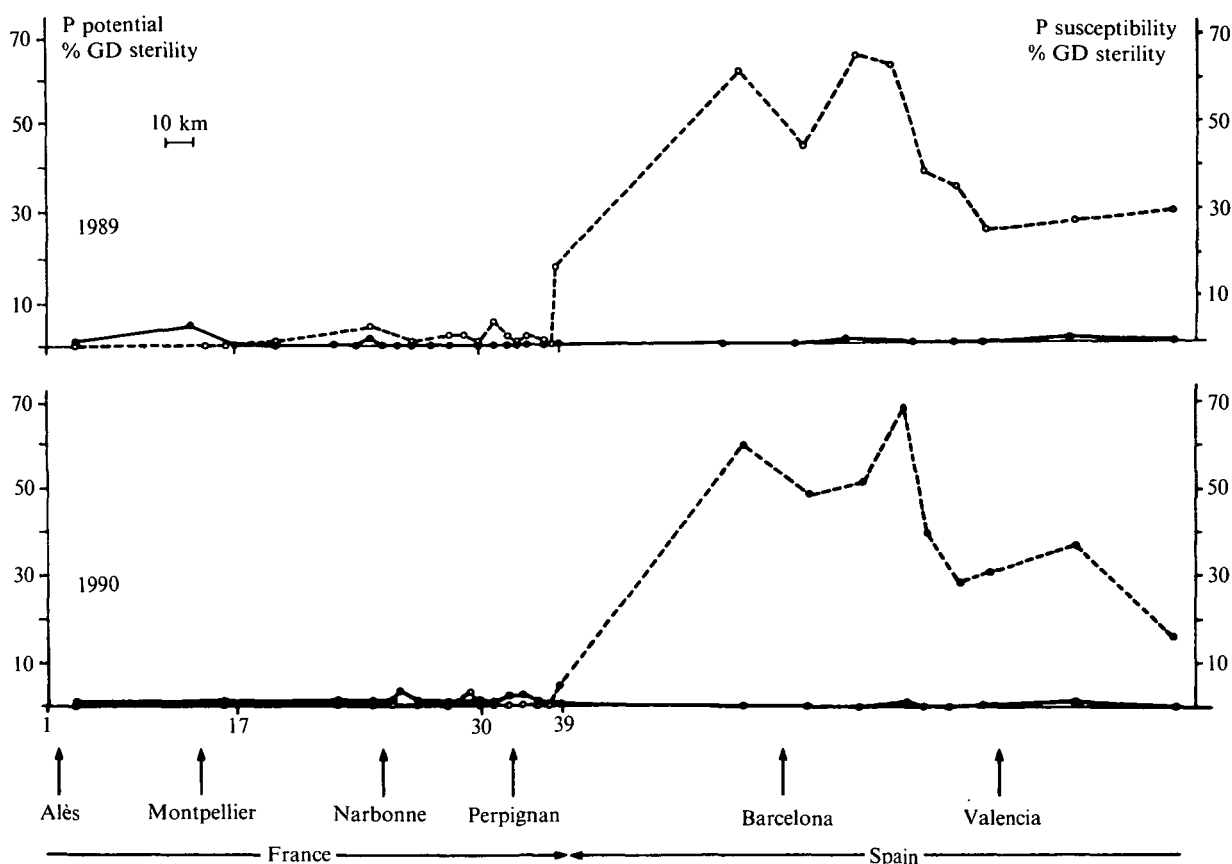


Fig. 3. Geographical and temporal distributions of strains collected in Languedoc area and Spain (P-M system). The data collected in 1991 were similar to those of 1990 (see Table 2) and are not presented in this figure. (Symbols as in Fig. 2).

of evolution of both systems examined. Some years will have to elapse before it is known whether the present geographical pattern is stable or whether Spanish populations will also evolve towards Q type as did the neighbouring French populations.

The main results on the P-M status of Languedoc populations show their slow but progressive evolution towards the Q type, leading to the disappearance of the previously observed geographical distribution. Previous analyses (Anxolabéhère *et al.* 1988a) showed that all these populations harbour P elements. No major differences in copy number or element structure were observed among these populations. The previous distribution was attributed to minor differences in the relative proportions of active P elements and deleted elements. Given the level of induced GD sterility, the complete elements may represent only a small proportion of the total set and be slightly more frequent in northern populations. The spread of these elements in the southern populations during the 1987-1991 period and the mixing with the other elements may explain the fate of these populations. This interpretation assumes that the complete elements have a slight advantage, as generally observed in experimental evolution (Anxolabéhère *et al.* 1986) and from the general model of the invasion hypothesis of *D. melanogaster* by P elements (Kidwell, 1983; Anxolabéhère *et al.* 1988b; Periquet *et al.* 1989; Daniels

et al. 1990). This model suggests that the invasion of the Americas preceded that of Europe by about a decade. It shows that France may have been invaded during the mid-1960s, leading to a majority of Q strains as found in the Rhône Valley, and that eastern and southern Europe with M' strains are still undergoing invasion. The present survey in Languedoc populations shows, for the first time, a significant variation in wild populations, which supports the invasion hypothesis in European populations.

The trend towards Q type strains instead of strong P strains is also in agreement with the model of innovative stepping-stone invasions (Anxolabéhère *et al.* 1986), in which most of the complete elements are deleted into non-autonomous elements during the transposition process. The mixture of these deleted elements along with rare autonomous elements is expected to continue spreading to the east, forming the M' strains presently found in eastern and southern Europe.

It now appears that invasion does occur in natural populations and that in Europe, and especially in Spain, it has not terminated. The presence of a few complete P elements and of numerous deleted elements, some having a regulatory role in suppressing P activity and dysgenic traits in their carrier (Black *et al.* 1987; Jackson *et al.* 1988) may intervene to slow down the transformation of these populations. Ultimately,



Fig. 4. Frequency of infected flies in the Rhône Valley. X, 1987; O, 1988; ●, 1989; △, 1990; ▼, 1991. M, Mâcon (site of sampling – Biziât); L, Lyon (site of sampling – Ste Foy); A, Alès (northernmost site of sampling in Languedoc).

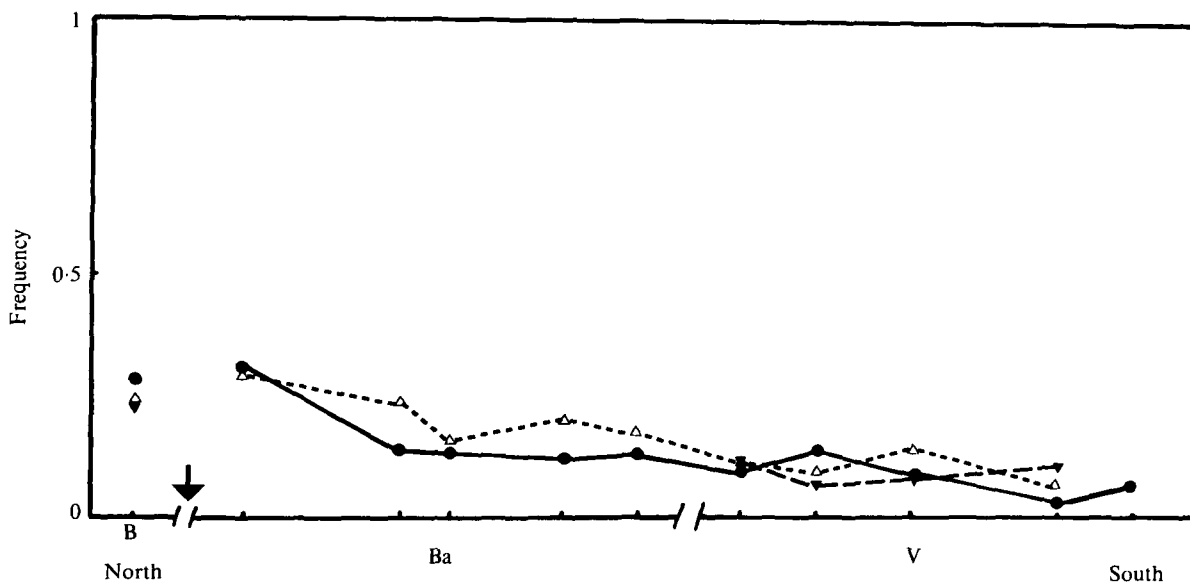


Fig. 5. Frequency of infected flies in Spain. ●, 1989; △, 1990; ▼, 1991; ↓, border. B, Banyuls (southernmost site of sampling in Languedoc); Ba, Barcelona; V, Valencia.

however, all the Eurasian populations may be expected to become of the Q type.

(ii) *Drosophila-sigma* system

(a) *Frequency of infected flies.* Measurements were made in 1988, 1989 and 1991 in the Rhône Valley and in 1989, 1990 and 1991 in Spain. Data are presented in Figs 4 and 5.

In the Rhône Valley, in 1988 and 1989, values were high (about 0.6), comparable to those observed in northern Languedoc (Fleuriet & Periquet, submitted) and remained so up to Lyon. The first hint of a decrease appeared north of Lyon (Biziât). Northwards, frequencies are thus expected to join the low values observed in northern and central France (about 0.15) (Fleuriet, 1990). No measurement could be done in 1990 but in 1991, this frequency clearly decreased as it did in Languedoc (Fleuriet & Periquet, submitted).

In Spain (Fig. 5), on the southern side of the border, the frequency (about 0.3) was similar to that observed in Banyuls, where the southernmost French population was examined (Fleuriet & Periquet, submitted). A steady decrease was observed from there southwards (about 0.05 at the south of Valencia). The few available data indicate that comparable low values of this frequency are prevailing in northern and central Africa (about 0.05) (unpublished results). No change over time was detected.

It thus appears that the main characteristic of Languedoc populations, that is the high frequency of infected flies, is also found northwards, in the Rhône Valley, up to Lyon, but not elsewhere. It is not known whether such high values there are as recent as they are in northern Languedoc, where a dramatic increase in the frequency of infected flies was observed from 1983 to 1988. Values as high as these were never observed in southern Languedoc, where the frequency of infected flies consistently decreased towards the

Table 3. Frequency of the ref(2)^P allele in Languedoc and surrounding regions (distributed from north to south, see Fig. 1)

	1987	1988	1989	1990	1991
Biziat	0.31 ± 0.12	—	0.37 ± 0.10	—	—
Ste Foy	0.35 ± 0.10	—	—	—	0.43 ± 0.08
Rhône Valley	—	0.33 ± 0.08	0.33 ± 0.10	—	0.41 ± 0.08
Languedoc	0.38 ± 0.04	0.44 ± 0.04	0.37 ± 0.08	0.42 ± 0.04	0.38 ± 0.04
Spain (Valencia)	—	—	—	0.20 ± 0.12	0.16 ± 0.10
	—	—	—	—	0.15 ± 0.08
	—	—	—	—	0.14 ± 0.08

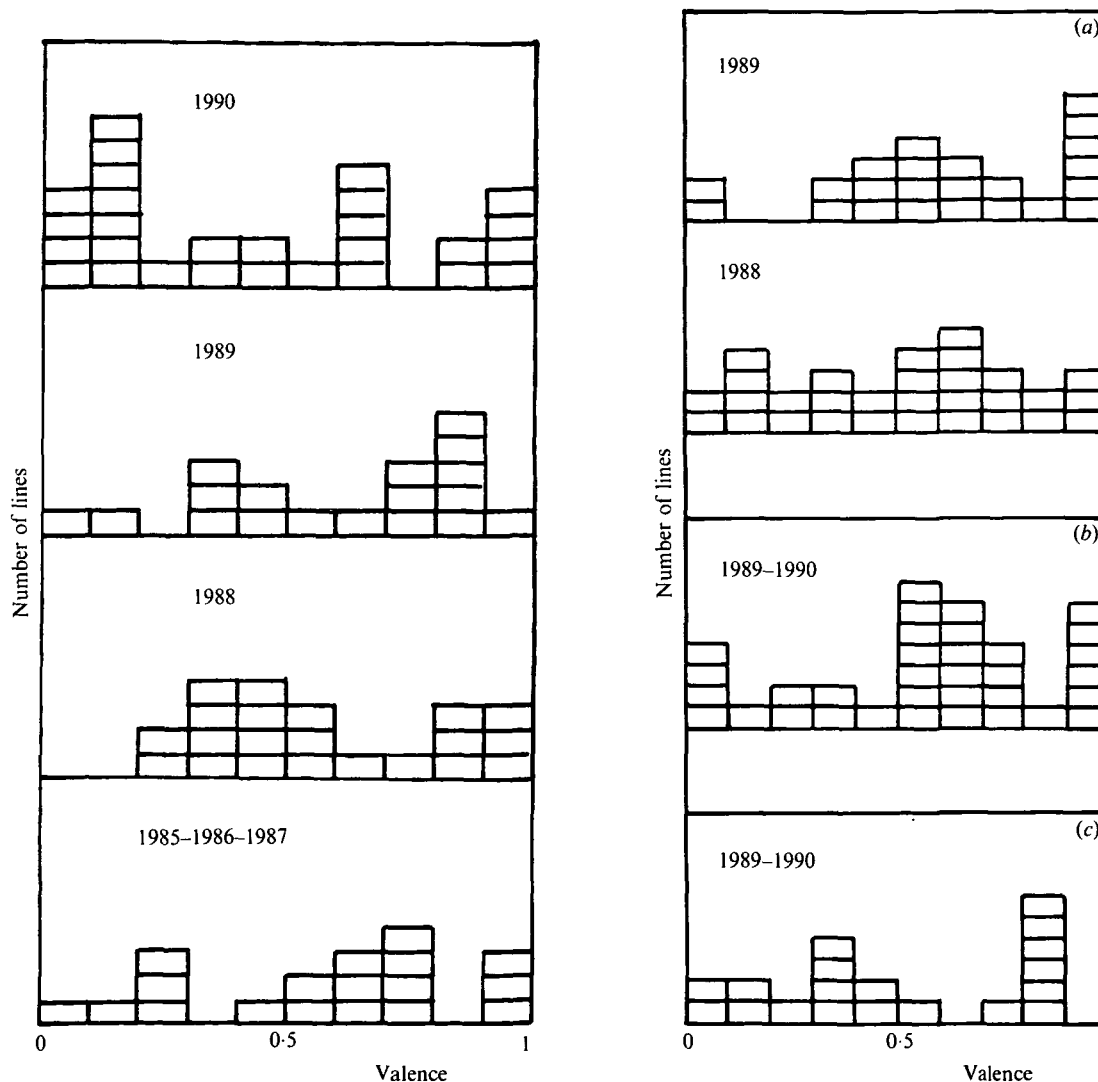


Fig. 6. Efficiency of transmission by males infected by viral type II. The valence of males (frequently of infected flies in their progeny) was measured in individual crosses with O/O uninfected females. Average values given here were obtained by pooling the valences of 5–10 males of the same line, infected by one clone. Left panel, Ste Foy. Right panel: (a) Rhône Valley; (b) Barcelona; (c) Valencia.

border. The steady decrease observed in Spain is thus only the continuation of the process initiated in France. The barrier of the Pyrenées mountains does not seem to alter this process.

Another trait connecting Rhône Valley populations with those of Languedoc is the decrease in the frequency of infected flies. This decrease occurred

from 1989 onwards in Languedoc and could be detected in the Rhône Valley in 1991. Its magnitude (Fig. 4) clearly shows that it is the continuation of the phenomenon which began in central Languedoc (Fleuriet & Periquet, submitted).

(b) ref(2)^P allele frequency. Only a few measurements were made each year, due to the difficult

Table 4. Distribution of viral clones collected in Languedoc and surrounding regions (distributed from north to south, see Fig. 1)

	1988		1989		1990		1991	
	No.	Freq.	No.	Freq.	No.	Freq.	No.	Freq.
Biziat	5	0.80 ± 0.36	24	0.92 ± 0.11				
Ste Foy	30	0.80 ± 0.14	28	0.64 ± 0.18	33	0.85 ± 0.12	31	0.68 ± 0.16
Rhône Valley	38	0.80 ± 0.14	25	0.96 ± 0.08			17	0.88 ± 0.16
Languedoc	119	0.95 ± 0.04	105	0.94 ± 0.04	150	0.97 ± 0.03	158	0.97 ± 0.03
Barcelona			28	0.96 ± 0.08	9	1.0		
Valencia			18	1.0	3	1.0	8	0.87 ± 0.24

No., number of collected clones; Freq, frequency of viral Type II clones.

method of determination of genotypes at the *ref(2)P* locus. The values observed northwards from Languedoc were very close to those reported in Languedoc during the same period (Table 3). This is not surprising since there seems to be no difference in *ref(2)P^p* allele frequency between Languedoc and the rest of France (Fleuriet, 1990).

The values observed in Spain are lower; this might be connected to the very low frequency of infected flies prevailing there.

(c) *Transmission of the virus by males in the absence of the ref(2)P^p allele.* Fig. 6 shows the valences of males (frequency of infected flies in their offspring). This parameter is a cornerstone for the maintenance of the virus in populations (Fleuriet, 1988). The measurement is made when males are mated with an uninfected *ref(2)P^o/ref(2)P^o* female (see Material and methods).

In the three regions sampled, the virus appeared to be transmitted, on average, to about half the offspring of males. This incidence of transmission is similar to that observed at present in Languedoc and the rest of France (a strong decrease in this parameter has been recorded since 1985) (Fleuriet *et al.* 1990; Fleuriet, 1990). But, while in Languedoc the transmission of most viral clones by males is only moderately high, a great heterogeneity among clones could be found in surrounding regions. A comparable heterogeneity might be also present now in northern and central France (Fleuriet, 1990). The situation prevailing there 15 years ago was completely different since measurements showed very high and homogeneous values of transmission by males for all the clones collected (Fleuriet, 1988).

In 1990 and 1991 the frequency of infected flies observed at Ste Foy was low (Fig. 4) and large numbers of clones were poorly transmitted (Fig. 6). The two phenomena may be causally related. It is not known whether the conjunction was fortuitous or whether it signals a long term change in population structure.

(d) *Type II frequency.* Two viral Types which differ in their sensitivity to the *ref(2)P^p* allele are known to coexist in populations: viral type I, very sensitive to

Table 5. Distribution of viral clones collected in Spain before 1989

Year	Site of collection	No. of type I clones	No. of type II clones
1976	Cordoba	1	—
1983	Barcelona	1	1
1984	Valencia	—	1
1986	Sevilla	—	1

ref(2)P^p and viral type II, more resistant (Fleuriet, 1988). The data are presented in Table 4. The results collected in Languedoc since 1988 are presented again. A dramatic increase in Type II frequency was observed there from 1983 onwards. Languedoc was initially divided into three zones, which contained different proportions of the two viral types (Fleuriet *et al.* 1990). However, from 1988 onwards there was little difference in the proportions between the zones, and so results from the whole region were pooled in Table 4. In the rest of France, a similar invasion of type II clones might have occurred in the seventies; viral type II is now in the majority (85–90% of collected clones) (Fleuriet, 1990).

Table 4 shows that viral Type II was predominant in all the regions examined. In the Rhône Valley, and particularly at Ste Foy, its frequency seems to be lower than it is in Languedoc. Type II frequency has been monitored for several years in Ste Foy, and, for unknown reasons, has remained slightly lower than in the rest of France (Fleuriet, 1990).

In Spain, type II frequency was very high. Table 5 presents the data collected previously in Spain. They are much too fragmentary to be significant but they are not incompatible with the hypothesis that Spanish populations were also recently invaded by viral type II.

The important point is that, in Languedoc, the increase in frequency of infected flies appeared to be clearly correlated with the increase in type II frequency (Fleuriet *et al.* 1990). This was the main distinguishing characteristic of Languedoc populations, since, in the

Table 6. Sensitivity of type II clones to the effect of the *ref(2)^{P^p}* allele in the region of Lyon and the Rhône Valley. For each region are given the number (upper value) and frequency (lower value) of clones presenting a given P/O ratio (see Material and methods for its calculation).

	1988	1989	1990	1991
Ste Foy				
P/O ≥ 1	1 0.04	1 0.06	6 0.24	9 0.45
0.5 ≤ P/O < 1	18 0.78	13 0.72	16 0.64	5 0.25
P/O < 0.5	4 0.17	4 0.22	3 0.12	6 0.30
Rhône Valley				
P/O ≥ 1	13 0.42	9 0.39	—	4 0.57
0.5 ≤ P/O < 1	14 0.45	10 0.44	—	3 0.43
P/O < 0.5	4 0.13	4 0.17	—	—

rest of France, the frequency of infected flies remained low, despite high type II frequencies. In this respect, the Rhône Valley is once again similar to Languedoc. This is clearly not the case in Spain where the frequency of infected flies is very low, while type II is predominant.

(e) Sensitivity of type II clones to the *ref(2)^{P^p}* allele.

Viral type II is less sensitive than type I to the effect of the *ref(2)^{P^p}* allele on transmission by males. But, among type II clones, there is a broad range of sensitivities to the *ref(2)^{P^p}* allele. This sensitivity can be expressed by a parameter, the P/O ratio (see Material and methods for its calculation). Type II clones, whose P/O ratio is lower than 0.5 are very sensitive to the effect of *ref(2)^{P^p}*. In contrast, when their P/O ratio is greater than, or equal to 1, viral clones are well or even better transmitted in the presence of *ref(2)^{P^p}*.

A very significant adaptation of type II clones to the *ref(2)^{P^p}* allele has been observed in Languedoc since 1983. This phenomenon was particularly clear in northern Languedoc where most clones are now insensitive to the effect of *ref(2)^{P^p}* (Fleuriet & Periquet, submitted). No comparable evolution has been observed in the rest of France (Fleuriet, 1990).

The data collected in the Rhône Valley and Ste Foy are presented in Table 6. Clones that were insensitive to the effect of the *ref(2)^{P^p}* allele were observed with high frequency in the Rhône Valley, but appeared later in the region of Lyon. As in northern Languedoc, the high frequency of infected flies in the Rhône Valley might be related to the fact that the *ref(2)^{P^p}* allele is no longer restrictive for many viral clones. The later decrease in the frequency of infected flies may be connected, at least in part, to the decrease in

transmission by males. This point is discussed with more details in Fleuriet and Periquet (submitted).

The data collected in Spain are not sizable enough to be clearly interpretable and will not be presented here.

In conclusion, the data presented in this paper confirm the peculiar status of the Languedoc region in the *Drosophila*-sigma system (Fleuriet & Periquet, submitted). Its main characteristic is the high frequency of infected flies, in all likelihood due to the fact that the *ref(2)^{P^p}* allele is no longer restrictive for many viral clones. There was two stages in this phenomenon: the first, the invasion of viral type II was qualitative, and the second, an improved adaptation of the type to the effect of *ref(2)^{P^p}* (P/O ratio increase) was quantitative. The problem is that, in the rest of France, the invasion by viral type II was not accompanied by any increase in the frequency of infected flies. From the present data, it appears that this specificity can be extended to the Rhône Valley, which in 1988 and 1989 exhibited characteristics comparable to those of northern Languedoc (high frequency of infected flies, high P/O values, i.e. very good adaptation of the sigma virus to the P allele). Another similarity is the decrease in frequency of infected flies which occurred later, both in Languedoc and in Rhône Valley. The region of Lyon might be considered as the northern limit of this situation, so far observed nowhere else. Southwards, the distinctive features begin to disappear before the border, since the frequency of infected flies has not increased in southern Languedoc, despite the invasion by viral Type II. In Spain, where the frequency of infected flies decreases steadily from north to south, while type II frequency is high, there is a continuation of the process initiated on the French side of the border.

The zone with specific characteristics might thus extend from the region of Lyon towards the centre of Languedoc (Narbonne). The factors responsible for this burst in the frequency of infected flies, biological or physical, or both, are completely unknown. Our results attest once again of the complexity of this kind of virus-host system. They also raise the problem of a sudden outbreak of infection in a limited area, due to prevailing conditions and which might have serious consequences in the case of a pathogenic virus (Culliton, 1990).

These results also confirm what previous results had already shown: the division of *D. melanogaster* populations into various zones, not necessarily very distant from one another, but with differing characteristics that may persist over time (Vouidibio *et al.* 1989; Fleuriet, 1990).

As reported previously (Anxolabéhère *et al.* 1988a), there is a striking correlation in Languedoc between the distribution of the frequency of sigma infected flies and the P-M status of populations. This led to the delimitation of similar geographical zones in the two systems, namely a difference between France and

Spain, and the subdivision of Languedoc into three similar regions, north, centre and south. From the data presented in this paper, it might be concluded that the highest frequency of infected flies is encountered when populations are of the Q type (Rhône Valley, Languedoc) and the lowest when they are of the M' type (Spain). However, such a correlation is not really consistent with the data when a broader geographical region is considered. For example, in northern and central France, where populations are also of the Q type, the frequency of infected flies is very low (Fleuriet, 1990). The geographical correlations observed between the two systems in southern France and Spain suggest therefore that there is a common but unknown factor that independently affects both systems, rather than a significant biological relationship. Further studies are required to elucidate this point.

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