dent *P* element subfamilies within the same genome (Clark et al. 1994).

Sequences homologous to *P* elements have been detected in all members of the *obscura* species group examined (Anxolabéhère et al. 1985; Daniels et al. 1990; De Frutos et al. 1992), but very little is known about the molecular structure of these sequences. The first *P* sequences characterized from this group were those of *D. bifasciata* (Hagemann et al. 1990). This species contains both internally deleted and complete *P* elements, and two types of complete elements have been described, the M and O types (Hagemann et al. 1992, 1994). The M-type elements are closely related to the *P* elements of *S. pal-*

*S.*Pse-

D. guanche G1 (Miller et al. 1992), *D. nebulosa* N10 (Lansman et al. 1987), *D. bifasciata* M and O types (Hagemann et al. 1992, 1994), *D. melanogaster* (O'Hare and Rubin 1983), and *Scaptomyza pallida* 2 and 18 (Simonelig and Anxolabéhère 1991).

PCR Amplification. Genomic DNA was prepared from adult flies of each species following the method described by Junakovic et al. (1984). Approximately 100 ng of this DNA was used as template in PCR amplifications with two degenerate primers: 2016 (5′-CGWRACCAITAYGTKGAITCCGG-3′), complementary to positions 1305–1327, and 2017 (5′-CCWTCMAGGGAWGCATTRTTSAC-3′), complementary to positions 1758–1780. These primers (see Fig. 2) amplify a 450-bp fragment within exon 2 of the canonical *D. melanogaster P* element [the first *P* element sequenced and the standard frame of reference for sequence comparisons (O'Hare and Rubin 1983)]. If amplification was not successful, an alternative primer, 2015 (5′-TGGTTTASCCATCCWRCRGAYG-3′), was substituted for primer 2016. Primer 2015 is complementary to *D. melanogaster P* element positions 1230–1251 and primers 2015 and 2017 amplify a 550-bp fragment. PCR was generally carried out in a 50-μl reaction using 200 μ*M* dNTPs, 5 pmol of each primer, and 2.5 U of Taq polymerase. Temperature cycling was done for 30 cycles, each consisting of 95°C for 1 min, 58°C for 1 min, and 72°C for 1 min; a final extension was done at 72°C for 10 min.

Cloning and Sequence Analysis. Purified PCR fragments were subcloned into pCRII (Invitrogen, San Diego, CA) or pCR-Script (Stratagene, La Jolla, CA) plasmid vectors and sequenced with T7 DNA polymerase (Amersham–US Biochemical, Pharmacia) using the dideoxy chain termination method (Sanger et al. 1977). For each species, between 4 and 14 individual clones were sequenced. DNA multiple alignments were obtained with the CLUSTAL V program (Higgins and Sharp 1988) and adjusted by eye to conform to codon assignments. Parsimony analysis of the aligned data matrix was performed (Fig. 3), with all characters weighted equally. Bootstrapping of 100 replicates was performed on a reduced data set of 35 sequences that included representative taxa from each of the major clades shown in Fig. 3. Neighbor-joining analysis (Saitou and Nei 1987) was performed on the same data set. In separate analyses, trees were constructed from distance matrices using the Kimura (1980) two-parameter model for substitution and the model of Tajima and Nei (1984), which compensates for unequal nucleotide base frequencies, and uncorrected distances. All taxa were included in the bootstrap analysis of 100 replicates.

Results

Amplification 1987)Mogoucons50is.

and 2017. Phylogenetic trees were constructed using maximum parsimony and neighbor-joining. For both methods, the *P* sequence from the blowfly, *L. cuprina*, was used as an outgroup. Parsimony searches were also performed using sequences from *D. melanogaster*, *D. willistoni*, and *D. nebulosa* as outgroups. This had no effect on the branching patterns within the ingroup. Of 448 characters, 169 were constant, 40 uninformative, and 239 parsimony informative. Since both neighbor-joining and parsimony analyses gave identical branching patterns for the major groups, only the parsimony tree is shown. However, bootstrap values for both methods are given in this tree, which is shown in Fig. 3. In order to provide a frame of reference for discussion, the major clades of *P* element sequences are designated *A–K*.

Clade *F* consists of two main lineages, clades *G* and *H*, and comprises sequences isolated only from species with Old World distributions. Sequences from four species, *D. guanche*, *D. madeirensis*, and *D. subobscura* of the *subobscura* subgroup and

from *D. ambigua* differ from one another by 9.7% and a likely explanation is the retention of an ancient polymorphism in this species. It is possible th7q% and a anation is the retention of an 9.7% and a

among the *P* elements sampled from *D. subsilvestris* (up to 5.2%) and *D. obscura* (up to 5.1%), reflecting differentiation within a given species.

Adjacent to clade *F* is clade *E,* which includes several sequences from *D. affinis* and *D. azteca* of the New World *affinis*

Discussion

The phylogenetic analysis described here provides the first broad description of the relationships among *P* elements of the *obscura* species group. The resulting *P* element phylogeny (Fig. 3) appears to differ in several respects from the phylogeny of the species themselves (Fig. 1). In order to understand better the events that may have been involved in the evolution of these elements, the results are discussed in the context of the evolutionary history and geographical distribution of the species within which they reside.

The *obscura* group was originally subdivided into the *obscura* subgroup and the *affinis* subgroup (Sturtevant 1942; Buzzati-Traverso and Scossiroli 1955). Subsequently, the Nearctic species of the *obscura* subgroup were placed in two subgroups, *pseudoobscura* and *affinis* (Lakovaara and Saura 1982). Within the Nearctic region the *pseudoobscura* subgroup is found in western continental areas of the New World from British Columbia to Mexico and Colombia. Some species of the *affinis* subgroup are distributed in western areas, from Oregon to Bolivia and in Haiti; others are found in eastern areas from southern Canada to Florida. Distinctions within the Old World members of the *obscura* subgroup were subsequently recognized, leading to a division of this subgroup into the *obscura* and *subobscura* complexes (Lakovaara and Saura 1982). Recent molecular studies suggest that these complexes are themselves distinct subgroups (Barrio et al. 1994; Acosta et al. 1995; Barrio and Ayala 1997); that proposal is followed here (see Fig. 1). A fifth subgroup, *microlabis*, which includes African species, has been described recently (Cariou et al. 1988). Its exact relationship to the *obscura*, *subobscura*, *affinis*, and *pseudoobscura* subgroups has not yet been determined (Gleason et al. 1997; O'Grady 1998).

Species from the Old World are distributed in overlapping areas throughout the Palearctic region from western Europe to eastern Asia. The range of distribution of the *obscura* and *subobscura* subgroups is fairly well-known for western Europe but poorly known for both eastern Europe and Asia. *D. bifasciata* is the most widely distributed species and is found throughout Europe, including Russia, and into northern Asia and Japan. One species, *D. subobscura*, distributed in Europe and North Africa, has been found in North and South America (Prevosti et al. 1985, 1988). However, this species appears to have colonized the Americas only relatively recently.

The widespread distribution of *P* sequences within the *obscura* species group is consistent with the results of earlier surveys based on Southern blots (Daniels et al. 1990; De Frutos et al. 1992). This broad distribution suggests that ancestral *P* element sequences were present at, or soon after, the divergence of the *obscura* species group from the proto-*melanogaster* lineage in the Old World. This proto-*obscura* lineage may have split into two lineages before any major migration occurred, one of them (*microlabis*) spreading in East Africa and the other (ancestral to the *affinis*, *pseudoobscura*, *subobscura*, and *obscura* subgroups) extending to temperate regions (Cariou et al. 1988). Diversification occurred in the Palearctic region and subsequently ancestors of the *pseudoobscura* and *affinis* subgroups spread to North America prior to the mid-Miocene, about 15 MYA (Throckmorton 1975).

Considerable diversity exists among the various subfamilies of *P* elements from the *obscura* species group described here. Although there is strong evidence for short-term vertical transmission, the overall phylogeny of the *P* sequences is not consistent with the phylogenetic relationships among the species themselves. Most of the species carry in their genomes sequences belonging to different *P* element clades, suggesting that the coexistence of divergent *P* sequences in the genomes of

the divergence of the *subobscura* subgroup from the ancestor of *D. ambigua* but before the diversification of *D. subobscura, D. guanche,* and *D. madeirensis.* There is a recent description of similarly truncated *P* elements from the *melanogaster* species group but it is not yet clear if this truncation was the result of an independent event or if it occurred in the common ancestor of the *melanogaster* and *obscura* species groups (Nouaud and Anxolabéhère 1997).

P elements from the three species, *D. affinis, D. algonquin,* and *D. azteca,* are expected to be monophyletic because they belong to the same subgroup. The radiation of the *affinis* subgroup is thought to have occurred within the past 2 million years or so (Beckenbach et al. 1993). Most phylogenies indicate that *D. affinis* is more closely related to *D. algonquin* than either is to *D. azteca* (Lakovaara and Saura 1982; Beckenbach et al. 1993; Barrio et al. 1992, 1994; Gleason et al. 1997; O'Grady 1998). However, the *P* elements from *D. algonquin* are clearly distinct from those of *D. affinis* and *D. azteca.* As discussed above, this could be attributed to PCR sampling.

from clade *F*, which represent the Old World species, are part of a larger clade (*E–K*) that includes some sequences from the *melanogaster*, *saltans*, and *willistoni* species groups. This clade may be equivalent to the T-type subfamily identified by Paricio et al. (1996), and its members may be extant representatives of an ancestral *Sophophora P* element