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Evolution of the *Drosophila melanogaster*-sigma virus system in a natural population from Tübingen

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Summary. In natural populations of *D. melanogaster*, usually, a minority of individuals are infected by a Rhabdovirus called sigma. This virus is not contagious but is vertically transmitted through the gametes. In *D. melanogaster*, a polymorphism for two alleles (O, permissive and P, restrictive) of a gene responsible for resistance to the virus is regularly observed in the wild. On the virus side two types are found, which differ in their sensitivity to the P allele: Type I is very sensitive, and Type II more resistant. Previous findings had led to the hypothesis that an invasion of Type II clones, starting from central France, might be spreading over European populations. This replacement of viral Type I by viral Type II in natural populations could be observed in Languedoc (southern France), where it led to a dramatic increase in the frequency of infected flies. The invasion hypothesis is confirmed by the data from samples collected at Tübingen, where the frequency of Type II clones increased from 0.27 to 0.93 over a 6-year period (1985–1991). However, over the same period, no increase in the frequency of infected flies was observed. The evolution of other viral characteristics is discussed.

Key words: *Drosophila melanogaster* – Rhabdovirus sigma – Natural populations – Polymorphism

Introduction

In practically all natural populations of *D. melanogaster*, a minority of individuals are infected by a Rhabdovirus called sigma (Fleuriet 1988). The virus is not contagious from fly to fly but is transmitted through male and female gametes (Brun and Plus 1980; Emeny and Lewis 1984). The virus does not integrate into the fly chromosomes but develops in the cytoplasm. The specific symptom of CO_2 sensitivity is a reliable character to determine whether a fly is infected. The fact that the virus is not contagious has to be emphasized. While most viruses can only be analysed through extensive study of a few laboratory strains, vertical transmission, CO_2 sensitivity and a good knowledge of the host make it possible to study the *Drosophila*-sigma system in natural populations.

A few loci of *D. melanogaster* are known to interfere with the virus cycle (Gay 1978). For one of the them, the *ref(2)P* locus, two alleles are known, O and P, which are permissive and restrictive for the virus, respectively. A polymorphism for both alleles is usually found in wild populations; the P allele is less frequent than the O allele (Fleuriet 1988). Two viral types, differing in their sensitivity to the P allele, are found in natural populations: viral Type I, very sensitive to the effect of P, and viral Type II more resistant (Fleuriet 1988).

Previous findings suggested that, in northern and central France during the seventies, viral Type I had been progressively replaced by Type II, but without complete elimination of Type I (Fleuriet 1986). A similar replacement could be observed later in populations from Languedoc (southern France). In this region, a dramatic increase in the frequency of infected flies was observed at the same time, apparently as a direct consequence of this substitution (Fleuriet et al. 1990).

To further corroborate the hypothesis that an invasion of Type II would spread from France and progressively affect other European populations, it was decided to analyse a wild *D. melanogaster* population from Tübingen (southwest Germany). This population was chosen because it is not too distant from France, and also because in a first study made in 1982 it contained a

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minority of Type II clones. The data obtained from the analysis of this population since 1982 are presented in this paper.

Materials and methods

Collection of samples

Samples of adult flies were trapped each September in the same garden, at the border of the city of Tübingen.

The characteristics of the viral clones were measured, as usual, immediately after fly collection. The frequency of infected flies is measured on Go flies (i.e., on flies from nature). The valence and viral Type are determined on G1 males (i.e., on the sons of wild females).

Culture conditions

In the laboratory, all flies were maintained on axenic food (David 1959) at 20 °C and under natural light conditions.

Determination of infected flies

The CO_2 test used to measure the frequency of infected flies is described in Plus (1954).

Genotypes at the ref(2)P locus

Males were classified as O/O, O/P or P/P at the *ref(2)P* locus, depending on whether, after crossing with a female of a reference strain, their adult progeny is entirely CO_2 sensitive, half CO_2 sensitive or entirely CO_2 resistant (Fleuriet 1976). This method is time consuming and allows determination of the genotype of only a few (about 40) males.

Valence and determination of the viral type

Isofemale lines were isolated from each sample; only CO_2 sensitive lines (i.e. infected lines) are kept. They are assumed to carry only one viral clone.

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 O/O uninfected females. The frequency of CO_2 sensitive flies was then measured in their offspring. The average valence of males from the same line was obtained, as a characteristic of that line, by pooling the values observed for each of the males.

The method used to determine the type of a viral clone (with respect to the P allele) has been described in detail in Fleuriet (1980). The valence of a male mated with a O/O female (see above) is taken first as a reference. Then, the valence of the same male with a P/P female is measured. If the latter value is zero (or close to zero) the male carries viral Type I; if it is only slightly lower than the reference value, it carries viral Type II.

Sensitivity of viral Type II to the P allele

It should be recalled that the effect of the P allele upon transmission by males is mainly maternal. In the presence of the P allele, viral clones can still be transmitted by males when they are of Type II, they are not transmitted by males they are of Type I. Even so, a wide range of sensitivities of Type II clones to the P allele exists. A parameter, designated as the P/O ratio in this paper, was calculated to express the sensitivity of a clone:

$$\text{P/O ratio} = \frac{\text{valence of males of a line mated with P/P females}}{\text{valence of the same males mated with O/O females}}$$

For viral Type I, this ratio is nearly zero. For viral Type II, it is usually less than 1. When P/O becomes equal to 1, it indicates that the P allele has no effect upon the transmission by males of the viral clone examined. If P/O is greater than 1, the viral clone is even better transmitted by males in the presence of the P allele.

Results

Proportion of infected flies and frequency of the P allele

The results are presented in Table 1. The proportion of infected flies at Tübingen was very low over the entire period; it even slightly decreased, from 0.10 to 0.04, from 1982 to 1991. The frequency of the P allele showed no detectable change over time; the values found for the Tübingen population are very similar to those usually observed in other European populations (Fleuriet 1988).

Frequency of Type II clones

Type I and Type II differ in their sensitivity to the P allele. In the presence of the P allele, transmission by males of viral Type I is almost completely stopped, whereas for viral Type II it merely decreases (for their identification, see Materials and methods). The frequency of infected flies at Tübingen is so low that it is difficult to obtain enough clones to make a quantitative comparison. In 1989, for example 1,500 isofemale lines had to be isolated in order to analyse 53 viral clones. This was only possible when very large samples of flies could be taken. It is nevertheless clear, from the data presented in Table 2, that the frequency of Type II clones steadily increased at Tübingen. The results for 1990 appear atypical. This is due to the small number of clones that could be analysed; because of mailing problems, the sample size was very small.

Efficiency of transmission by males (valence) in the absence of P allele

The efficiency of transmission by males (also called the valence of males) is the proportion of infected flies observed in the progeny of an infected male mated with an uninfected female. With reference to the *ref(2)P* locus, uninfected O/O females were used for this measurement. Table 3 gives the average values, separately for viral Type I and II, found for the clones collected each year. From 1985 to 1991, transmission efficiency values for viral Type II proved medium and remained constant over time. For viral Type I, transmission by males was significantly lower in 1985 and 1986 than that of Type II. Unfortunately, measurements could not be made in 1987, 1988 and 1991. In 1989, similar values were observed for Type I and Type II clones.

Table 1. Frequency of infected flies and of the P allele in the Tübingen population

Year	Frequency	
	Infected flies	P allele
1982	0.10±0.08	0.29±0.16
1985	0.12±0.03	0.34±0.06
1986	0.08±0.02	0.29±0.06
1987	0.06±0.02	0.33±0.07
1988	0.04±0.01	0.25±0.08
1989	0.05+0.01	0.36±0.09
1990	0.04±0.02	
1991	0.04±0.02	0.23±0.06

Table 2. Evolution of Type II frequency in the Tübingen population

Year	No.[a]	Type II frequency
1982	1	0
1983	1	0
1985	26	0.27±0.18
1986	50	0.30±0.12
1987	28	0.46±0.18
1988	19	0.84±0.16
1989	53	0.87±0.10
1990	3	0.33
1991	15	0.93±0.13

[a] Number of analyzed clones

Table 3. Efficiency of transmission by males in the absence of the P allele. This table presents the average value of transmission by males in lines carrying viral Type I or viral Type II (see Materials and methods). Values were significantly different (t test) in 1985 ($0.01 < P < 0.02$) and 1986 ($0.02 < P < 0.05$)

Year	Viral Type I	Viral Type II
1985	0.39±0.13	0.64±0.22
1986	0.37±0.06	0.54±0.21
1987		0.73±0.25
1988		0.57±0.31
1989	0.60±0.19	0.56±0.07
1991		0.56±0.12

Table 4. Evolution of the sensitivity of Type II clones to the effect of the P allele upon transmission by males in the Tübingen population. (For calculation of P/O ratio, see Materials and methods.) The table presents the number (upper value) and frequency (lower value) of Type II clones with a given P/O ratio

Year	1985	1986	1987	1988	1989	1990	1991
P/O≥1					8		2
					0.19		0.26
0.5≤P/O<1	2	2	3	5	14	1	3
	0.29	0.25	0.50	0.56	0.33		0.37
P/O<0.5	5	6	3	4	20		3
	0.71	0.75	0.50	0.44	0.48		0.37

Sensitivity of Type II clones to the P allele

Viral Type II is less sensitive than Type I to the effect of the P allele on transmission by males. However, there is a broad range of sensitivities to the P allele among Type II clones. A parameter, the P/O ratio, was calculated to express this sensitivity (see Materials and methods). Type II clones whose P/O ratio is lower than 0.5 are very sensitive to the effect of P: their transmission by males is greatly hindered in the presence of the P allele. When the P/O ratio is equal or superior to 1, viral clones are equally well or even better transmitted in the presence of P.

In spite of the scarcity of the data, (frequency values are given just as an indication), a clear pattern does emerge (Table 4). Viral clones whose P/O ratio was equal or superior to 1 were not found at Tübingen earlier than 1989. In contrast, the frequency of clones that were very sensitive to P ($P/O < 0.5$) decreased continuously from 1985 to 1991. It thus appears that the adaption of Type II clones to the presence of the P allele in the genome of the flies improved over time; this is demonstrated by the upward shift in the P/O ratio.

Discussion

The data presented in this paper clearly demonstrate that viral Type I has been replaced by viral Type II in the Tübingen population of *D. melanogaster*. It is not clear whether viral Type I will ever be completely eliminated (and this has not yet happened in France either). The same trend could also be observed in southern French populations from Languedoc (Fleuriet et al. 1990). In addition, there is a strong likelihood that a similar phenomenon, which could not be precisely documented, also occurred in northern and central France in the seventies (Fleuriet 1986). All this supports the hypothesis that an invasion by viral Type II, initially originating in northern France, is likely to spread to other European populations of *D. melanogaster*.

The presence of the P allele in natural populations is one of the factors that might be responsible for the invasion. Type II clones are better adapted to the effect of P since, unlike Type I, their transmission by males is not completely prevented. Another observation might explain why Type II clones were favoured. In the Tübingen population (Table 3), when Type II frequency started to increase, Type II clones were better transmitted by males than Type I clones, even in the absence of the P allele. This was also the case in Languedoc (Fleuriet et al. 1990). This corroborates the idea that the invasion started in northern and central France (where transmission by males was higher than elsewhere in 1982) (Fleuriet 1986). It should be noted that these two components of fitness (resistance against P and higher transmission rate) work

transmission by males, which is a cornerstone for viral maintenance in populations (Fleuriet 1988).

The fact that a polymorphism, where viral Type I is rather rare, persists in France suggests that Type I might possess some other (unknown) selective advantage.

Another question arises from the comparison of the Languedoc and Tübingen populations. In Languedoc, a dramatic increase in the frequency of infected flies (from approximately 0.10 to approximately 0.70) appeared together with the invasion of viral Type II (and more generally as a consequence of the improved adaption of the virus to the P allele) (Fleuriet et al. 1990). An increase in P allele frequency was also observed (Fleuriet and Periquet 1992). No such consequences can be seen at Tübingen: the frequency of infected flies remained very low and even tended to decrease. If the invasion of viral Type II did start from northern and central France, it was not accompanied there by any not notable change in the frequency of infected flies (though the P allele frequency did increase slightly) (Fleuriet 1990). Languedoc is thus the only place where, for some biological or physical reason (or both), an increase in the frequency of infected flies has ever been observed in natural populations. Another illustration of this problem is the observation that, in Spanish population, a low frequency of the P allele (less than 0.2) is accompanied by (and interpreted as a consequence of) a low frequency of infected flies (Fleuriet et al. 1992). In the Tübingen population, an even lower frequency of infected flies is accompanied by a more usual (approximately 0.3) value of P frequency.

These data once again illustrate the complexity of such a host-parasite system. It is clear that other parameters, as yet unknown, affect the relations between the virus and its host. The results also corroborate an idea conveyed by previous observations (Fleuriet 1990): even in populations geographically close together, in which the prevailing conditions are apparently very similar, different equilibria may be reached in the *Drosophila*-sigma system, suggesting that different selective forces are at work. To predict the equilibria, we would need to know all the parameters involved, an impossible task even in this apparently simple system of two so well known coevolving organisms.

There is an apparent contradiction between the rapid spread of viral Type II (its frequeny rose from 0.53 to 0.91 between 1983 and 1987 in Languedoc, and from 0.27 to 0.93 over a 6 year period in Tübingen) and the existence of many genetic differences between populations of *D. melanogaster*. The former observation would mean a high rate of exchange by migration between populations while the latter, on the contrary, would suggest that gene flow is rather low. Sigma virus, like transposable elements, is more efficiently transmitted than a Mendelian allele (L'Heritier 1970; Hickey 1982). Both information-carrying systems are characterized by high invading capacities, and a high rate of exchange between populations appears not to be required for them to spread. Invasions of transposable elements have already been recognized on a worldwide scale (Bregliano and Kidwell 1983).

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