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Developmental expression of the *412* retrotransposon in natural populations of *D. melanogaster* and *D. simulans*

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Summary

We analysed the pattern of expression of retrotransposon *412* through developmental stages in various populations of *Drosophila simulans* and *D. melanogaster* differing in *412* copy number. We found that the *412* expression pattern varied greatly between populations of both species, indicating that such patterns were not entirely species-specific. In *D. simulans*, total transcripts increased with number of *412* copies in the chromosomes when this number was low, and then decreased for high copy numbers. *D. melanogaster*, which has a higher *412* copy number than *D. simulans*, had overall a lower global *412* expression, but again showed variation in *412* expression pattern between populations. These results suggest that in populations of *D. simulans* with low *412* copy number, the expression pattern of this element depends not only on copy number but also on host cellular regulatory sequences near which the elements were inserted. In *D. simulans* populations with high copy number overall transcription was on the contrary globally repressed, as observed in *D. melanogaster*. A population from Canberra (Australia) which had a very high *412* copy number was found to be associated with very high expression of *412* over all developmental stages, suggesting that the above *412* expression regulation processes are overcome in this population sample. The analysis of hybrids between geographically distinct populations of *D. simulans* showed that *412* expression was trans-regulated differently according to developmental stages, implying complex interactions between the *412* element and stage-specific host genes.

1. Introduction

Transposable elements (TEs) are ancient components of the genome of many organisms, and because of their capacity of promoting mutations, recombination and chromosome rearrangements, they are a serious threat to the genome. Mechanisms restraining their transposition property have thus been selected. Methylation, chromatin-mediated silencing, homology-dependent gene silencing (co-suppression) and other kinds of global repression have been proposed as defensive responses of host genomes to TE invasion (Yoder *et al.*, 1997; McDonald, 1998; Jensen *et al.*, 1999). Among these mechanisms, regulation of transcription is increasingly recognized, implying host factors, chromatin conformation or internal cis-acting elements. Many TEs are transcribed in a stage-dependent manner with transcript level modulated during development (Flavell *et al.*, 1980; Parkhurst & Corces, 1987). For example, in *Drosophila melanogaster*, retrotransposons *412*, *mdg1* and *17·6* are mainly transcribed at embryonic and pupal stages (Parkhurst & Corces, 1987), and in the visceral mesoderm and gonads of the embryos (Brookman *et al.*, 1992; Ding & Lipshitz, 1994). *Roo/B104* is almost solely transcribed in embryos (Parkhurst & Corces, 1987), especially in the somatic mesoderm and yolk nuclei of early embryos (Ding & Lipshitz, 1994). Its expression patterns are nearly identical in embryos of *D. yakuba* (Brönner *et al.*, 1995), a species closely related to *D. melanogaster*. The *17·6* element is expressed at the anterior margin of the lamina anlage and its expression depends on an enhancer element in the LTR and a repressor element within the retrotransposon (Mozer & Benzer, 1994). Transcription

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of the *F* element is detected in specific cells of female and male germ lines and in various tissues during embryogenesis (Kerber *et al*., 1996). The *doc* element is transcribed in both male and female germ lines (Zhao & Bownes, 1998), while the *copia* element is highly transcribed in testes (Pasyukova *et al*., 1997; Filatov *et al*., 1998*b*), the *I* factor only in the female germ line cells (Lachaume *et al*., 1992; Udomkit *et al*., 1996) and the *gypsy* element in the somatic follicle cells surrounding the oocytes (Pélisson *et al*., 1994). *1731* transcripts are detected in embryos, ovaries and testes, but the gag-pol proteins are detected in testes and gag proteins only in ovaries, indicating possible translation and post-translational control of this element (Haoudi *et al*., 1997). Regulation of expression of endogenous retrovirus-like elements is also tissue-specific and age-dependent in murines (Gaubatz *et al*., 1991), and regulation of the LTR of such elements in humans is known to depend on adjacent cellular genes (Anderson *et al*., 1998).

Expression patterns of TEs during embryonic and larval development and in adults appear highly conserved between different wild-type strains (Ding & Lipshitz, 1994; Haoudi *et al*., 1997; Parkhurst & Corces, 1987) or even different species (Brönner *et al*., 1995). This suggests that the regulation of TE expression is not entirely dependent on host cellular regulatory sequences of actively transcribed cellular genes near which the elements are inserted, but depends on cis-regulatory sequences of the TEs (Cavarec & Heidmann, 1993) that interact with host transcription factors. Transposable elements, known to be associated with actively transcribed regions (Taruscio & Manuelidis, 1991), seem to be regulated like other genes under a developmental cis-regulatory network (Yuh *et al*., 1998). Transcript abundance of various TEs in *Drosophila* thus depends to a large extent on host genes, such as the genes *Lip* (Lighten up: Csink *et al*., 1994*a*), *Wow* (Weakener of white: Birchler *et al*., 1994), *Msu* (Mosaic suppressor: Csink *et al*., 1994*b*), *Doa* (Darkener of apricot: Rabinow *et al*., 1993) and *su*(*Hw*) (suppressor of Hairy wing: Corces & Geyer, 1991). Expression of *412* is even proposed to be a direct consequence of transcriptional activation by the UBX transcription factor (Ding & Lipshitz, 1994), and is decreased by 20-OH ecdysone applied to cultured *D. melanogaster* Kc cells (Micard *et al*., 1988; Becker *et al*., 1991). Expression of the *blastopia* transcripts in blastoderm embryo is under the control of the *Drosophila* morphogen *bicoid* (Frommer *et al*., 1994).

It is known, however, that transcription of solo LTRs of the endogenous retrovirus *HERV-K* is probably directed by external cellular promoters (Leib-Mösch *et al*., 1993). The quantity and type of *IAP* (Intracisternal A-particle), integrated mammary tumour virus DNA and Moloney leukaemia virus (*M-MuLV*) transcripts vary according to strains of mice (Jähner & Jaenisch, 1980; Jaenisch *et al*., 1981; Feinstein *et al*., 1982; Kuff & Fewell, 1985). The *copia* transcript level varies between natural populations of *D. melanogaster* analysed but is not associated with copy number (Csink & McDonald, 1990). All these results suggest a possible link between TE insertion sites and some characteristics of the host region (chromatin conformation, presence of cellular regulatory sequences) in which they are inserted. We have, however, little information on variation in the pattern of expression for many TEs in natural populations. By analysing populations of *D. simulans* presenting a very large variation in *412* copy number, we show that the expression pattern of this retrotransposon depends on the population analysed and varies according to the number and localization of insertions of this element along the chromosomes. In populations with a low copy number, the linear relationship between *412* total expression and copy number suggests that a large part of *412* transcription is regulated during development by cis or host sequences in the vicinity of the *412* element insertions. For high copy number, however, *412* expression decreased as though global repression was efficient only when a threshold value of copy number was attained. Crosses between geographically distinct populations of *D. simulans* showed that regulation of *412* expression in the F1 hybrid offspring differed with developmental stage, and suggest additive, dominant and over-dominant trans-acting action of stage-specific host regulatory genes.

2. Experimental procedure

(i) *Natural populations collected*

We studied 14 populations of *D. melanogaster* and 10 populations of *D. simulans* from various geographically distinct regions. The populations of *D. melanogaster* were from Arabia, Argentina (Virasoro), Bolivia, China (Canton 5, 7, 8 and 9), Congo (Brazzaville), France (St Cyprien, Valence), Indian Ocean (Reunion BNF and SW), Portugal (Chicharo) and Senegal. The population of *D. simulans* were from Australia (Canberra, Cann River, Eden), Kenya (Kwale), Madagascar, New Caledonia (Amieu, Noumea), Polynesia (Papeete), Portugal (Madeira) and Russia (Moscow). The population samples were maintained in the laboratory as isofemale lines with around 50 pairs every generation. The populations were chosen according to their *412* diploid copy numbers, ranging from 4 to 65 as determined from *in situ* hybridization on polytene chromosomes (Vieira & Biémont, 1996*a*). Hybrids between populations were obtained by crossing 50 virgin females from an isofemale line of one population with 50 males from an isofemale line of another population. Flies from

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both *D. melanogaster* and *D. simulans* were maintained in the laboratory under 18 °C, and the experiments were done at this temperature.

(ii) *RNA extraction and northern blotting*

Total RNA was extracted by the guanidium chloride method (Cox, 1968) from 500 0–8 h embryos, 200 second and 60 third instar larvae, 80 pupae, 60 male and 60 female adult flies either 5, 15 or 25 days old. The poly-A^+ fraction was purified from 250 μg of total RNA with the Oligotex mRNA mini kit (Qiagen) according to the manufacturer's recommendations. Agarose–phosphate gel electrophoresis and transfer were made as in Heddi *et al.* (1993) with 20 μg of total RNA per lane. Pre-hybridization and hybridization were at 42 °C as in Heddi *et al.* (1993). The *412* genomic clone (Finnegan *et al.*, 1978) used as probe was labelled with a Megaprime kit (Amersham). The exposure times ranged from overnight to 3 days with intensifying screens at −80 °C. The autoradiographs were then scanned and analysed with Optilab software (Loevenbruck *et al.*, 1991). The spots were delimited by automatic search. Background was considered separately for each lane, imaged under each spot, and subtracted from the spot intensity. The RNA extraction was performed two times independently to test for reproducibility. For northern quantification, we used rRNA as reference for normalization. Indeed, it has been shown that rRNA expression does not vary during *Drosophila* development (Borie *et al.*, 1999), or in vertebrates (De Leeuw *et al.*, 1989). RNA amount was estimated with ethidium bromide (EtBr) staining of rRNAs as described in Bonini & Hofmann (1991), after verification of the linear relationship between the RNA amounts estimated with EtBr and radioactivity.

3. Results

(i) *Developmental expression of the* 412 *element in* D. simulans *populations*

For all populations tested, only one transcript, about 7 kb long and corresponding to a putative complete element, was detected on northern blots with total RNA (Fig. 1). Because *412* RNAs are known to be polyadenylated (Schwartz *et al.*, 1982; Micard *et al.*, 1988) we purified poly-A^+ RNAs from flies of the Canberra and Amieu populations to detect other possible transcripts. No transcript other than that of 7 kb was detected on these two populations (Fig. 1), suggesting that truncated copies of the *412* element were silent or that the complete transcript was not spliced. The amount of 7 kb transcript was therefore determined in the different stages of development.

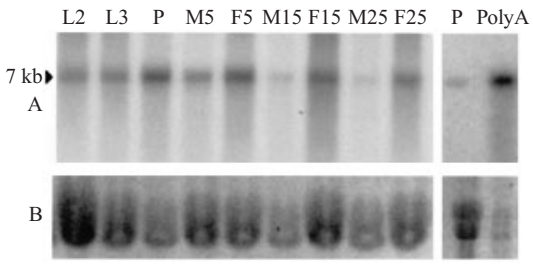

Fig. 1. (*A*) Developmental expression for the *412* retrotransposon in *Drosophila simulans* from Canberra: second (L2) and third (L3) instar larvae, pupae (P), adult males (M) and females (F), 5, 15 and 25 days old. On the right is shown the 7 kb *412* transcript in total RNA and in the poly-A^+ fraction from pupae. (*B*) RNA amount and integrity verified with rRNA.

Fig. 2 shows the patterns of the *412* element for 10 populations of *D. simulans*. Patterns differed greatly between populations, with transcripts found either only at some developmental stages or at all developmental stages.

In the populations from Papeete, Noumea, Amieu, Kwale and Madagascar, which had a restricted pattern, the 7 kb transcript was detected mainly at pupae and young adult stages. In the Papeete, Noumea, Madagascar and Kwale populations, *412* expression was maximum at the pupal stage, then decreased in young adults, and was not detected in other pre-imaginal stages. In the Amieu population expression was also found in embryos. In the populations from Moscow, Eden, and Canberra, the *412* transcript was observed at all stages of development, but the patterns differed greatly between populations. The Canberra population differed from the others by showing much higher expression at all stages. In the Eden population expression was strong in all pre-imaginal stages and then decreased in adults. Hence, the expression pattern of the *412* retrotransposon during development depended on the population, but was mostly observed at pupal stage and in young adults.

An analysis of variance (Table 1) on the two replicates of data from each *D. simulans* population revealed a strong population effect ($p < 0{\cdot}001$) as well as a strong stage effect ($p < 0{\cdot}001$). There was no effect of the replicates, suggesting that variation in patterns was not due to poorly reproducible RNA estimations. The same population and stage effects were still observed ($p < 0{\cdot}001$) when the Canberra population was removed from the analysis, indicating that this particular population was not alone responsible for the effects. To gain more information on which stages the *412* expression differed between populations, we did analyses of variance on data from each developmental stage individually. Stages L2, L3, pupae and adult males showed a strong population effect

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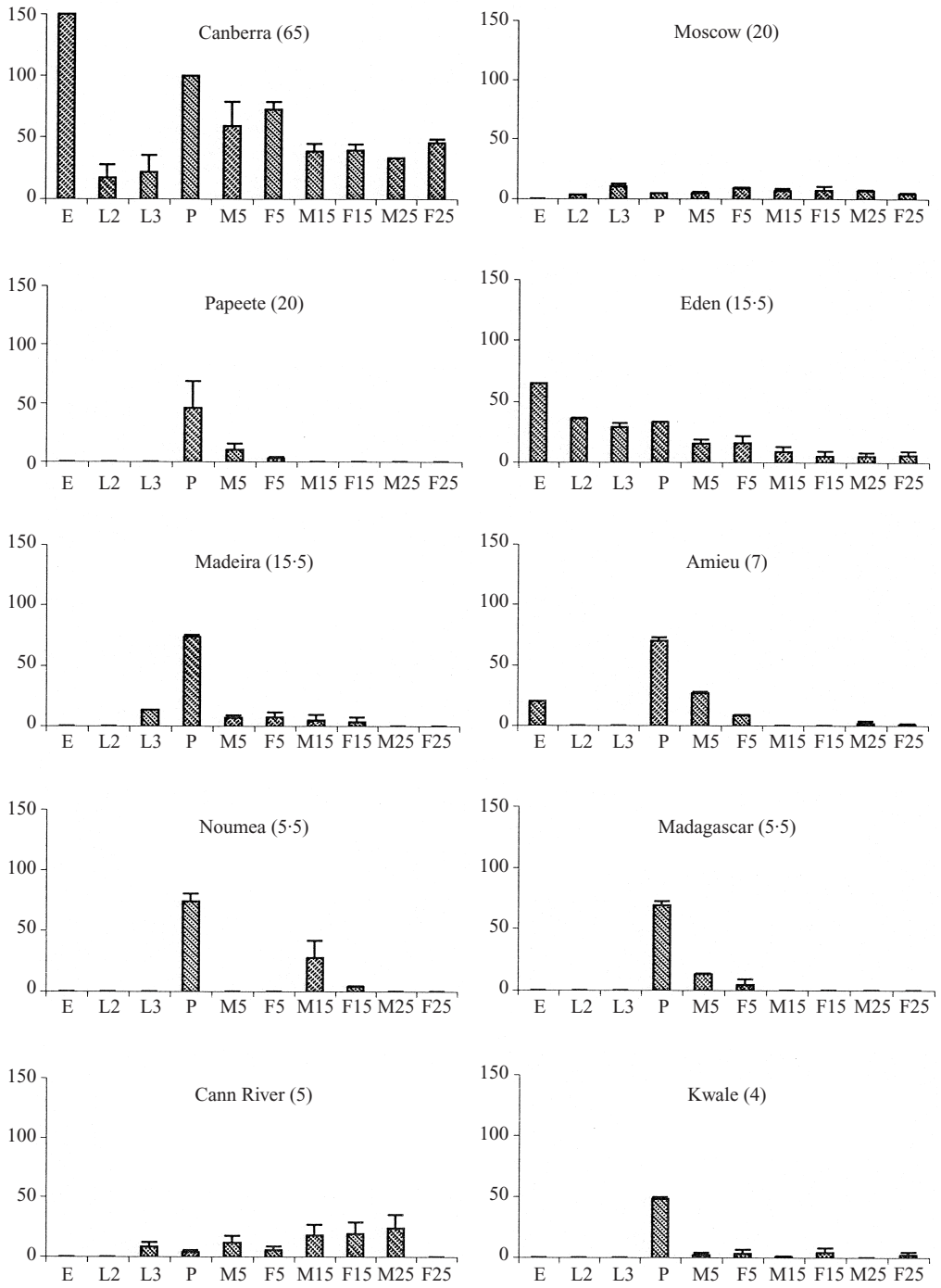

Fig. 2. Expression patterns for the *412* retrotransposon in natural populations of *D. simulans*. Average *412* copy numbers are indicated in parentheses. All data are expressed as a percentage of the amount of *412* transcript in Canberra pupae, and standardized with rRNA. See Fig. 1 for abbreviations. Bars correspond to standard errors calculated from the two replicates.

($p < 0{\cdot}01$), and the populations concerned differed for the corresponding stage. For example, among the populations, Eden presented a significantly stronger expression at the L2 stage, while Moscow, Eden and Madeira showed stronger expression at the L3 stage. These results strongly suggest that each population had its own expression pattern.

We analysed the influence of sex and age in the adults by non-parametric statistics on data pooled over the populations. A mean comparison test (Mann–Whitney's *U*-test) showed that there was no significant difference in *412* expression between male and female adult flies for each age group ($p > 0{\cdot}6$ for each group) but *412* expression decreased significantly

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Table 1. *Two-way analysis of variance on* 412 *expression data for* Drosophila simulans *populations*

Source	Degree of freedom	Sum of squares	Mean squares	*F* value	*p* value
Population	9	24178·68	2686·52	91·45	< 0·001
Stage	8	34438·58	4304·82	146·53	< 0·001
Interaction	72	23226·70	322·59	10·98	< 0·001
Error	90	2644·03	29·38		
Total	179	77990·41			

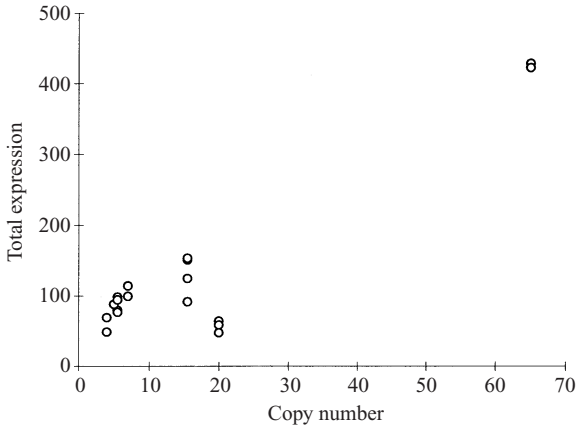

Fig. 3. Relationship between total expression of the *412* retrotransposon during development and the *412* euchromatic copy number for the 10 *D. simulans* populations analysed.

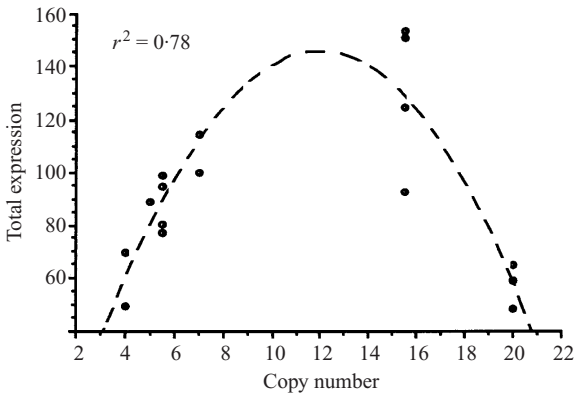

Fig. 4. Statistical relationship between total expression and copy number for *D. simulans* populations except Canberra.

in both males and females with age (Friedman test, $\chi^2 = 10{\cdot}5$, $p = 0{\cdot}004$).

(ii) *Relationship between* 412 *copy number and amount of* 7 *kb transcript*

We compared *412* euchromatic copy number with total *412* expression during development by summing the amount of 7 kb transcript at each developmental stage. Fig. 3 confirms that the Canberra population is very different from the other populations as regards both *412* expression and *412* copy number. It has been shown that Canberra is the only population among a set of 72 populations to have a *412* copy number higher than 20 (Vieira & Biémont, 1996*a*), and this population has a high copy number for many TEs (Vieira *et al.*, 2000). Since the Canberra population appears to be quite different from the others, we have excluded it from the regression analysis of the total expression over copy number. This regression analysis, done on data from the 9 populations (Fig. 4), revealed that the total expression increased with copy number for low copy numbers, then decreased in the populations from Madeira, Moscow and Papeete, which had higher copy numbers. A second-degree polynomial curve, found to be highly significant $r^2 = 0{\cdot}78$; $p < 0{\cdot}005$), suggests a maximum level of *412* expression for copy number around 10–12. It is evident that the Canberra population does not follow this pattern, showing instead high *412* expression associated with high copy number. Canberra thus fits the expression expected under an hypothesis of a linear relationship between expression and copy number as observed for the populations with low copy number.

(iii) *Interpopulation hybrids*

We analysed *412* expression during *Drosophila* developmental stages on two replicates of hybrid crosses: Amieu × Noumea, Moscou × Madeira and Moscow × Madagascar. The parental populations were analysed simultaneously. Hybrid offspring from crossing Amieu females with Noumea males and Amieu males with Noumea females showed an expression equal to the mean of the two parents (the mid-value), in agreement with co-dominant host genes regulating *412* expression in an additive fashion (data

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Table 2. 412 *mean expression in the Moscow and Madeira populations and their F1 hybrids*

Stages	Moscow	Madeira	♀Mo × ♂Md	♂Mo × ♀Md	Expected
L2	21·08	5·57	2·13	9·13	13·32
L3	100·00	18·17	35·90	65·94	59·08
P	115·48	57·58	24·50	13·49	86·53
M	24·02	2·00	4·92	26·38	13·01
F	14·21	2·26	8·92	28·95	8·23
Total	274·79	85·58	76·37	143·89	180·18

The last row presents the total expression summed over developmental stages. The last column gives the expression in hybrids under the hypothesis of a co-dominant model of inheritance. See Fig. 1 for abbreviations.

not shown). In hybrid offspring generated by crossing the Moscow and Madeira populations (Table 2), and the Moscow and Madagascar populations (data not shown), regulation of *412* expression depended on developmental stage. The *412* expression of the F1 hybrids corresponded to the average expression between the two parents in most stages, suggesting additive effects due either to difference in numbers of transcriptionally active *412* copies or to trans-acting regulatory factors.

The cross between the Moscow and Madeira populations showed varying *412* expression according to the direction of the cross, independent of the stage, Hybrids from female Moscow × male Madeira showed low *412* expression similar to that of the Madeira parents, indicating the effect of dominant trans-acting repressor, while the hybrids from the reciprocal cross showed a result close to the mid parental value, suggesting additive effects. This is illustrated in Table 2, which summarizes the values of *412* expression, summed over all developmental stages.

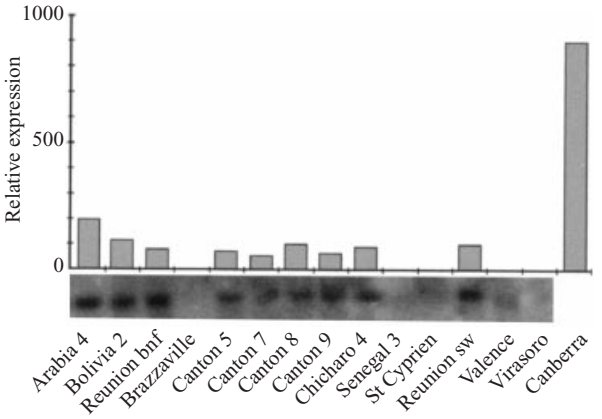

Fig. 5. Expression of the *412* retrotransposon at the pupal stage in *D. melanogaster*. Data are expressed as a percentage of the *412* transcript amount in Canton 8 pupae. The amount of *412* transcript in pupae of the Canberra population of *D. simulans* is represented on the right for comparison. Below is the corresponding northern blot.

(iv) *Developmental expression of the* 412 *element in* D. melanogaster *populations*

According to Parkhurst & Corces (1987) *412* element expression in *D. melanogaster* is detected in embryos, pupae and young adults. However, since the expression in *D. simulans* was highly variable, depending on the population considered, we analysed 14 natural populations of *D. melanogaster* for *412* expression at the pupal stage. As for *D. simulans* populations, only the complete 7 kb transcript was observed. Surprisingly, the *D. melanogaster* populations showed high variability in *412* expression at that stage (Fig. 5), and there was no relationship with copy number value, which varied only slightly around 30 (Vieira & Biémont, 1996*b*). The populations from Valence, St Cyprien, Virasoro, Senegal and Brazzaville had no detectable 7 kb transcript, while this transcript was detected in all other populations, the population from

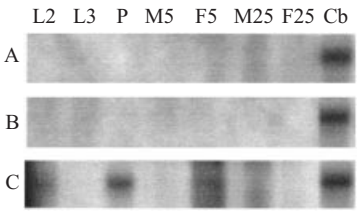

Fig. 6. Northern blots show developmental expression of the *412* retrotransposon in three populations of *D. melanogaster*: St Cyprien (*A*), Valence (*B*) and Canton 8 (*C*). On the right is *412* transcript amount in *D. simulans* Canberra pupae (b). See Fig. 1 for abbreviations.

Arabia having the highest *412* expression. Consequently we determined the *412* expression pattern throughout development in the populations from Valence, St Cyprien and Canton 8. As seen in Fig. 6, no transcript was detected in Valence or St Cyprien, whatever the developmental stage considered. In

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Canton 8, the main expression was observed for the pupal stage, with a faint signal in adults. Overall, expression in *D. melanogaster* populations was lower, when detected, than in populations of *D. simulans*. No transcript other than the 7 kb one was observed either in the St Cyprien or Canton 8 populations after purification of the poly-A^+ fraction (data not shown).

4. Discussion

Although it is usual to consider that a pattern of expression for a TE determined in one or a few lines characterizes the species as a whole (Parkhurst & Corces, 1987), differences between strains and lines have been reported. For example, Csink & McDonald (1990) have shown that *copia* transcript level in adult flies varies in natural populations of *D. melanogaster*. No experiment has been done, however, on populations with such a large variation in copy number as observed here for *412* in *D. simulans*.

The 10 populations of *D. simulans* analysed present strong differences in their *412* developmental expression pattern. This is particularly evident for populations with a low copy number, which showed a restricted expression pattern, with transcripts detected only in some specific stages. This can be interpreted as the influence of host genes near which elements are inserted. Such a position effect was previously described in eukaryotes (Wilson, 1990) and was shown for some TEs (Feinstein *et al*., 1982; Kuff & Fewell, 1985; Medhora *et al*., 1991; Lozovskaya *et al*., 1995). For example, in *Drosophila P* element regulation depends on *P* copies localized in X chromosome subtelomeric heterochromatin (Ronsseray *et al*., 1997), and this regulation disappears when copy localization is modified. In mice, 50% of *IAP* total expression is due to only one copy localized on chromosome 3 (Puech *et al*., 1997). Furthermore, TE expression may be directed by the gene within which the element is inserted. This was reported for a *412* element inserted into an exon of the vermilion gene, which was expressed like the gene and then excised like an intron (Fridell *et al*., 1990; Pret & Searles, 1991). When *412* expression was summed over developmental stages, it appeared positively correlated with *412* copy number in these populations with a low copy number. These results suggest that even if the amount of *412* transcripts increases with copy number, developmental stages at which *412* is expressed depend on the population and on copy localization. Note that this relationship between *412* expression and copy number disappears when only adults are considered. This could explain the absence of a relationship between *copia* expression and copy number in the study of Csink & McDonald (1990) in *D. melanogaster*, although a difference in behaviour between TEs or between species cannot be ruled out.

The picture is different in the populations from Moscow, Papeete and Madeira, which had around 15–20 copies of *412* but a low *412* total expression, this expression being weaker when copy number increased. Such low expression was also observed in populations of *D. melanogaster*, in which *412* copy number is around 30 (Vieira & Biémont, 1996*b*). Downregulation of *412* expression in flies with a copy number of 15–20 may result from host defence against invasion by TEs. This repression may depend on cis-regulatory sequences of the TEs themselves (Cavarec & Heidmann, 1993; Ding & Lipshitz, 1994; Zhao & Bownes, 1998; Jensen *et al*., 1999) that could interact with host genes, as proposed by Ding & Lipshitz (1994) for the *412* element. Numerous genes have been reported to modify TE expression (Csink *et al*., 1994*a*; Birchler *et al*., 1994). Some of these modifiers are developmentally regulated (Birchler *et al*., 1994) and induce different regulation of *copia* expression according to developmental stages. This is in accordance with our results on hybrids, which generally present an expression corresponding to the average expression of the parents but sometimes, at the pupal stage, show different regulation, suggesting trans-acting modifiers developmentally regulated. Such strong interaction between host genes and TE expression agrees with *412* expression decreasing in ageing flies, since it is known that host gene expression decreases or remains constant in ageing flies (Balazs & Haranghy, 1965). Such a decrease in *412* expression in ageing flies differs from previous results on *copia* retrotransposon, which found maximal expression for 10- to 12-day-old flies (Filatov *et al*., 1998*b*), suggesting that the two elements may be differently regulated. Repression may also depend on interaction between copies of the same TE based on chromatin-mediated or homology-dependent gene silencing. Such a phenomenon could involve specific chromatin conformation, as suggested for the *I* factor (Jensen *et al*., 1999). It has indeed been shown in *Drosophila* that when many copies of transgenes are inserted on the same chromosome, they have a lower expression than when they are scattered over the genome (Wolffe, 1997). Hence, in populations with low copy number, insertions may have been too distant to interact with each other.

It could be argued that differences in amount of *412* transcript between populations are due to differences in *412* sequences, some sequences being more transcriptionally active than others. Such a sequence variation was observed for the *gypsy* and *copia* elements, which present size-variants for their UTR region corresponding to variation in the number of enhancer motifs (Csink & McDonald, 1995; Matyunina *et al*., 1997). It has also been shown by restriction site analysis that *D. simulans* presents many rearranged sequences of the *412* element (Cizeron &

Biémont, 1999), but it is difficult to understand how such rearranged sequences could account for the relationship between *412* expression and copy number.

The Canberra population is a special case of interest since it has an unusually high *412* copy number associated with very high expression. This population may be derepressed for *412* expression, as if it had overcome the repression pathway postulated for populations having 15–20 copies, having instead an expression that followed the increase in copy number. Presence of rearranged copies could be involved in this deregulation, although no specific copy could be found for this population alone (Cizeron & Biémont, 1999). However, the fact that Canberra appears to have high copy number for many other TEs (Vieira *et al*., 2000) suggests that the specific characteristics of this population concern different TEs and not only *412*. We cannot, of course, exclude the possibility that drift, associated with weak purifying selection against *412* insertions in a population of dramatically reduced size (Charlesworth & Charlesworth, 1983), or against inbreeding, was responsible for the accumulation of *412* elements in Canberra, as suggested in *D. melanogaster* (Nuzhdin *et al*., 1998).

The populations of *D. simulans* still present such a large variability for their *412* copy number that different kinds of regulation of expression can be observed, while in *D. melanogaster* only a global repression is acting. In *D. simulans*, *412* expression is mainly regulated at the level of copy number with a positive relationship in low copy number populations, and a global repression in high copy number populations. In all such populations, position effect and modifier allelic frequencies account for variability during development among populations. Of course, our observation does not exclude a specific, more precise regulation of transposition and transcription in the germ line, independently of what was observed for the whole organism. Differences in expression regulation were indeed observed for the *copia* and *F* elements in *D. melanogaster* (Kerber *et al*., 1996; Pasyukova *et al*., 1997). One last point raised by our results on the *412* element is that the genome, which contains many families of TEs, seems to regulate them mostly individually in a stage-specific way. In addition to cis-acting sequences of the elements, we expect many host genes to be involved in TE regulation, at the transposition, transcription and translation levels, clearly placing these traits under the complex rules of quantitative inheritance.

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