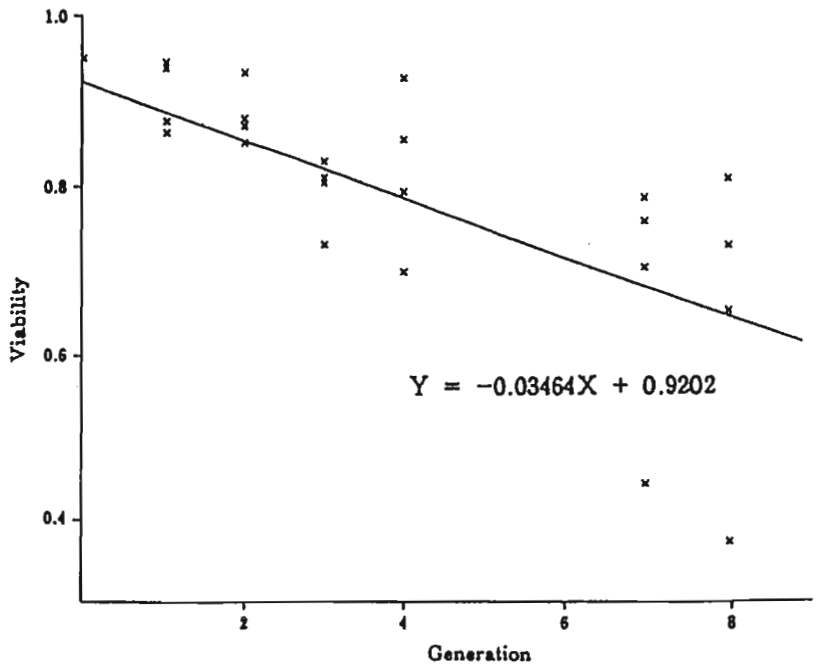


Figure 3. Regression line of viability on generations in selection lines.

facts imply that the artificial selection for light and heavy body weight has deleterious effects on egg to adult viability and suggest the existence of the stabilizing selection for body weight in *D. melanogaster*.

Regner, L.P.,[1] A. Zaha,[2] E. Abdelhay,[3] and V.L.S. Valente[1]. [1]Departamento de Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul (UFRGS). Caixa Postal 15053, CEP 91501 - 970. Porto Alegre, RS, Brazil; [2]Departamento de Biotecnologia, 1. Biociências, UFRGS, CEP 91501 - 900 Porto Alegre, RS, Brazil; [3]Laboratório de Biologia Molecular, Instituto de Biofísica, Universidade Federal do Rio de Janeiro (UFRJ). CEP 21949 - 900. Rio de Janeiro, RJ, Brazil. *P* elements in natural populations of *Drosophila willistoni* from different geographical origins.

The present study involved a screening of several strains of *Drosophila willistoni* from different places of its geographical distribution, analyzed by Southern blot for the presence of *P* elements.

Drosophila willistoni is among the most abundant drosophilid species inhabiting the hot, humid South American forests, with a wide Neotropical distribution extending from Mexico and Florida to North Argentina and from the Atlantic to the Pacific Oceans (Ehrman and Powell, 1982).

P elements have been shown to be widely distributed in this species, as well as in several others of the subgenus *Sophophora* (Lansman *et al.*, 1985; Daniels and Strausbaugh, 1986; Daniels *et al.*, 1990; Kidwell, 1994). It has been noticed that virtually all strains of *D. willistoni* studied show the presence of *P* elements, in contrast to *D. melanogaster*, where strains may (P strains) or may not (M strains) have the complete *P* sequences.

Molecular analysis of *D. melanogaster P* elements has permitted the identification of two structurally distinct types: complete elements and defective ones (O'Hare and Rubin, 1983). The complete *P* elements are 2.9 kb in length and encode two known polypeptides. Depending on the pattern of pre-mRNA splicing, a complete element may produce a transposase or a transposition-repressor protein (for a review, see Rio, 1990). Defective elements are deletion-derivatives of complete ones, and have lost their capability to encode transposase themselves but can be mobilized if a source of transposase is provided to them. It has been suggested that some truncated forms of transposase produced by internally deleted elements can act as negative regulators of transposition in *D. melanogaster* (Black *et al.*, 1987; Robertson and Engels, 1989).

The behavior of *P* elements and their effects on the host provide means by which evolutionary changes may come about, but it is not yet completely known how *P* elements behave in *D. willistoni*. Pursuing this issue we asked how widespread *P* elements are in *D. willistoni* populations and if it is possible to found any *P* free population among samples from different origins than those screened by the former authors.

In an attempt to contribute to the knowledge of the evolutionary history of this mobile element system, we performed a survey of geographically distinct strains available in our laboratory, searching for the presence or the absence of *P* elements in *D. willistoni*.

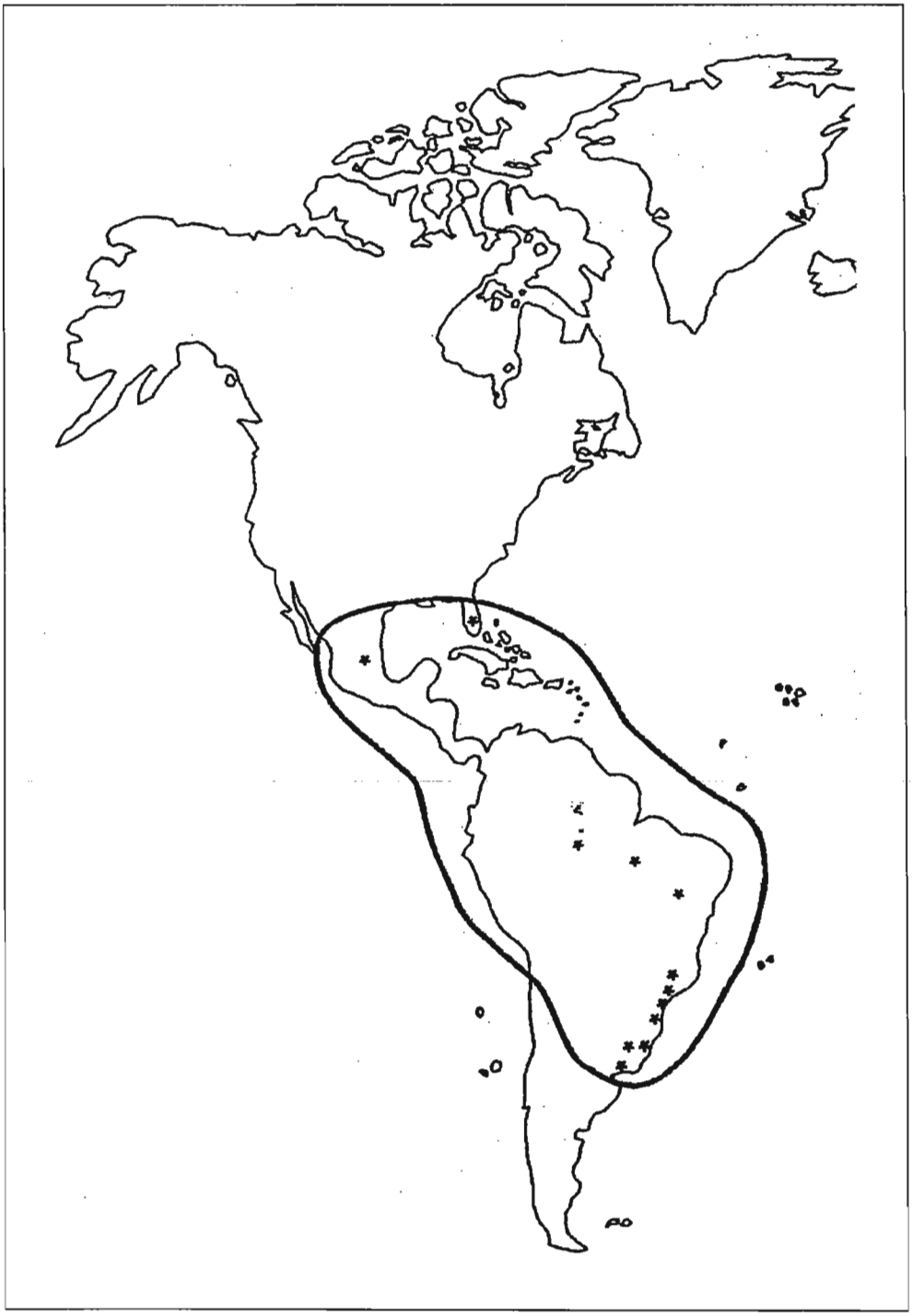


Figure 1. Geographical distribution of the *Drosophila willistoni* populations (Table 1), screened for the presence of *P* sequences. The species distribution range (according to Spassky *et al.*, 1971) is delimited by the line.

The *D. willistoni* stocks employed in this study are listed in Table 1. All of them were screened for the presence of *P* homologous sequences by Southern blotting. The fly stock cultures were maintained by mass matings on standard *Drosophila* culture medium (Marques *et al.*, 1966) at 17 ± 1°C. The Southern blot analysis was performed as follows: to assay for *P*-homologous sequence, *Pst*I, *Pvu*II, *Eco*RI/*Sal*I, or *Ava*II digests of genomic DNA were probed with the 2.4 kb *Acc*I internal fragment derived from the *P* element contained in the plasmid pπ25.1 kindly provided by Dr. Alfred M. Handler (USDA-ARS, Gainesville, USA). Genomic DNA was prepared from approximately 200 adult flies (Jowett, 1986) and digested with appropriate enzymes. The fragments were separated on 0.8% or 0.9% agarose gels, transferred to nylon membranes, and hybridized to the DNA probe labelled with ^{32}P-α-dATP and ^{32}P-α-dCTP by random priming. The specific activity of the probes was about 10^8 cpm/μg DNA. Hybridizations were carried out at 42°C for 24 h in the presence of 50% formamide. Filters were washed with 0.5×SSC and 0.1%

SDS, at 50°C and exposed to X-ray film for 48 h.

Figure 1 shows a map with the geographical distribution of *D. willistoni* according to Spassky *et al.* (1971). The strains analyzed for the occurrence of *P* elements in this study came from representative places of the distribution range of the species and their origins are plotted on the map.

Sequences homologous to *P* elements were found in all stocks examined. Southern blot analysis (Figure 2) showed DNA segments with the expected sizes for the complete canonical *P* element. Also noticeable is the systematic occurrence in almost all *D. willistoni* stocks of smaller DNA fragments besides the one expected for a complete *P* element when DNAs were digested with *Pst*I and *Ava*II. These DNA fragments may reflect the presence of internally deleted elements that might play a role in the genomic positions of *P* elements in *D. willistoni*. There is a great deal of intraspecific variation in the genomic positions of *P* elements in *D. willistoni*. In all cases, the hybridization patterns indicate a reduced number of elements relative to the number in a *D. melanogaster* P strain (compare with the first lane in each autoradiogram).

Table 1. Fly stocks of *Drosophila* employed in the present study.

Stock	Location	Collection Date
(1) *D. melanogaster* Harwich Positive Control		
(2) *D. willistoni* Manaus	Amazonas State, Brazil	1986
(3) *D. willistoni* Marabá	Pará State, Brazil	1984
(4) *D. willistoni* WIP-4	Bahia State, Brazil	1961
(5) *D. willistoni* WITA	Rio de Janeiro State, Brazil	1971
(6) *D. willistoni* WE27	São Paulo State, Brazil	1987
(7) *D. willistoni* WIC	Paraná State, Brazil	1983
(8) *D. willistoni* WUBA	Santa Catarina State, Brazil	1972
(9) *D. willistoni* WSPe	Rio Grande do Sul State, Brazil	1960
(10) *D. willistoni* Florida	Florida, USA, Bowling Center	?
(11) *D. willistoni* Mexico	Mexico, Bowling Center	?
(12) *D. willistoni* Montevideo	Montevideo, Uruguay	1991
D. melanogaster Canton S Negative Control	Bowling Center	

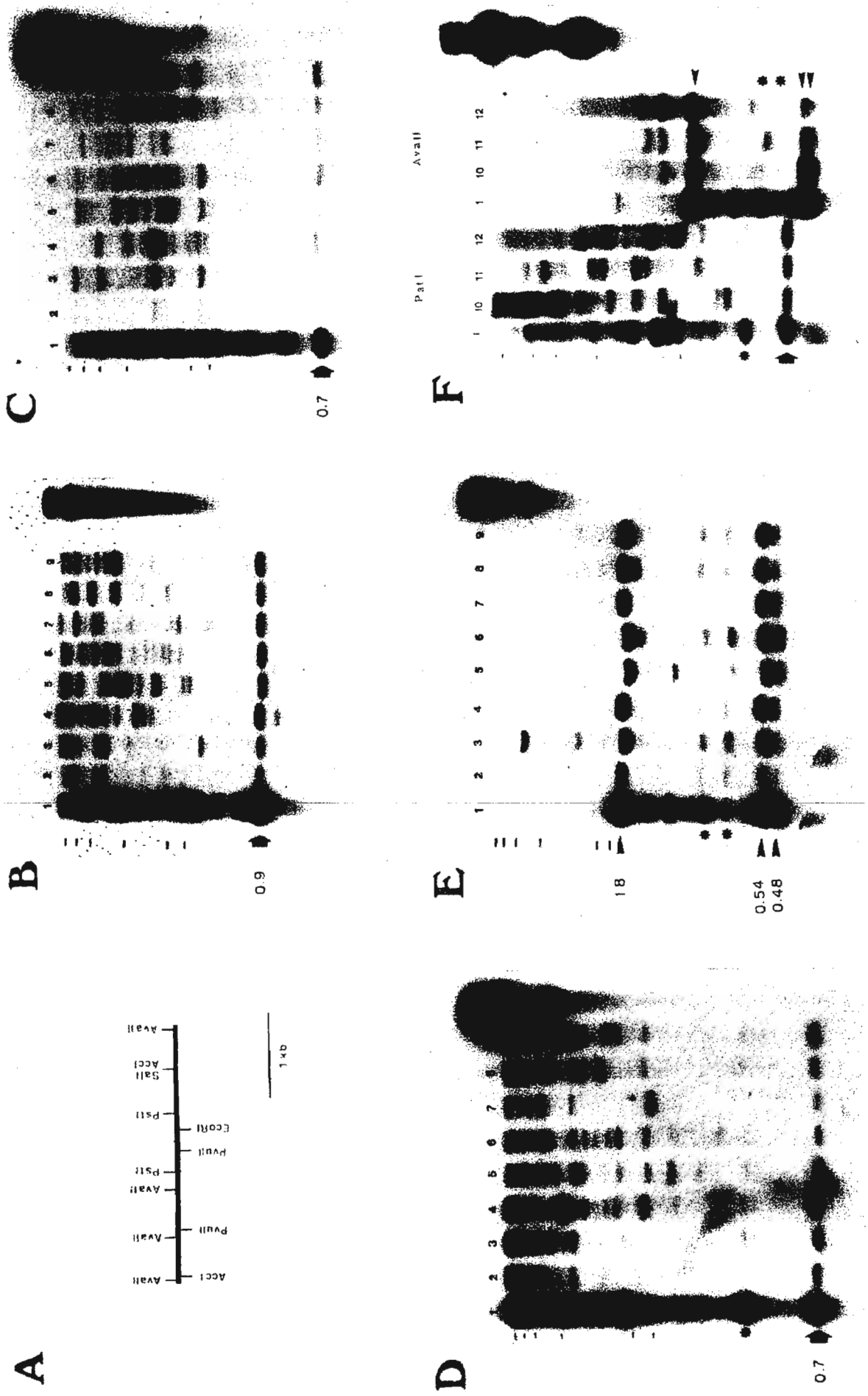
An especially careful analysis of *Ava*II restriction fragments hybridized to the *P* element probe shows that all strains analyzed presented at least one complete element in addition to a probably deleted *P* element (asterisk marked on the figure) and some elements polymorphic for *Ava*II. Particularly interesting is the presence of two fragments (about 6 kb and about 3 kb) in the Marabá strain (lane 3, Figure 2E) that are not present in the other strains. The same can be said for fragments of about 1 kb and 5.9 kb in lane 5 (WIT A); fragment of about 1.7 kb in lanes 6 and 8 (WE27, WV BA); fragment of about 2.3 kb in lanes 10, 11, 12, and corresponding to samples from Florida, Mexico and Uruguay, respectively (Figure 2F); and a fragment of approximately 3 kb in lanes 11 and 12 (samples from Mexico and Uruguay, in opposite borders of the geographical distribution of the species).

By roughly analysing these findings with the geographical origin of the strains, it appears that strains more distant from the putative center of *D. willistoni* distribution (Central Brazil) show a higher degree of polymorphism while strains closer to the origin have large numbers of deleted elements. It is also interesting to note that strain WIC (lane 7) contains only the canonical *P* element. Looking at the results of other restriction hybridizations it is possible to observe that in this strain the element has at least 3 insertion sites in the *D. willistoni* genome (lane 7, Figure 2B, C, D).

Certain other similarities were also observed among strains from geographically closer locations. The samples in lanes 2, 3 and 4 of Figure 2 correspond to strains from the States of Amazonas, Pará and Bahia (North and North East regions of Brazil); the samples in lanes 5 and 6 correspond to strains from the States of Rio de Janeiro and São Paulo (South East region), and the samples of lanes 8 and 9 are of strains from the States of Santa Catarina and Rio Grande do Sul (South region). Strain WIC (lane 7) seems to be more distantly related to the other strains, a fact that may be partially explained by its place of origin (Ilha das Cobras), an Atlantic island.

From all studies performed until now (Daniels and Strausbaugh, 1986; Daniels *et al.*, 1990; Lansman *et al.*, 1985), including our own, it is clear that the number of copies of *P* elements in *D. willistoni* is much lower than in *D. melanogaster*. The presence of complete *P* elements in all studied populations adds support to the idea that in the absence of a homologous M strain, those elements cannot undergo transposition, a fact that ould explain the poor

Figure 2 (see facing page). Southern blots of *Drosophila willistoni* populations. (A) Map of pertinent restriction enzyme sites of 2.9 kb intact *P* element contained in the pπ25.1 plasmid. Genomic DNA samples were digested with *Pvu*II (B), *Eco*RI and *Sal*I (C), *Pst*I (D and F), and *Ava*II (E and F), and probed with the 2.4 kb *Acc*I fragment of the *P* element. Fly stocks are designated by the numbers listed in Table 1. The approximate positions of the 23.1, 9.4, 6.6, 4.4, 2.3, 2.0 kb fragments generated from a *Hind*III digest of lambda DNA are indicated on the left. The last lane in each panel correspond to the pπ25.1 plasmid. The arrows indicate the internally derived fragments that are produced whenever a complete *P* element is digested. The asterisks point to other low molecular weight bands peculiar to most populations.


A
AvaII
AccI
SalI
PstI
EcoRI
PvuII
PstI
AvaII
AvaII
PvuII
AccI
AvaII
1 kb
B
0.9
C
0.7
D
0.7
E
18
0.54
0.48
F
AvaII
PstI

number of insertions in the genome. However, previous results from *in situ* hybridization analysis have shown some differences in the position of *P* elements in several *D. willistoni* subgroup species (Daniels and Strausbaugh, 1986; Lansman *et al.,* 1985). In some cases *P* mapped at the chromocenter while in others at few euchromatic sites.

Recently we have analyzed two other *D. willistoni* strains (17A2 and WIP 11A) by Southern blot and by *in situ* hybridization with *P* elements and we found strong differences between a freshly collected strain (17A2) and an old laboratory stock (WIP 11A) concerning *P* element genomic position (Regner *et al.,* 1996). These differences are reflected by the finding of 24 euchromatic insertion sites in the 17A2 strain and the unique chromocenter mapping of *P* in the old stock WIP 11A. These studies suggested that wild strains are still capable of transposition, while old stocks are not, probably because of the insertion of *P* elements in heterochromatin, as proposed by Stofford (1976), Spradling and Rubin (1983), and Devlin *et al.* (1990).

If transposition is still able to occur in *D. willistoni* under certain special conditions, is it possible that invasion and re-invasion of the populations by *P* elements would result in periods of genomic disturbances proportional to the amount of complete elements, followed by their accumulation in heterochromatic "hot spots"? Responses to such questions probably should be done by studies including a wide spectrum of *D. willistoni* natural populations, and the present is a preliminary attempt to do that. By the other hand, strains coming from places closer to the limits of the *D. willistoni* geographical distribution show higher polymorphism in *P* sequences probably because they are subject to lower selective pressures when in heterochromatin. In contrast, those strains coming from Central and South Brazil show lower polymorphism, but a large quantity of deleted elements. Those deleted *P* elements may be a potential source for transposition induction under appropriate environmental conditions.

Recently, however, Clark *et al.* (1995) found the presence of four major *P* element families in the genome of *D. willistoni*, being possible the coexistence of more than one member of these subfamilies in the same genome. Such findings need to be considered in order to establish evolutionary relationships among species, groups of species, and other upper taxa, as done by Clark and Kidwell (1997). So, a finer characterization of the *P*-homologous sequences present in the natural populations of this species needs to be done before raising hypotheses to explain variability such as that here described.

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Singh, B.K., and R.S. Fartyal. Cytogenetic Laboratory, Department of Zoology, Kumaun University, Naini Tal, India. Drosophilidae collected from Chaubatiya Garden, Ranikhet, Kumaun, India.

The Drosophilidae is a large family of world wide distribution. In recent years, our studies particularly in Kumaun region, which is located in the north-eastern periphery of the state Uttar Pradesh of the Indian subcontinent, have yielded considerable data on the Indian species (Singh and Bhatt, 1988; Singh and Negi, 1989, 1992; Singh and Dash, 1993). However, the authors believe that these data in no way furnish a complete picture of the Drosophilid species inhabiting this region since a vast area still awaits exploration. This note deals with the Drosophilid survey of Chaubatiya garden from May 1996 to April 1997.

Chaubatiya garden is located in Ranikhet, Almora district of the Kumaun region at an elevation of about 7025 feet from the sea level. It has an area of about 30 acres and is mainly characterized by the presence of *Quercus* sp.,