Characterization of a Chlamydomonas Transposon, *Gulliver*, Resembling Those in Higher Plants

Patrick J. Ferris

Biology Department, Washington University, St. Louis, Missouri 63130

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ABSTRACT

While pursuing a chromosomal walk through the mt^+ locus of linkage group VI of *Chlamydomonas reinhardtii*, I encountered a 12-kb sequence that was found to be present in approximately 12 copies in the nuclear genome. Comparison of various *C. reinhardtii* laboratory strains provided evidence that the sequence was mobile and therefore a transposon. One of two separate natural isolates interfertile with *C. reinhardtii*, *C. smithii* (CC-1373), contained the transposon, but at completely different locations in its nuclear genome than *C. reinhardtii*; and a second, CC-1952 (S1-C5), lacked the transposon altogether. Genetic analysis indicated that the transposon was found at dispersed sites throughout the genome, but had a conserved structure at each location. Sequence homology between the termini was limited to an imperfect 15-bp inverted repeat. An 8-bp target site duplication was created by insertion; transposon sequences were completely removed upon excision leaving behind both copies of the target site duplication, with minor base changes. The transposon contained an internal region of unique repetitive sequence responsible for restriction fragment length heterogeneity among the various copies of the transposon. In several cases it was possible to identify which of the dozen transposons in a given strain served as the donor when a transposition event occurred. The transposon often moved into a site genetically linked to the donor, and transposition appeared to be nonreplicative. Thus the mechanism of transposition and excision of the transposon, which I have named *Gulliver*, resembles that of certain higher plant transposons, like the *Ac* transposon of maize.

TRANSPOSONS are invaluable tools for molecular genetics. Diverse mutations in a variety of organisms are the result of transposon insertions, and the presence of a transposon near or within a gene of interest can expedite cloning—for example the *opaque-2* gene of maize (SCHMIDT, BURR and BURR 1987) and the *Caenorhabditis elegans unc-22* gene (MOERMAN, BENIAN and WATERSTON 1986). Specially engineered versions of the yeast *Ty* and the Drosophila *P* element have been constructed to make such transposon-mediated mutagenesis and cloning more efficient (COOLEY, KELLEY and SPRADLING 1988; GARFINKEL *et al.* 1988). Transposons can serve as genetic markers (FEDEROFF 1983), and both the Drosophila *P* element (SPRADLING 1986) and the yeast *Ty* element (BOEKE, XU and FINK 1988) have been used as integrative transformation vectors. The unavailability, until very recently (DAY *et al.* 1988), of any transposons in the unicellular green alga Chlamydomonas has been a limitation in the molecular genetics of this system.

Transposons fall into two categories based on their mechanism of transposition. The first group, which includes maize *Ac* and *Spm*, Drosophila *P* elements and probably the *C. elegans Tc1*, appears to transpose via a DNA intermediate. The second group, termed retroposons, transposes via an RNA intermediate. Retroposons fall into two subgroups. The more familiar viral-like retroposons are so named because they resemble retroviral proviruses (BOEKE *et al.* 1985; VARMUS 1982). Members of this group have long terminal direct repeats at their ends, and transpose by reverse-transcribing a full-length RNA copy, initiated from a promoter within the terminal repeat, and integrating the DNA copy back into the genome. Viral-like retroposons generally encode the enzymes required for their own transposition. Included in this group are the yeast *Ty* element, Drosophila *copia* and the Chlamydomonas *TOC1* (DAY *et al.* 1988). The nonviral retroposons are a heterogeneous group of elements, including pseudogenes and *Alu* sequences, which are formed by the reverse transcription of various cellular RNAs. These elements frequently terminate in a poly A tract at the original 3′ end, produce target-site duplications of variable length, and rarely encode their own transposition enzymes (WEINER, DEININGER and EFSTRATIADIS 1986), although some nonviral retroposons, like the vertebrate LINEs and the Drosophila *jockey* element, encode a protein with some homology to reverse transcriptase (MIZROKHI, GEORGIEVA and ILYIN 1988; HATTORI *et al.* 1986).

During the course of a chromosome walk through the mating type region of linkage group VI of *Chlamydomonas reinhardtii* I encountered a sequence that hybridized to roughly a dozen fragments on a genomic

Southern blot. Slight variations in the pattern of hybridizing fragments among various Chlamydomonas strains suggested that the element might be mobile. Molecular cloning and genetic analysis showed that the element was part of a discrete 12-kb unit dispersed about the genome. DNA sequence analysis of copies of the element and corresponding genomic regions before its insertion or after its excision indicates that transposition produces an 8-bp target duplication and excision completely removes the element, leaving both copies of the target duplication. The element often inserts into a new site genetically linked to the site it excises from. The only sequence homology at the ends of the element is an imperfect 15-bp inverted repeat. Thus this Chlamydomonas element, which I have named *Gulliver* (SWIFT 1726), resembles DNA-mediated transposons, particularly those of higher plants.

MATERIALS AND METHODS

Chlamydomonas strains and culture conditions: Most of the Chlamydomonas strains used (Table 1) were obtained from the *Chlamydomonas* Culture Collection, Duke University, Durham, NC. *Fus* was supplied by Y. MATSUDA, *bs-37* by R. KAMIYA, *mbo-1* alleles and *fla-6* by D. J. L. LUCK, *shf-1* by J. JARVIK and CC-1952 (S1-C5) by P. LEFEBVRE. Strain S1-C5 was isolated from the same soil sample as S1-D2 (GROSS, RANUM and LEFEBVRE 1988) and the two strains are probably identical (P. LEFEBVRE, personal communication). Cells were maintained on solid TAP medium (GORMAN and LEVINE 1965), supplemented as necessary with 4 μg/ml nicotinamide, 100 μg/ml arginine or 5 μg/ml thiamine. Chlamydomonas crosses were performed using standard protocols (LEVINE and EBERSOLD 1960).

Southern blots: Chlamydomonas DNA was prepared according to WEEKS, BEERMAN and GRIFFITH (1986), except that cells were grown on solid medium and the DNA was phenol extracted several times after recovery from CsCl. Restriction enzyme-digested genomic DNA was electrophoresed on agarose gels prepared with Tris-borate or Tris-acetate buffer (MANIATIS, FRITSCH and SAMBROOK 1982), blotted to nitrocellulose (WAHL, STERN and STARK 1979) and hybridized with ^{32}P-labeled probes (CHURCH and GILBERT 1984). Probes were prepared by nick-translation of restriction fragments purified on low-melting point agarose gels (MANIATIS, FRITSCH and SAMBROOK 1982).

Library construction and screening: Chlamydomonas DNA was partially digested with *Sau*3AI and fragments 15–22 kb in length were isolated from a low melting agarose gel. The purified fragments were then ligated into *Bam*HI/*Eco*RI-cut EMBL3 λ DNA (ProMega), packaged *in vitro* according to manufacturer's instructions (ProMega) and plated on *Escherichia coli* strain CES200 (NADER *et al.* 1985). The libraries were not amplified before use. Plating of phage, preparation of plaque lifts on nitrocellulose for screening by hybridization, and purification of phage DNA were performed as in MANIATIS, FRITSCH and SAMBROOK (1982).

The original transposon, *O*, was isolated from a library constructed from strain CC-620. Additional copies of *Gulliver* were isolated from a library constructed using DNA from one of the *mbo-1.1A* × CC-1952 progeny (progeny 4 of tetrad 1 in Figure 2). This strain contained only 7 copies of *Gulliver* (those at *B*, *C*, *G*, *H*, *I*, *M*, *N*), all derived from the *mbo-1.1A* parent. Hybridization of approximately 60,000 plaques from this library with probe *a* (see Figure 3) yielded 56 positive phage. By restriction mapping some of these phage, and by classifying the rest based on cross-hybridization to the non-transposon flanking sequences of the mapped phage, eight groups were identified. Seven of these corresponded to the 7 transposon copies; the eighth group comprised clones from within the *mt*$^+$ locus, isolated by their homology to the short region of flanking sequence in probe *a*. In strain *mbo-1.1A* there is no transposon at this position.

To isolate the transposons at *J* and *K* from the CC-620 EMBL3 library, the J_L and K_L junction fragments were first selected from subgenomic plasmid libraries prepared as follows. Genomic DNA (strain CC-621) was digested with *Hin*dIII and size fractions cut out of a low melting agarose gel. An aliquot of each size fraction was electrophoresed on an agarose gel with an unfractionated control sample, blotted to nitrocellulose, and hybridized with probe *a* to identify the transposon fragments. The fractions containing the J_L or K_L junction fragments were separately ligated into *Hin*dIII-cut, calf alkaline phosphatase-treated pUC13 (MESSING 1983), transformed into *E. coli* strain TG1, and clones containing the transposon sequences identified by colony hybridization (MANIATIS, FRITSCH and SAMBROOK 1982). The non-transposon portion of the J_L plasmid (*i.e.*, a unique sequence) was then used as the probe to isolate the complete *J* transposon copy from the CC-620 phage library. The K_L plasmid had too little flanking DNA to use as a probe, so another subgenomic plasmid library was prepared from the size fraction containing the K_R junction fragment, identified using probe *b*. The K_R plasmid had enough flanking DNA to provide a probe for isolating the complete *Gulliver* at *K*.

The excision point of the *Gulliver* at *N* was isolated from the CC-620 phage library, and the excision point of *K* from the *mbo-1.1A* × CC-1952 progeny library. (Restriction fragment length polymorphism analysis had demonstrated that this progeny had inherited the *K* region of the genome from the *mbo-1.1A* parent.) The excision point of *M* was isolated by preparing a subgenomic plasmid library (as above) by ligating the size fraction of *Hin*dIII/*Xho*I-digested CC-421 DNA that contained the excision point, identified using a probe flanking *M*, into *Hin*dIII/*Sal*I-cut pUC13. A plasmid containing the desired fragment was isolated from the plasmid library with the same probe. The *mt*$^-$ DNA corresponding to the empty target site of *N* was isolated from a Charon 30 library (BRUNKE *et al.* 1982) kindly supplied by K. BRUNKE.

DNA sequencing: Sequencing was performed using the chemical cleavage method, essentially as in MAXAM and GILBERT (1980). DNA restriction fragments were labeled at the 3′ end using reverse transcriptase and the appropriate [α-^{32}P]-dNTP (SMITH and CALVO 1980).

RESULTS

Evidence of a transposon: During the course of a chromosome walk, a 12-kb sequence was found to be present in the *mt*$^+$-linked DNA of strain CC-620 but absent from the corresponding location in several other strains. One interpretation of this observation was that the sequence was a transposon that had undergone a recent insertion within the *mt*$^+$ locus of CC-620. Since transposons are generally present in many copies per genome, each integrated at a unique

TABLE 1

Strain list

Strain designation	Genotype	Source
CC-620	R3, mt^+, high efficiency mating wild type	HARRIS (1989)
CC-621	NO, mt^-, high efficiency mating wild type	HARRIS (1989)
CC-124	mt^-, wild type	HARRIS (1989)
CC-125	mt^+, wild type	HARRIS (1989)
CC-1373	*C. smithii*, mt^+	BELL and CAIN (1983)
CC-1952	S1-C5, mt^-	GROSS, RANUM and LEFEBVRE (1988)
—[a]	*mbo-1.1A*, mt^+	SEGAL *et al.* (1984)
—	*mbo-1.4D*, mt^+	SEGAL *et al.* (1984)
CC-1158	*imp-1*, mt^+	GOODENOUGH, HWANG and MARTIN (1976)
CC-463	*imp-2*, mt^+	GOODENOUGH, HWANG and MARTIN (1976)
CC-470	*imp-5*, mt^-	GOODENOUGH, HWANG and MARTIN (1976)
CC-471	*imp-6*, mt^+	GOODENOUGH, HWANG and MARTIN (1976)
CC-472	*imp-7*, mt^+	GOODENOUGH, HWANG and MARTIN (1976)
CC-473	*imp-7*, mt^-	GOODENOUGH, HWANG and MARTIN (1976)
CC-475	*imp-8*, mt^+	GOODENOUGH, HWANG and MARTIN (1976)
CC-476	*imp-8*, mt^-	GOODENOUGH, HWANG and MARTIN (1976)
CC-1146	*imp-9*, mt^+	ADAIR, HWANG and GOODENOUGH (1983)
CC-1147	*imp-10*, mt^-	HWANG, MONK and GOODENOUGH (1981)
CC-1148	*imp-11*, mt^-	GOODENOUGH, DETMERS and HWANG (1982)
CC-1149	*imp-12*, mt^-	HWANG, MONK and GOODENOUGH (1981)
CC-1394	*fla-6*, mt^-	ADAMS, HUANG and LUCK (1982)
CC-2062	*fus*, mt^+	MATSUDA, TAMAKI and TSUBO (1978)
CC-85	*nic-7*, mt^+	EBERSOLD *et al.* (1962)
CC-350	*nic-7, ac-29a*, mt^-	SMYTH, MARTINEK and EBERSOLD (1975)
CC-421	*nic-7, ac-29a, spr-u-1-27-3*, mt^-	*Chlamydomonas* Culture Collection
CC-44	*ac-29a*, mt^-	SMYTH, MARTINEK and EBERSOLD (1975)
CC-45	*ac-29*, mt^+	EBERSOLD *et al.* (1962)
CC-1062	*ac-212*, mt^+	GIRARD *et al.* (1980)
CC-2001	*arg-9-1*, mt^+	LOPPES and HEINDRICKS (1986)
—	*bs-37*, mt^+	FOREST (1987)
CC-1370	*fa-1*, mt^+	LEWIN and BURRASCANO (1983)
CC-123	*thi-10*, mt^+	EBERSOLD *et al.* (1962)
—	*shf-1-253*, mt^-	JARVIK *et al.* (1984)
CC-410	*C. reinhardtii*, mt^-, Lewin Caroline Islands, SAG 11-32c	*Chlamydomonas* Culture Collection
CC-1374	*C. reinhardtii*?, France, SAG 77.81	*Chlamydomonas* Culture Collection
CC-1418	*C. reinhardtii*, mt^-, red tide, Florida, SAG 18.79	*Chlamydomonas* Culture Collection
CC-1871	*C. sp.*, from Pringsheim, SAG 11-31	*Chlamydomonas* Culture Collection

"CC" strains are available from the *Chlamydomonas* Culture Collection, Department of Botany, Duke University, Durham, North Carolina.
[a] — strains not in the *Chlamydomonas* Culture Collection.

chromosomal site, a probe from one end of the sequence was hybridized to a southern blot of *Hin*dIII-digested CC-620 genomic DNA. As shown in Figure 1A, 11 fragments have visibly hybridized with the probe, indicating that it is indeed a multicopy sequence. Moreover, each fragment was of a unique size, as expected for a collection of "junction fragments," each representing one end of a transposon and its adjoining genomic DNA extending to the first available *Hin*dIII site.

Since a transposon should move, evidence of transposition was sought by analyzing the pattern of junction fragments in other laboratory strains. As documented in Figure 1A, the original transposon copy detected in CC-620, represented by the junction fragment designated *O*, is indeed absent from the other strains. Similarly, the fragment labeled *C* is present only in strain *mbo-1.1A*. Reciprocally, fragment *H* is present in most strains but absent from CC-621, and fragment *M* is uniquely absent from CC-421. (Fragment *N* is absent in several strains because it is mt^+-linked and therefore absent from all mt^- strains.)

The aforementioned *C. reinhardtii* laboratory strains all derive from a single natural isolate (HARRIS 1989). If the sequence were a transposon, one might expect more dramatic differences in the pattern of bands when *C. reinhardtii* is compared with other natural isolates. This is indeed the case for *C. smithii* (CC-1373), a strain interfertile with *C. reinhardtii* (BELL and CAIN 1983): while it also displays roughly 12 junction fragments (Figure 1A), many of these migrate differently, and in fact those with the same apparent mobility have been shown to segregate independently in crosses (data not shown).

The most dramatic result is obtained with a second natural isolate, CC-1952 (also called S1-C5—see MA-

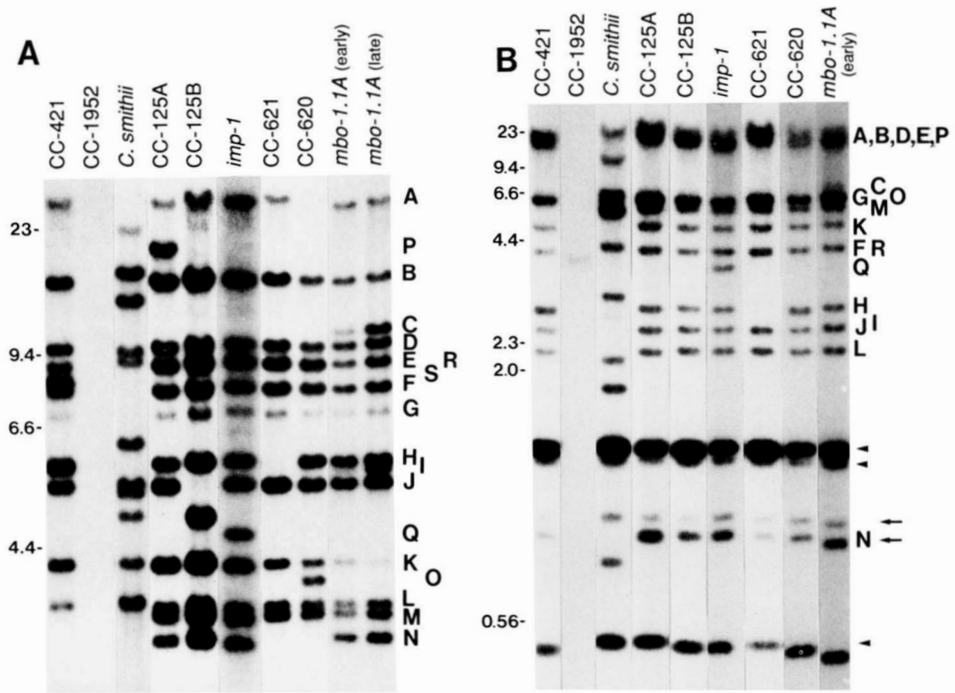

FIGURE 1.—A mobile, multicopy sequence in the nuclear genome of *C. reinhardtii*. Southern blots of genomic DNA from indicated Chlamydomonas strains digested with *Hin*dIII and probed with the left and right ends of the *Gulliver* transposon. Panel A, a 0.7% agarose/Tris-acetate gel, blotted to nitrocellulose and hybridized with a probe from the left end of the transposon (probe *a*; see Figure 3). Panel B, a 0.8% agarose/Tris-borate gel, blotted and hybridized with a probe from the right end of the transposon (probe *b*; see Figure 3). In both panels, the junction fragments corresponding to the different copies of *Gulliver* are labeled to the right. Although unresolved in panel B, fragments *A*, *B*, *D*, *E* and *P* have been resolved on gels run for longer times. The comigration of fragments *E* and *R* (panel A) and *F* and *R* (panel B) in *imp-1* was discovered while analyzing progeny from *imp-1* × CC-1952, some of which contained only one or the other copy from each pair. The indicated comigration of *G* and *O* (panel B) in CC-620 is an inference based on the size of fragment *O* as determined by restriction mapping the cloned *Gulliver* at *O*. *Hin*dIII-cut phage λ size standards are shown to the left (sizes in kb). In B, three arrowheads mark internal *Hin*dIII fragments present in most copies of the transposon (the 1.25 kb fragment is fainter because the probe only partly overlaps it). Note that although the junction fragments in *C. smithii* differ from those in *C. reinhardtii* strains, the three internal fragments are the same. The arrows mark two fragments (one comigrating with *N*) that represent internal transposon sequences present in only one or a few copies. Probe *a* hybridizes to an internal *Hin*dIII fragment of 2.2 kb, which has been cut off the gel in A. Both probe *a* and *b* contain flanking, non-transposon sequence. For probe *a*, this sequence is too short to produce a visible band; for probe *b*, the flanking DNA hybridizes a 6.3-kb band in *C. reinhardtii* strains (obscured by the *G* band) and a 4.0-kb band visible in CC-1952. The DNA in the *mbo-1.1A* (early) lanes was prepared 9 months before that in the *mbo-1.1A* (late) lane. The CC-620 strain carries a copy of *Gulliver* at *A* which did not transfer well in the blot used in panel A.

TERIALS AND METHODS). This strain, also interfertile with *C. reinhardtii*, lacks the sequence altogether (Figure 1A). It is also absent from two more distantly related species, *C. monoica* and *C. eugametos*. Hence this sequence is clearly not essential for Chlamydomonas viability. Taken together, these data suggest that the sequence is a transposon which I shall call *Gulliver*. More definitive evidence in this regard is presented in later sections.

Evidence that *Gulliver* is a discrete 12-kb transposon: The probe used in Figure 1A, called probe *a*, derived from one end (arbitrarily referred to as the left end) of the 12-kb *Gulliver* sequence (Figure 3). A second probe, probe *b* (Figure 3), was prepared from the right end and hybridized to *Hin*dIII-cut genomic DNA from the same set of strains. As would be expected if *Gulliver* were a discrete 12-kb element, a similar number of junction fragments is detected in each strain with probe *b* as with probe *a*, although they of course display different electrophoretic mobilities (Figure 1B). As with probe *a*, *C. smithii* displays a different pattern of bands than *C. reinhardtii* and CC-1952 again shows no hybridization at all (except for a faint band due to homology with the non-transposon, flanking DNA present in probe *b*).

If they represent opposite ends of a transposon, genetic linkage between pairs of bands seen by the left and right probes should be demonstrable. Genetic crosses were performed between various laboratory strains and CC-1952, which contributes no copies of *Gulliver* to the progeny. Figure 2 shows representative results. In Figure 2A, a southern blot of DNA from the progeny of two tetrads was hybridized with the left probe *a*; in Figure 2B, the same progeny were analyzed with the right probe *c*. In each tetrad, each junction fragment segregates 2:2. Moreover, when the two blots are compared, co-segregating fragments can be identified. For example, fragment *F* in Figure

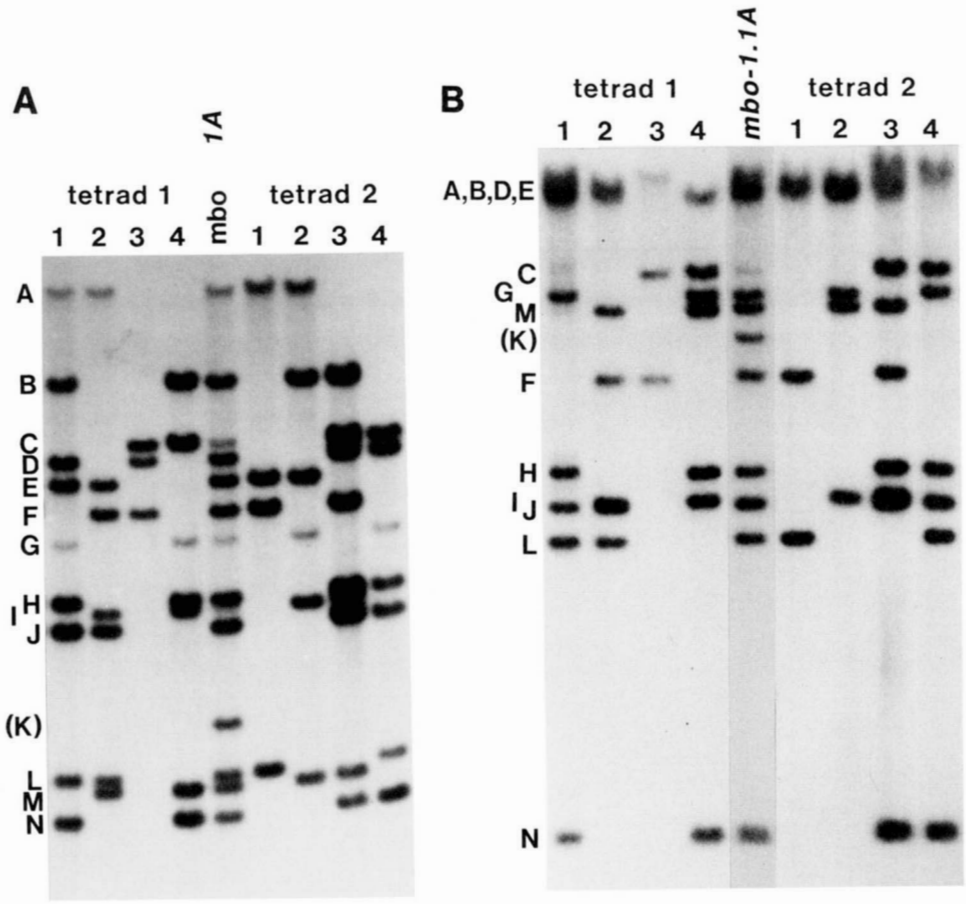

FIGURE 2.—Inheritance of the transposon junction fragments. Southern blots were prepared from *Hin*dIII-digested DNA from the progeny of two tetrads of the cross *mbo-1.1A* × CC-1952 as in Figure 1. The blot in panel A was hybridized with probe *a*, which identifies the left end of the transposon (Figure 3); panel B was hybridized with probe *c*, which identifies the right end (Figure 3). Probe *c*, isolated from *Gulliver* at *N*, contains only the last 500 bp of the transposon and about 100 bp of flanking DNA and therefore, unlike probe *b* in Figure 1, does not hybridize to any internal *Hin*dIII fragments. The CC-1952 parent is not shown since it does not contain any copies of the transposon. The pairs of junction fragments that segregate together and therefore presumably represent opposite ends of the same transposon are labeled with the same letter in each panel. Note that the *mbo-1.1A* strain is a mixed population of cells, some containing the transposons at *C*, *I* and *K*, and some not. The *mbo-1.1A* parent gametes that were the progenitors to each of these two tetrads apparently contained *C* and *I*, but not *K* (see text).

2A is present in progeny 2 and 3 of the first tetrad and progeny 1 and 3 of the second. In Figure 2B, the right probe identifies one fragment inherited by these same four progeny, which is consequently designated *F*. Extending this analysis to 4 tetrads and 34 random progeny, it was possible to establish linkage of each left junction fragment with a right junction fragment; the molecular weights of the cosegregating pairs of junction fragments are presented in Table 2. To simplify nomenclature, pairs of fragments are referred to by the same letter, as is the case in Figures 1 and 2, but when a distinction is important, they are designated with the subscripts L and R, respectively. In subsequent sections, moreover, an individual copy of *Gulliver* will usually be designated by a letter—*e.g.*, the transposon at *O*—when it should correctly, but more awkwardly, be designated "the transposon flanked by the O_L and O_R junction fragments."

Consistent with the linked fragments being opposite ends of a transposon is the observation that strains in which one of the left fragments is missing, presumably due to transposon excision, are missing the corresponding linked right fragment. For example, CC-421 is missing the M_L and M_R bands (Figure 1).

In addition to scoring crosses for cosegregation of transposon ends, progeny were also analyzed for linkage between copies of *Gulliver*. Fifteen transposon copies (*A*–*N*, *P*) that were scored in a variety of crosses are largely unlinked, with the following exceptions: *N* (like *O* in CC-620) is linked to the mt^+ locus; *I* (which is present only in *mbo-1.1A*) and *M* are linked; *J* is approximately 20-cM centromere-proximal of *mt* (an assignment later confirmed by data from crosses with *arg-9*, also 20 cM from *mt* (LOPPES and HEINDRICKS 1986), to which *J* is closely linked; data not shown); and *K* is roughly 20 cM from *C*. Because of its widely scattered locations about the genome, *Gulliver* should be a useful genetic marker.

Mutagenic potential of *Gulliver*: A variety of existing *C. reinhardtii* mutant strains were analyzed to determine whether their mutations were caused by insertion of *Gulliver*. The mutants selected were largely those with mating defects or linked to *mt*, due to my interest in this region of linkage group VI. In some cases there were new transposon insertions in these mutant strains (Table 2), including: *Gulliver* at *U* in *imp-7* mt^+, *T* in *imp-8*, *C* and *I* in *mbo-1.1A*, *Q* and *R* in *imp-1*, *V* in *fla-6*, *S* in *nic-7*, and *W* in *ac-29a*. However, in all cases the novel *Gulliver* either fails to segregate with the mutant gene in genetic crosses or is not present in all strains carrying a particular mutant allele (data not shown). Therefore, the novel transposon copies in these mutants are insertions that have occurred independently of the mutations, probably

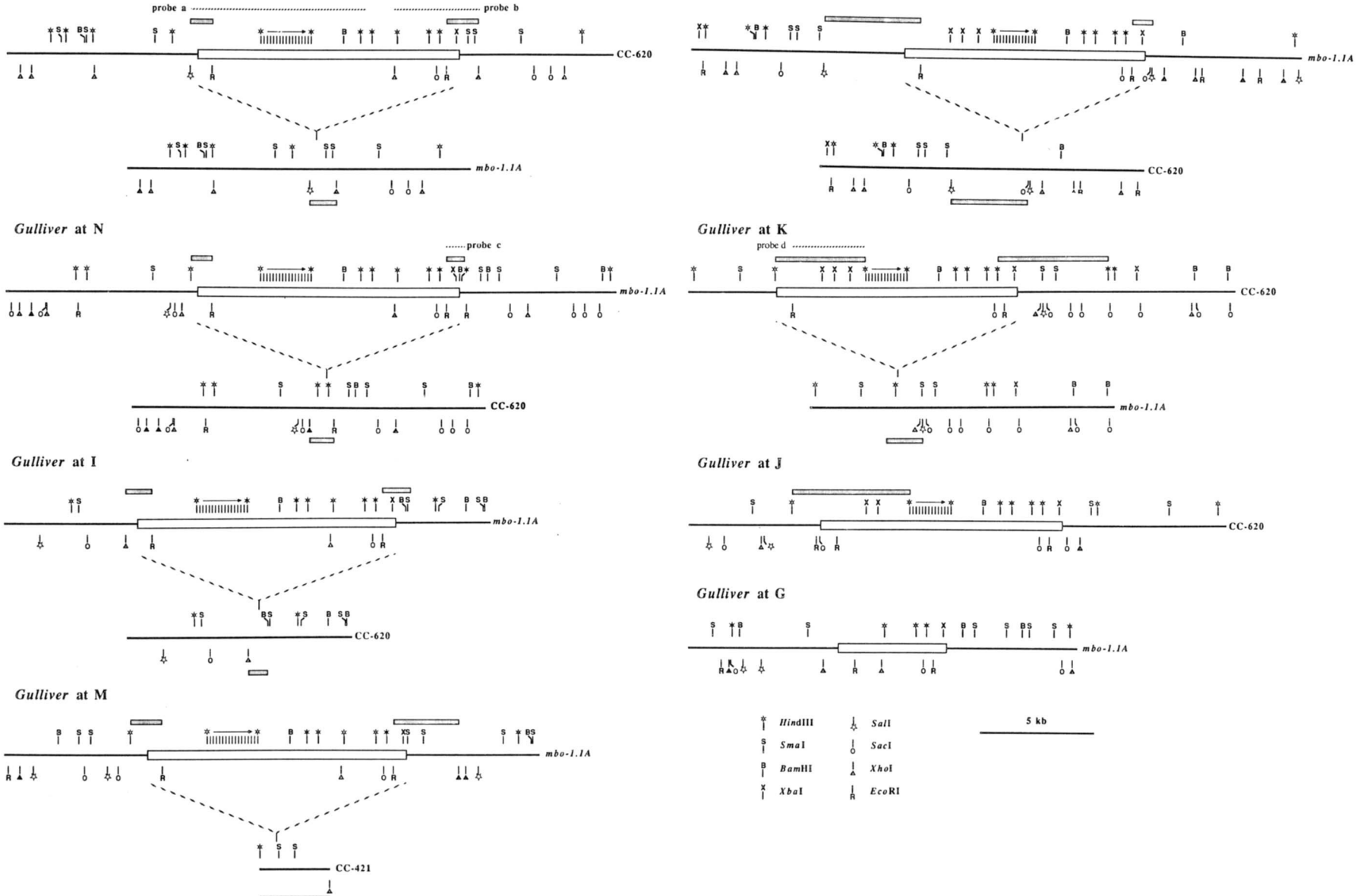

FIGURE 3. Cloning individual transposons. The restriction maps of eight transposon copies are shown. *Gulliver* is represented by a box, the flanking DNA by a solid line. Three of the transposon copies (*C*, *I*, *O*) are new insertions and the restriction map of the corresponding empty target site is also shown. Another three (*K*, *M*, *N*) have been excised in one strain, and the restriction map of the corresponding region in that strain is shown. At the right of each map, the strain from which the DNA was derived is indicated. Broken lines above the appropriate transposon maps show the source of the four hybridization probes used (probes *a*–*d*). The gray boxes above or below the maps indicate regions subcloned into pUC13 for DNA sequencing. Four of these plasmid clones were actually cloned directly from Chlamydomonas genomic DNA (see text)—the left and right junctions from *K*, the left junction from *J*, and the excision product at *M*. The cluster of *Hin*dIII sites in each transposon is indicated by a horizontal arrow. Note that the *Xba*I site near the right end of the transposon is actually a pair of sites 37 bp apart (*cf.* the sequence at *N* in Figure 6).

TABLE 2

Gulliver in *C. reinhardtii* strains

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X
Left (kb)	30	15	10.4	9.8	9.0	8.0	6.9	5.7	5.6	5.4	4.2	3.5	3.4	3.2	3.9	19	4.6	9.0	8.3	4.8	4.4	19	9.3	7.7
Right (kb)	19	17[a]	7.4	30	22[a]	4.2	6.2	2.8	2.55	2.5	4.9	2.2	5.8	0.85	6.3	15	3.6	4.2	—[b,c]	2.45	—[b]	11	—[b]	1.4
mbo-1.1A	+	+	+[d]	+	+	+	+	+	+[d]	+	−[d]	+	+	+	−	−	−	−	−	−	−	−	−	−
CC-620	+	+	−	+	+	+	+	+	−	+	+	+	+	−	+	−	−	−	−	−	−	−	−	−
imp-1	+	+	−	+	+	+	+	+	−	+	+	+	+	+	−	−	+	+	−	−	−	−	−	−
CC-124	+	+	−	+	+	+	+	+	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
CC-125A[e]	+	+	−	+	+	+	+	+	−	+	+	+	+	+	−	+	−	−	−	−	−	−	−	−
CC-125B[e]	+	+	−	+	+	+	+	+	−	+[f]	+	+	+	+	−	−	−	−	−	−	−	−	−	−
imp-7 mt^{+g}	+	+	−	+	+	+	+	+	−	+	+	+	+	+	−	−	−	−	−	−	+	−	−	−
imp-7 mt$^-$																								
CC-621																								
shf-1-253																								
imp-5 mt$^-$	+	+	−	+	+	+	+	−	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
imp-10																								
imp-11																								
imp-12																								
imp-8 mt^{+h}	+	+	−	+	+	+	+	+	−	+	+	+	+	+	−	−	−	−	−	+	−	−	−	−
imp-8 mt$^-$	+	+	−	+	+	+	+	−	−	+	+	+	+	−	−	−	−	−	−	+	−	−	−	−
fla-6	+	+	−	+	+	+	+	+	−	+	+	+	+	−	−	−	−	−	−	−	−	+	−	−
CC-1374																								
fus	+	+	−	−	+	+	+	+	−	+	+	+	+	+	−	−	−	−	+	−	−	−	−	−
CC-85																								
CC-350	+	+	−	+	+	+	+	+	−	+	+	+	+	−	−	−	−	−	+	−	−	−	−	−
CC-421	+	+	−	+	+	+	+	+	−	+	+	+	−	−	−	−	−	−	+	−	−	−	−	−
ac-29a mt$^-$	+	+	−	+	+	+	+	+	−	+	+	+	+	−	−	−	−	−	−	−	−	−	+	−
ac-29 mt$^+$																								
ac-212																								
arg-9-1																								
bs-37																								
fa-1	+	+	−	+	+	+	+	+	−	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
imp-2																								
imp-6																								
imp-9																								
mbo-1.4D																								
thi-10																								
CC-410																								
CC-1418	+	+	−	+	+	−	+	−	−	+	+	+	−	−	−	−	−	−	−	−	−	−	−	+
CC-1871																								

This table shows which *Gulliver*-generated junction fragments (indicated by their letter names) are present (+) or absent (−) in the listed strains. See Table 1 for complete descriptions of the strains' genotypes. Immediately below each letter is the size, in kilobases, of the left and right *Hin*dIII junction fragments diagnostic of that *Gulliver* insertion. The junction fragments present in *C. smithii* are not tabulated.

[a] Identification complicated by a comigrating band which has no apparent left counterpart.

[b] The right junction fragment could not be identified, presumably because it is comigrating with another fragment.

[c] The identity of *S* was also demonstrated using the unique size of its internal *Eco*RI/*Hin*dIII fragment.

[d] Present/absent in only a subpopulation of cells (see text).

[e] CC-125 from the GOODENOUGH laboratory is designated CC-125A; from the *Chlamydomonas* Stock Center, CC-125B.

[f] Left *Hin*dIII fragment has changed to 4.8 kb (see text).

[g] Faint left junction fragment at 6.2 kb.

[h] Two faint left junction fragments at 5.2 and 4.7 kb; two faint right junction fragments at 11 and 28 kb.

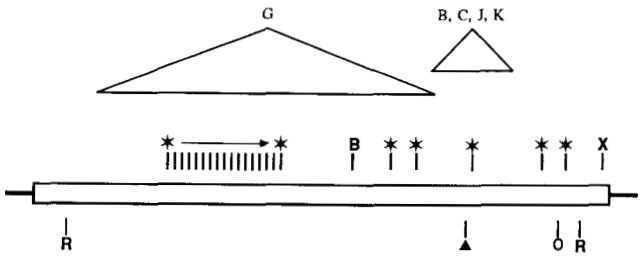

FIGURE 4.—Internal deletions. A restriction map of the presumed full length *Gulliver* is pictured, above which are shown the approximate locations of the internal deletions in the copy at *G*, and at *B*, *C*, *J* and *K*.

arising in the strains during routine subculturing over the years. To date, I have not identified any mutations caused by insertion of *Gulliver*.

With the goal of finding a *Gulliver*-induced mutation, and looking for possible dysgenic effects, I also screened for spontaneous mutants among the mass progeny of a CC-125 × CC-1952 mating. *Pf* (paralyzed flagella) and *bald* (flagella-less) mutants were sought because many genes can mutate to give these phenotypes and the traits are easily scored: nonmotile mutants produce small colonies when grown on soft agar (WARR *et al.* 1966). Nine *pf/bald* mutants were isolated from among the CC-125 × CC-1952 progeny, and two more from a control cross of CC-125 × CC-124. All 11 mutants were screened for the presence of new *Gulliver* insertions; only one of the mutants (one of the two from the control cross) had a new copy, and this proved to be unlinked to the new *pf* mutation. These results, and the infrequent identification of new junction fragments in progeny of CC-1952 crosses (Figure 2), suggest that crossing *Gulliver* into the transposon-lacking CC-1952 strain does not result in a spectacular rise in transposition frequency as observed with the *P* element in *Drosophila* crosses (KIDWELL 1986), although a modest increase cannot be ruled out.

Cloning the transposons: To characterize *Gulliver* in more detail, several copies, with their flanking DNA, were cloned and restriction mapped using the strategies outlined in MATERIALS AND METHODS. Figure 3 presents the results. At each genomic location *Gulliver* has a similar (or identical) restriction map, while the maps of the flanking DNA are, as expected, completely different. The sizes of the *Hin*dIII junction fragments at both ends of the transposons at *C*, *G*, *I*, *J*, *K*, *M*, and *N*, as determined from these restriction maps, are consistent with the sizes determined by genetic analysis (Figures 1 and 2 and Table 2). The transposons at *I*, *M*, *N* and *O* have the longest (and identical) unique sequence regions, and may represent full-length transposons. The other transposons are shorter, as summarized in Figure 4. Copies at *B*, *C*, *J*, and *K* are missing a 1.7-kb region (including an *Xho*I and a *Hin*dIII site) near the right end and *G* contains only 4 kb of transposon sequence, which explains why the G_L fragment is relatively faint on southern blots (see Figure 1A and 2A). Other defective copies of *Gulliver* may exist but remain unidentified because they are missing the parts of the transposon that have been used as probes.

Unstable sequences in the transposon: As shown in Figure 3, most copies of *Gulliver* carry a cluster of *Hin*dIII sites. Digestion of this region of the transposon with *Hin*dIII produces only two fragments, of 180 and 200 bp, implying that these *Hin*dIII sites are regularly spaced and comprise a repetitious sequence, a sequence restricted to *Gulliver* and not found elsewhere in the genome. Not surprisingly, this sequence occasionally undergoes size changes in *E. coli*, presumably as the consequence of recombination within misaligned sequences (data not shown).

The sequence to the left of the *Hin*dIII cluster also appears to be repetitious in that its length can also change during growth of the phage in *E. coli*: when clones of *Gulliver* and its flanking DNA are digested with *Hin*dIII and examined after electrophoresis and ethidium bromide staining, a faint ladder of regularly spaced bands usually surrounds the left junction fragment. To ensure that the cloned copies of *Gulliver* accurately represent the copies in the Chlamydomonas genome, the sizes of the *Hin*dIII junction fragments in the phage were compared with those in the genome on adjacent lanes of a southern blot (data not shown). In the restriction maps of Figure 3, the sizes of the *Hin*dIII junction fragments are those ascertained for the Chlamydomonas genome.

Since the left-end sequences of *Gulliver* can rearrange in *E. coli*, it was important to establish that similar rearrangements in Chlamydomonas were not generating size changes in the left *Hin*dIII junction fragments that could be misinterpreted as simultaneous excision/transposition events. Described below is the one example of such a rearrangement encountered in the course of this work, which illustrates that rearrangement can in fact be readily distinguished from true excision/transposition.

DNA was prepared from two independent cultures of CC-125, one derived from the Goodenough lab stock, the other from a stock more recently provided by the *Chlamydomonas* Culture Collection at Duke University; the two stocks had been propagated separately for at least three years. Three differences between the two DNA samples were noted using probe *a* (see Figure 1A): CC-125 from the Goodenough lab (called CC-125A in Figure 1 and Table 2) contains a new transposon copy (*P*), while the sample from the *Chlamydomonas* Culture Collection (CC-125B) is missing the 5.4 kb J_L fragment and has a new fragment at 4.8 kb. When CC-125B was analyzed with probe *b*, however, the J_R fragment was still present (Figure 1B),

suggesting that *Gulliver* at *J* had not been excised and that the new 4.8 kb fragment might be J_L, changed in size by unequal crossing-over. This was confirmed by showing that non-*Gulliver* DNA flanking *J* hybridizes only to the 5.4-kb J_L fragment in CC-125A and hybridizes only to the 4.8-kb fragment in CC-125B. Hence, although loss of one fragment and simultaneous appearance of a novel fragment can result from recombination within the presumed repetitive domain at the left end of *Gulliver*, such an event can be detected by ascertaining whether or not the left and right junctions of a particular transposon have disappeared simultaneously.

Identifying the donor elements of new transposon insertions: Most copies of *Gulliver* can be distinguished from one another by minor differences in their restriction maps: some contain internal deletions, some have *Xba*I sites to the left of the *Hin*dIII cluster, and some differ in the length of the *Eco*RI/*Hin*dIII restriction fragment near the left end (corresponding to probe *d* in Figure 3). Using these criteria, all the cloned transposon copies are distinguishable, with the following exceptions: *I* is identical to *M*; *N* to *O*; and *B* and *C* to *K*. Significantly, each of these three groups includes a transposon copy that has only recently transposed: *Gulliver* is present at *I*, *O* and *C* in only one strain, but is present at *M*, *N*, *B*, and *K* in most strains. Therefore, the likely explanation for this identity is that the new transposon copies derived from their "standard" counterparts by transposition.

Since all copies of *Gulliver* have not been cloned, it was possible that the new transposon copies were also identical to some of the uncloned copies and might have derived from them instead. To investigate this possibility, DNA from progeny of various crosses with CC-1952 was digested with *Hin*dIII and *Eco*RI, blotted to nitrocellulose, and hybridized with probe *d* to see if all copies of *Gulliver* could be distinguished based on the size of this internal *Hin*dIII/*Eco*RI fragment (data not shown). The transposons at *A* through *O* all proved to have a *Hin*dIII/*Eco*RI fragment of a different size except at *I* and *M*, and *O* and *N*, as already mentioned, and at *B*, *C*, *J* and *K*. This latter group presumably all derive from one another since they share a 3.3 kb *Hin*dIII/*Eco*RI fragment and have the same 1.7-kb internal deletion. (*J* is slightly different, however, having only two *Xba*I sites to the left of the *Hin*dIII site cluster rather than three.) The simplest interpretation of these results, then, is that *Gulliver* at *I* could only have been derived from *M*, *O* only from *N*, and *C* from either *B* or *K*.

The transposon copy at *C* appears to have derived from *K*, not *B*. DNA was prepared from *mbo-1.1A* on two occasions about 9 months apart (Figure 1A). The later time point was shortly after the strain was used in the cross with CC-1952, whose progeny are shown in Figure 2. During the 9-month period, the C_L and I_L fragments were found to have become more pronounced, and the K_L fragment less so, suggesting that the *mbo-1.1A* strain originally contained a small subpopulation of cells in which two new transposons (*C* and *I*) had appeared (not necessarily simultaneously) and one (*K*) had excised. During the nine months in culture, this subpopulation came to dominate the stock. Thus, the four gametes that gave rise to the four tetrads analyzed from the *mbo-1.1A* × CC-1952 cross all had *Gulliver* at *C* and *I* but no longer at *K*.

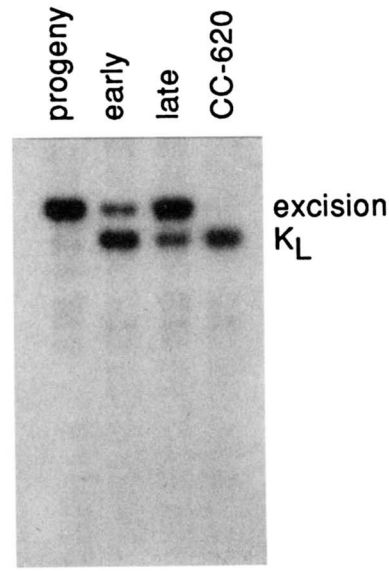

FIGURE 5.—Excision of the *Gulliver* at *K* in *mbo-1.1A*. DNA was prepared from *mbo-1.1A* on two occasions, the second (late) 9 months after the first (early). A Southern blot was prepared from *Sma*I/*Eco*RI-digested DNA and hybridized with a probe flanking the left end of *Gulliver* at *K*. This probe hybridizes to two fragments in the early and late *mbo-1.1A* lanes—a 2.4-kb fragment that represents the left junction at *K* (K_L) and a fragment of 2.7 kb (designated *excision*) containing the flanking genomic sequence but lacking the transposon component of K_L because *Gulliver* has excised from *K*. Strain CC-620 contains *Gulliver* at *K* and exhibits the K_L fragment; the left-most lane shows a progeny strain from the cross *mbo-1.1A* × CC-1952 that has inherited the excision fragment from the *mbo-1.1A* parent. The fraction of cells containing the excision product has increased over the nine month period, presumably due to stochastic processes. The lanes are not strictly quantitative; hybridization intensities should only be compared within each lane.

To confirm the presence of a mixed population of cells within the strain, *mbo-1.1A* DNA was hybridized with probes from sequences flanking the transposons at *C*, *I* and *K*. The *C* and *I* flanking probes each hybridize to two bands, the C_L or I_L fragment and a fragment which represents DNA lacking the transposon—the "empty target site" (data not shown). The *K* flanking probe hybridizes to the K_L junction fragment as well as to a fragment corresponding to this region of the genome after *Gulliver* excised from *K* (Figure 5). The increasing intensity of the C_L fragment (Figure 1A) parallels the increasing intensity of the fragment

representing excision from *K* (Figure 5). Since *Gulliver* at *C* must derive from either *K* or *B*, I suggest that the simultaneous appearance at *C* and disappearance from *K*, occurring in a similar fraction of the cells, is best explained as the result of a single event in which the *Gulliver* at *K* was excised and inserted at *C*.

Sequencing the transposon termini: In order to analyze transposition events in more detail, six copies of *Gulliver* were selected for closer analysis. Three (those at *O*, *C* and *I*) were chosen because they are present in only one strain; as argued above, these presumably represent relatively recent transposition events. The empty target site was isolated for each, and a comparison of the restriction maps before and after these three transposon copies inserted (Figure 3) demonstrates that Gulliver indeed inserted into the preexisting DNA sequence. The other three (at *N*, *K* and *M*) were chosen because they are present in most strains, but missing in one. These presumably represent excision events, and the DNA remaining after excision was isolated from those strains (see MATERIALS AND METHODS). Comparison of the restriction maps before and after these transposons excised (Figure 3) indicates that, at this resolution, excision cleanly removes the transposon, leaving no sequences behind. (The only exception to this is the *Bam*HI site just to the right of *N* which is not present after excision. As documented below, this site is eliminated because it is immediately adjacent to the transposon.) Finally, the region of the mt^- locus corresponding to the location of the mt^+-linked *Gulliver* at *N* was isolated since it should also represent an empty target site.

Having isolated the six transposon copies and the corresponding empty target sites and excision points, the details of transposition and excision at the DNA sequence level could be investigated. For all six, the restriction fragments spanning the two transposon junctions and the fragment containing the excision/insertion breakpoint were subcloned into pUC13 and at least 80 bp of DNA sequence was determined, on both strands, spanning each end of the six transposon copies, and covering the four target sites and the three excision points. In addition, the sequence of roughly 250 bases was determined at both ends of *Gulliver* at *N*. These data are shown in Figure 6, where for each transposon copy, line 1 gives the genomic sequence before insertion, line 2 the sequence after insertion, and, where relevant, line 3 indicates the sequence after excision.

Comparing the sequence of the termini from all six transposon copies allows for an unambiguous identification of the exact ends of *Gulliver* (shown in capital letters in Figure 6). All six transposon copies are flanked by an eight base pair direct repeat (boxed in Figure 6). From the sequence of the target sites for the three recently inserted transposon copies (at *C*, *I* and *O*) and for the target site of *N* from the mt^- locus, it is clear that the eight base pair repeats represent target site duplications, since these sequences occur only once in the target DNA (Figure 6, lines 1). A target sequence is unavailable for transposon copies at *K* and *M*, but the eight base pair duplications flanking them likely resulted from their original transposition (Figure 6, lines 1).

The final entry in Figure 6 gives 250 bases of sequence from each end of *Gulliver* at *N*. No extensive homologies are evident, and since there is no visible cross-hybridization of the ends on southern blots, it appears that the transposon does not contain terminal direct repeats as are found in viral-like retroposons. The 15 bp at either end of the transposon form an inverted repeat (indicated by arrows in Figure 6) containing two mismatches. Terminal inverted repeats are found in most transposons (DÖRING and STARLINGER 1986).

The sequences present after *Gulliver* excised from sites *N*, *K* and *M* indicate that excision of the transposon is not perfect (Figure 6, lines 3). The transposon sequences are completely removed, but both copies of the target duplication remain. In each case a sequence change has occurred in one or both of the bases at the junction of the duplicated target sites (Figure 6, lines 3, underlined).

DISCUSSION

Within a chromosomal walk of sequences linked to mt^+ on chromosome *VI* of Chlamydomonas, I encountered a 12-kb sequence that has the characteristics of a transposable element, which I have named *Gulliver*. The sequence is multicopy and scattered throughout the genome, rather than clustered at a single locus as a simple repetitive sequence might be. Differences in its locations among strains suggested that the element is capable of moving. Using probes representing the two ends of the transposon to analyze progeny from crosses, I showed that the sequence exists as a conserved unit at most locations. Molecular cloning of several copies confirmed that the 12-kb element is present at each location as a discrete structure. DNA sequence analysis of the termini of four insertions and their corresponding target sites demonstrated that transposition creates an 8-bp target site duplication and the transposon termini form a 15-bp inverted repeat containing two mismatches (Figure 7). It is intriguing that the terminal 7 bp of *Gulliver* ($CAGGG^G/_CT$) resembles that of the maize transposon *Ac* ($^C/_TAGGGAT$), and that both elements create an 8-bp target site duplication upon insertion (POHLMAN, FEDEROFF and MESSING 1984).

Excision of viral-like retroposons usually leaves behind one copy of the direct repeat present at the ends

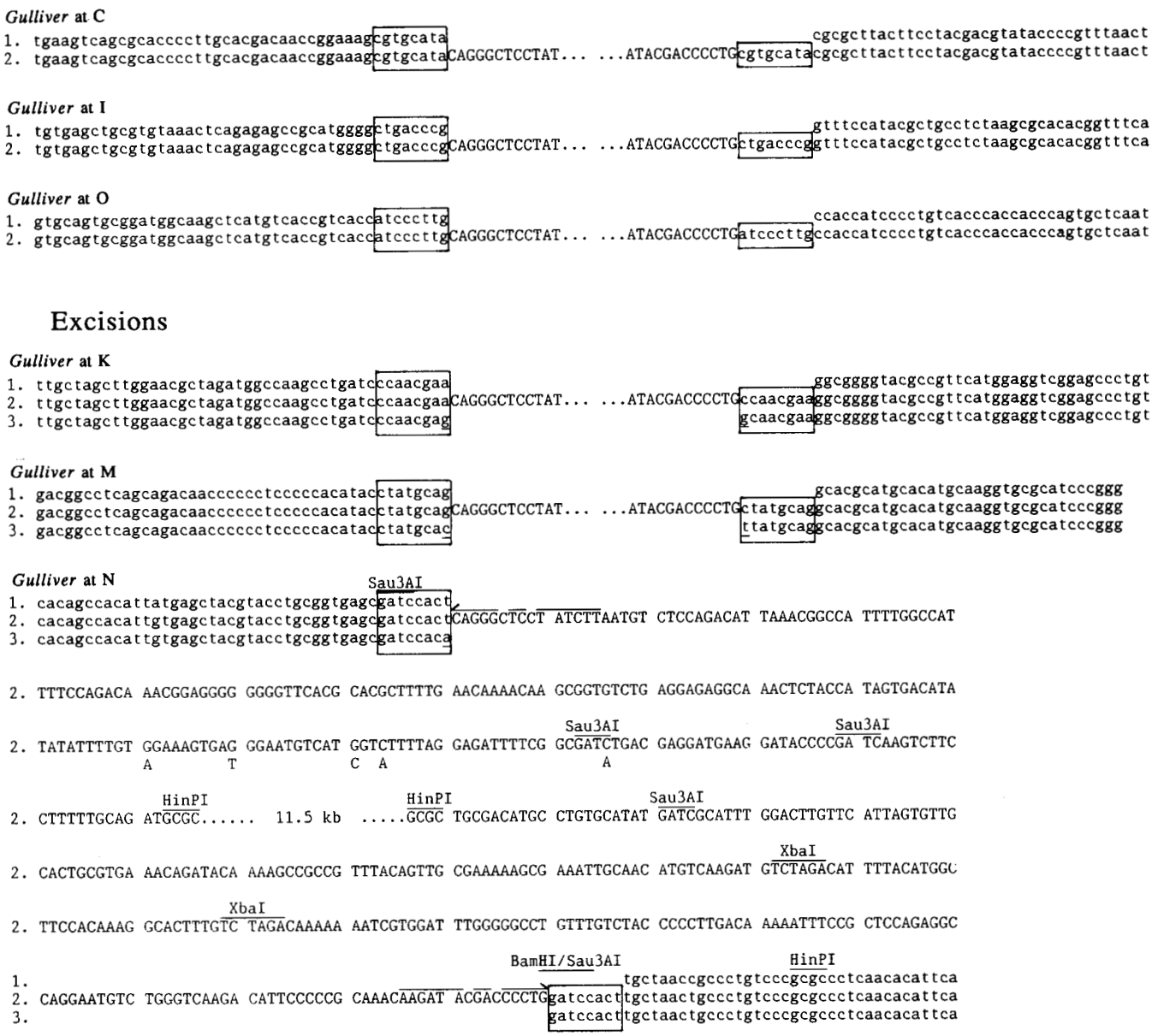

FIGURE 6.—Sequence analysis. The DNA sequence of the left and right transposon junctions for six copies of *Gulliver* is shown in line 2. The sequence of *Gulliver* is shown in capital letters; flanking DNA in lower case. Line 1 is the sequence present in the genome before the transposon inserted. The 8 bp target site duplication present at each end of the transposon and once in the target DNA is boxed. For *K* and *M* this target sequence is merely postulated based on the sequence in line 2. The sequence present after excision from *K*, *M*, and *N* is shown in line 3. The base changes seen in the duplicated target site after excision are underlined. Roughly 250 bp of sequence were determined at each end of the transposon at *N*. Restriction sites are indicated only for this sequence. The 15-bp terminal inverted repeat is overlined. Five base changes were noted, relative to the sequence at *N*, in the first 200 bp at the left end of *M*. These changes are indicated below line 2. Within the limited extent sequenced (50–100 bp at each end), no other differences were observed among the transposons or the flanking DNA except for the region of mt^- corresponding to the mt^+ linked copy (*N*, line 1), which has two changes in the region pictured.

(ROEDER and FINK 1983; DAY *et al.* 1988), whereas excision of DNA-mediated transposons generally results in complete removal of the transposon sequences. Occasionally, the original target sequence is restored, as seen for *P* element (TSUBOTA and SCHEDL 1986) and *Tc1* excisions (RUAN and EMMONS 1987), but often insertions or deletions are created. In higher plant transposable elements, like *Ac* and *Spm*, excision of the transposon generally leaves both copies of the target site duplication, although these are altered—one or a few bases have changed, or been deleted, at the junction between the duplications (SAEDLER and NEVERS 1985; DENNIS *et al.* 1986). The three excisions of *Gulliver* resemble those in higher plants—both copies of the target site duplication remain, and base pair changes occur where the duplications meet. Un-

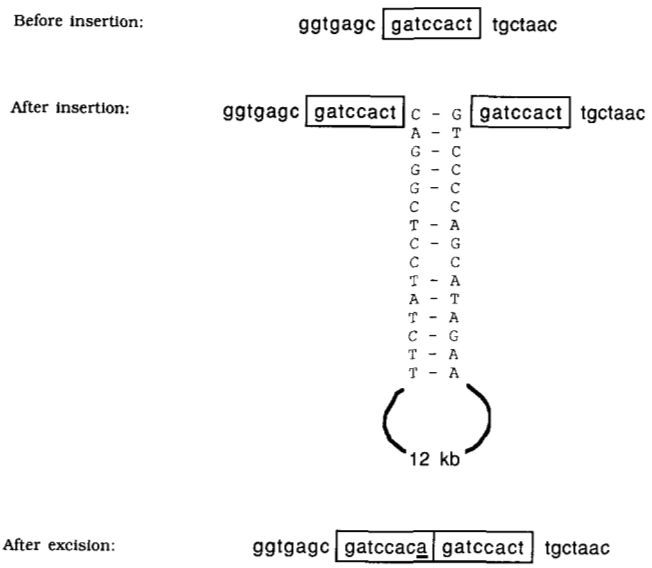

FIGURE 7.—Insertion and excision of *Gulliver*. Using the sequence at *N* as an example, this figure shows that the insertion of *Gulliver* into a new location creates an 8-bp target site duplication (boxed). Subsequent excision completely removes the transposon, leaving both copies of the duplicated target site. One or both of the bases at the junction between the duplications may be altered (underlined). Only the terminal 15 bp of the transposon are indicated (upper case), drawn to show the inverted repeat.

like the higher plant examples, no deletions within the target site duplication have been observed with *Gulliver* and changes only occur in the two bases at the junction of the duplicated target sites. Obviously, if excision of *Gulliver* always leaves an extra 8 bp, mutations caused by insertion of *Gulliver* into the coding region of a gene will not revert upon excision because of the resulting frameshift.

Discovery that the transposon was absent from the CC-1952 strain, a natural isolate distinct from the standard *C. reinhardtii* laboratory strains, made genetic analysis of *Gulliver* practical. The genetic data indicate that the majority of the transposons are unlinked and therefore would be useful additions to the RFLP markers already mapped in *Chlamydomonas* (RANUM *et al.* 1988). At present the locations of only three of the transposons are known—*Gulliver* at *N* and *O* are both in the mt^+ locus on linkage group VI, and *J* is on the same arm of linkage group VI, just centromere-proximal of *arg-9* (LOPPES and HEINDRICKS 1986), which is roughly 10 cM from the centromere (P. J. FERRIS, unpublished data).

Comparison of the transposons present in various *C. reinhardtii* strains (Table 2) suggests that transposition may be relatively infrequent. Chlamydomonas strains are cultured continuously (they do not survive in frozen storage), so many of these strains have been growing independently for hundreds to thousands of generations, yet there are only minor differences in their transposon complements: for example, *thi-10*, *ac-29* and *imp-2* have the same copies of *Gulliver*, even though they have been cultured separately for more than 20 yr. Nevertheless, new transpositions are detected in a few strains, and occasionally in the progeny from crosses. Faint bands are sometimes seen on Southern blots using the junction probes, indicating that a subpopulation of cells within a stock has acquired a copy of *Gulliver* at a new location. Therefore, transposition is occurring, if slowly.

For *Gulliver* to be a useful tool for mutagenesis, a faster rate of transposition is necessary. A variety of approaches have been used in other organisms to manipulate the rate of transposition. Some of these methods exploit the transposon's own control mechanisms. For example, when *P* element-bearing *Drosophila* males are crossed with females lacking the transposon (M strains), transposition occurs at a high frequency (KIDWELL 1986). Crossing *Gulliver*-containing *C. reinhardtii* strains to CC-1952, however, does not seem to increase greatly the transposition frequency in this way, as shown by the screening of such progeny, including spontaneous *pf* and *bald* mutations, for the presence of new *Gulliver* insertions. Nonetheless, there may be regulators of transposon copy number that produce more modest effects. Transposition of the maize *Ac* element, for example, decreases with increasing active copy number, although *Spm* shows no such effect and *Dt* has the opposite effect (FEDEROFF 1983). Whether transposition frequency of *Gulliver* is influenced by growth conditions, stage of the life cycle (levels of *Ty* promoted RNA are reduced in MAT**a**/MATα diploids relative to haploids—ERREDE, COMPANY and HUTCHISON 1987) or DNA methylation (CHOMET, WESSLER and DELLAPORTA 1987) remains for future investigation. It has been possible in the *Tc1* system to increase germ-line transposition frequency by mutation (COLLINS, SAARI and ANDERSON 1987), and in both the *P* element and *Ty* systems, transposase function has been put under the control of strong inducible promoters (STELLER and PIRROTTA 1986; BOEKE *et al.* 1985). Investigating similar approaches for *Gulliver* is complicated by the fact that, at present, we have no mutations whose reversion could be used to assay transposition frequency. Since an excision by *Gulliver* may always leave a nonrevertible frameshift, a suitable mutant might require insertion of *Gulliver* into an intron or promoter instead of the coding sequence.

The pattern of transposon copies inserted in different *Chlamydomonas* strains can sometimes indicate how they are interrelated. For example, a number of strains in Table 2 are missing *Gulliver* at *H*. This copy probably excised in the wild-type strain CC-621, since most other strains missing *H* are either mutants derived from CC-621 (*shf-1*, *imp-10/11/12*), or progeny from crosses to CC-621 (*imp-5* mt^-, *imp-7* mt^-, *imp-8* mt^-). A number of strains held in the *Chlamydomonas*

Culture Collection are described as separate natural isolates (CC-410, -1374, -1418, -1871). Three of these strains (CC-410, -1418, -1871) have the same complement of transposon copies, including the novel transposon *X* (Table 2; E. ORR, unpublished data). The transposon complement also clearly resembles that seen in the *C. reinhardtii* laboratory strains. In addition, the chloroplast DNA restriction maps of these strains all resemble *C. reinhardtii* (E. HARRIS, personal communication). Therefore, these three strains, at least as they exist in the *Chlamydomonas* Culture Collection, are actually the same strain, and related to all the other *C. reinhardtii* strains. CC-1374 contains the same set of transposon copies as *fus* and CC-85, including the novel *Gulliver* at *S*, and has chloroplast DNA resembling *C. reinhardtii*. Therefore, CC-1374 and the other four strains with *Gulliver* at *S* are probably related.

Five of the cloned transposon copies contain internal deletions (Figure 4). The one at *G* has lost about 8 kb, while those at *B*, *C*, *J* and *K* have all lost 1.7 kb. The occurrence of internally deleted members of a transposon family has been observed among *P* elements (O'HARE and RUBIN 1983), for *Ac* (some *Ds* elements are internally deleted copies of *Ac*—POHLMAN, FEDEROFF and MESSING 1984), and for *Spm* (DÖRING and STARLINGER 1986). These deleted elements are no longer able to transpose autonomously; however, if a full-length element is present in the genome to provide necessary functions in *trans*, these deleted elements can often be mobilized. Since *Ds1*, which has little more homology to *Ac* than the inverted repeats, can be mobilized (SUTTON *et al.* 1984), this is perhaps not too surprising. Whether or not the five deleted copies of *Gulliver* can function autonomously is unknown; however, since the copy at *K* moved recently to *C*, and since those at *B*, *C*, *J* and *K* are probably all derived from the one element that first suffered the 1.7-kb deletion, it appears that the short deletion, at least, has not destroyed the transposon's ability to move.

Genetic evidence from the *Ac/Ds* and the *Spm* systems in maize suggests that transposition of these elements is nonreplicative—that is, when an element transposes, it is removed from its original location and inserted into a new site (FEDEROFF 1983; GREENBLATT 1984). Moreover, this new site is often genetically linked to the original site. The genetic data pertaining to five recent transposition events (Table 3) indicate that *Gulliver* behaves similarly, as detailed below.

1. The *Gulliver* at *O* derives by transposition from *N*, and the strain containing the copy at *O* (CC-620) no longer possesses the copy at *N*. Either a later, separate event fortuitously excised the copy at *N* or, more likely, it was excised when it transposed to site *O*. Since both copies are located at the mt^+ locus, transposition was to a linked site. *N* and *O* are located at opposite ends of a small multigene family coding for a zygote-specific mRNA (class III, FERRIS and GOODENOUGH 1987). The exact size of this multigene family is uncertain, but I estimate that *N* and *O* are about 100 kb apart, with the *Gulliver* at *O* having inserted in inverted orientation relative to that at *N*.

TABLE 3

Linkage of new *Gulliver* insertions to their donor

Strain	Donor site	New site	Linkage	Excision of donor?
CC-620	N	O	Both at mt^+	Yes
mbo-1.1A	M	I	2-3 cM[a]	No
mbo-1.1A	K	C	20 cM[a]	Yes
CC-125A	B, J or K	P	None[b]	No
imp-1	D	R	10–20 cM[c]	No

[a] Based on four tetrads and 34 random progeny.
[b] Based on 25 random progeny.
[c] One recombinant among nine random progeny from *imp-1* × CC-1952.

2. The *Gulliver* at *I* arose from *M*, and they are also linked, some 2–3 cM apart. In this case, however, both the *M* and *I* copies are present in the *mbo-1.1A* strain.

3. The *Gulliver* at *C* apparently derives from *K*, and they are more loosely linked, 20 cM apart. Since only a subpopulation of the *mbo-1.1A* strain carried the *Gulliver* at *C* and a subpopulation was excised at *K*, both must have been recent events, and it seems likely that the insertion at *C* and loss from *K* was a single event.

4. The *Gulliver* at *P* is a new insertion in CC-125A. It has not been cloned, but the *Hin*dIII/*Eco*RI fragment at the left end of *P* is 3.3 kb (determined by analyzing several CC-125A × CC-1952 progeny containing different subsets of the transposon), implying that it derived from *B*, *J* or *K*. Since CC-125A contains the usual complement of transposons, including the *B*, *J*, and *K* copies, there is no evidence of a coupled excision event, and there is no obvious linkage of *P* to any of the other copies of *Gulliver* in CC-125A.

5. The *Gulliver* at *R*, a new insertion in *imp-1*, has also not been cloned. However, using the same analysis of *Hin*dIII/*Eco*RI-digested progeny DNA, *R* apparently derives from *D*, the only transposon to which *R* shows linkage.

Taken together, these examples document two cases in which transposition and excision were probably coupled, and three cases in which both the donor and the new transposon remain. The latter cases could be examples of replicative transposition. However, it is possible to explain the retention of both the donor and the new transposon even if transposition is nonreplicative. If one postulates that shortly after DNA replication the transposon copy on one chromatid moves (and is simultaneously excised) into a site that

has not yet replicated, then after mitosis one daughter cell will contain both the excision product and the new insertion, while the other will contain both the donor and the new transposon. Data from twin sectors on maize ears support such a model (FEDEROFF 1983; GREENBLATT 1984; CHEN, GREENBLATT and DELLAPORTA 1987), and the same mechanism could apply as well to *Gulliver*.

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