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

**ANNE FLEURIET**, **ROBERT KALMES**, **LUIS PASCUAL** and **GEORGES PERIQUET**

1. Laboratoire de Génétique, Université de Clermont Ferrand II, 63177 Aubière, Cedex, France
2. Laboratoire de Biocénotique Expérimentale des Agrosystèmes, Université de Tours, 37200 Tours, France
3. Departamento de Genética, Universitat de València, Doctor Moliner 50, 46100 Burjasot, Spain

(Received 21 October 1991 and in revised form 2 April 1992)

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⁺ and restrictive ref(2)P⁻ alleles, with the ref(2)P⁻ allele in the minority. Two viral types, differing in their sensitivity to the ref(2)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⁻ 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 – Biziat); L, Lyon (site of sampling – Ste Foy). Rhône Valley: nine populations sampled (from north to south Peage de Roussillon, St Desirat, Sarras, Tain, Loriol, St Gervais, Alba, Suze, St Alexandre). Languedoc: A, Ales; N, Narbonne; B, Banyuls. Spain: Ba, Barcelona (five populations sampled – Perelada, Artés, Sant Sadurni, Montbrió, Gandesa); V, Valencia (five populations sampled – Sant Mateu, Villafamè, Villar, Albaida, Monovar); C, Cordoba; S, Sevilla.

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 Fleuriet 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 Gl 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.
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 a male when it is mated with a uninfected female, was determined by mating the males individually with their progeny, was determined by mating the males

| North (Les Fumades-Gigean) | Centre (Mèze-Salses) | South (Tautavel-Cerbère) |
|----------------------------|----------------------|-------------------------|
| No. pop. | P potential* | P susceptibility* | No. pop. | P potential* | P susceptibility* | No. pop. | P potential* | P susceptibility* |
| 1983 | 14 | 58±1.6 | 0.9±0.2 | 12 | 08±0.6 | 44±2.2 | 9 | 10±0.7 | 21±9.84 |
| 1984 | 8 | 81±3.0 | 0.4±0.6 | 5 | 49±3.4 | 1.1±13 | 9 | 12±1.3 | 14±6.9 |
| 1985 | 5 | 50±6.0 | 0.8±12 | 13 | 10±0.6 | 18±14 | 8 | 1±1.3 | 12±0.1 |
| 1986 | 15 | 38±1.5 | 0.7±0.6 | 13 | 07±0.8 | 1.5±12 | 8 | 0±0.6 | 8±0.2 |
| 1987 | 11 | 54±2.7 | 2.2±1.9 | 11 | 17±1.7 | 3.2±21 | 8 | 04±0.5 | 51±26 |
| 1988 | 9 | 28±1.5 | 0.4±0.5 | 7 | 06±0.7 | 1.4±15 | 8 | 1±1.6 | 56±46 |
| 1989 | 5 | 10±1.5 | 0.0±0.0 | 6 | 02±0.3 | 1.3±12 | 8 | 0±1.0 | 35±39 |
| 1990 | 3 | 00±0.0 | 0.0±0.0 | 10 | 04±0.7 | 02±0.4 | 8 | 05±0.6 | 05±9.0 |
| 1991 | 6 | 03±0.7 | 0.7±0.9 | 6 | 00±0.0 | 07±0.8 | 8 | 01±0.2 | 01±0.2 |

* 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.

\[ P/O \text{ ratio} = \frac{\text{valence of males of a line mated with ref(2)P}^/\text{ref(2)P}^* \text{ females}}{\text{valence of the same males mated with ref(2)P}^/\text{ref(2)P}^* \text{ females}} \]

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^ 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^ allele.

(d) Genotypes at the ref(2)P locus. Males are classified as ref(2)P^/ref(2)P^, ref(2)P^/ref(2)P^ or ref(2)P^/ref(2)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: 54%) and the lowest P susceptibility (0.7%); a central zone with low
Annie Fleuriet and others

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)

|             | Rhone 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 | 24±2±2 | — | — | — | — | — | — |
| 1989        | 7 | 0±0    | 0±0    | 19 | 0-3±0-6 | 1-9±1-7 | 4 | 0-2±0-4 | 59±5±83 | 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±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 (Fleuriet & 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...
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,
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 (Biziat). 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
Comparative evolution of P–M system and infection by sigma virus in D. melanogaster

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

| Region          | 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    | 0.15±0.08    |
|                 | —            | —            | —            | 0.14±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/Q 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.

The steady decrease observed in Spain is thus only the continuation of the process initiated in France. The barrier of the Pyrenees 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 begun in central Languedoc (Fleuriel & Periquet, submitted).

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

(d) Type II frequency. Two viral Types which differ in their sensitivity to the ref(2)P allele are known to coexist in populations: viral type I, very sensitive to ref(2)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. Distribution of viral clones collected in Languedoc and surrounding regions (distributed from north to south, see Fig. 1)

|      | 1988  | 1989  | 1990  | 1991  |
|------|-------|-------|-------|-------|
| Site | No.   | Freq. | No.   | Freq. | No.   | Freq. | No.   | Freq. |
| Biziat | 5     | 0.80±0.36 | 24   | 0.92±0.11 | 33   | 0.85±0.12 | 31   | 0.68±0.16 |
| Ste Foy | 30    | 0.80±0.14 | 28   | 0.64±0.18 | 25   | 0.96±0.08 | 17   | 0.88±0.16 |
| Rhône Valley | 38 | 0.80±0.14 | 105  | 0.94±0.04 | 150 | 0.97±0.03 | 158 | 0.97±0.03 |
| Languedoc | 119   | 0.95±0.04 | 28   | 0.96±0.08 | 9    | 1.00 | 3    | 1.00 |
| Barcelona | 18    | 1.00    | 8    | 0.87±0.24 |

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

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                    |
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^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

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

The authors thank M. H. Hamelin, I. Grolleau and J. C. Landré for their technical assistance and Dr R. Allemand, Dr Serra and his colleagues for collecting flies in Ste Foy and Barcelona. This work was supported by grants from the CNRS (URA 360, URA 1298), the Ministère de l’Éducation Nationale (DRED Evolution), the Action Intégrée Franco-Espagnole and the Foundation pour la Recherche Médicale Française.

References
Anxolabéhere, D., Nouaud, D., Periquet, G. & Ronsseray, S. (1986). Evolution des potentialités dysgénésiques du système P—M dans des populations expérimentales mixtes, P, Q, M et M’ de D. melanogaster. *Genetica* 69, 81–95.
Anxolabéhere, D., Charles-Palabost, L., Fleuriet, A. & Periquet, G. (1988a). Temporal surveys of French populations of D. melanogaster: P—M system, enzymatic polymorphism and infection by the sigma virus. *Heredity* 61, 121–131.
Anxolabéhere, D., Kidwell, M. G. & Periquet, G. (1988b). Molecular characteristics of diverse populations are consistent with the hypothesis of a recent invasion of D. melanogaster by mobile P elements. *Molecular Biology and Evolution* 5 (3), 252–269.
Black, D. M., Jackson, M. S., Kidwell, M. G. & Dover, G. A. (1987). KP elements repress P-induced hybrid dysgenesis in D. melanogaster. *EMBO Journal* 6, 4113–4123.
Brun, G. & Plus, N. (1980). The viruses of Drosophila. In *The Genetics and Biology of Drosophila*, (ed. M. Ashburner and T. R. F. Wright), pp. 626–702. New York: Academic Press.
Culliton, B. J. (1990). Emerging viruses, emerging threat. *Science* 247, 279–280.
Daniels, S. B., Peterson, K. R., Strausbaugh, L. D., Kidwell, M. G. & Chovnick, A. (1990). Evidence for horizontal transmission of the P transposable element between Drosophila species. *Genetics* 124, 339–355.
David, J. (1959). Etude quantitative du développement de la Drosophile élevée en milieu axénique. *Bull. Soc. Biol. Fr. Belg.* 93, 472–505.
Emeny, J. M. & Lewis, M. J. (1984). Sigma virus of *Drosophila* as a vector model. In *Vectors in Virus Biology*, (ed. M. A. Mayo and K. A. Harrap), pp. 93–112. New York: Academic Press.
Engels, W. R. (1979). Hybrid dysgenesis in *D. melanogaster*: rules of inheritance of female sterility. *Genetical Research* 33, 219–236.
Engels, W. R. (1989). P elements in *Drosophila*. In *Mobile DNA*, (ed. D. Berg and M. Howe), pp. 437–484. ASM Publications.
Fleuret, A. (1976). Presence of the hereditary Rhabdovirus sigma and polymorphism for a gene for resistance to this virus in natural populations of *Drosophila melanogaster*. *Evolution* 30, 735–739.
Fleuriet, A. (1980). Polymorphism of the hereditary sigma virus in natural populations of *D. melanogaster*. *Genetics* 95, 459–465.
Fleuriet, A. (1988). Maintenance of a hereditary virus, the sigma virus in populations of its host, *D. melanogaster*. In *Evolutionary Biology*, vol. 23, pp. 1–30 (ed. M. K. Hecht and B. Wallace, New York: Plenum.
Fleuriet, A. (1990). Evolution of natural populations in the *D. melanogaster*—sigma virus system. II. Northern and Central France. *Genetica* 81, 33–41.
Fleuriet, A., Periquet, G. & Anxolabéhere, D. (1990). Evolution of natural populations in the *D. melanogaster*—sigma virus system. I. Languedoc (Southern France). Submitted.
Gay, P. (1978). Les gènes de la Drosophile qui interviennent dans la multiplication du virus sigma. *Mol. gen. Gen.* 159, 269–283.
Jackson, M. S., Black, D. M. & Dover, G. A. (1988). Amplification of KP elements associated with the repression of hybrid dysgenesis in *D. melanogaster*. *Genetics* 120, 1003–1013.
Kidwell, M. G. (1983). Evolution of hybrid dysgenesis determinants in *D. melanogaster*. *Proceedings of the National Academy of Sciences, U.S.A.* 80, 1655–1659.
Periquet, G. (1980). ‘Atrophie gonadique’ character and hybrid dysgenesis in *D. melanogaster*. *Biol. Cellulaire* 39, 7–12.
Periquet, G., Ronsseray, S. & Hamelin, M. H. (1989). Are *D. melanogaster* populations under a stable geographical differentiation due to the presence of P elements? *Heredity* 63, 47–58.
Plus, N. (1954). Etude de la multiplication du virus de la sensibilité au gaz carbonique chez la Drosophile. *Bull. Soc. Biol. Fr. Belg.* 88, 1–46.
Voutilainen, J., Capi, P., Delafaye, D., Pla, E., Sandrin, J., Csink, A. & David, J. R. (1989). Short range genetic structure of *D. melanogaster* populations in an Afro-tropical urban area and its significance. *Proceedings of the National Academy of Sciences U.S.A.* 86, 8442–8446.