The Translocation-Associated *Tox1* Locus of *Cochliobolus heterostrophus* Is Two Genetic Elements on Two Different Chromosomes

M. Kodama,[1] M. S. Rose,[2] G. Yang, S. H. Yun, O. C. Yoder and B. G. Turgeon

Department of Plant Pathology, Cornell University, Ithaca, New York 14853

Manuscript received July 13, 1998
Accepted for publication October 30, 1998

ABSTRACT

Previously, *Tox1* was defined as a single genetic element controlling the difference between races of *Cochliobolus heterostrophus*: race T is highly virulent on T-cytoplasm corn and produces the polyketide T-toxin; race O is weakly virulent and does not produce T-toxin. Here we report that *Tox1* is two loci, *Tox1A* and *Tox1B*, on two different chromosomes. Evidence for two loci derives from: (1) the appearance of 25% Tox^+ progeny in crosses between induced $Tox1^-$ mutants, one defective at *Tox1A*, the other at *Tox1B*; (2) the ability of $Tox1A^- + Tox1B^-$ heterokaryons to complement for T-toxin production; and (3) electrophoretic karyotypes proving that $Tox1^-$ mutations are physically located on two different chromosomes. Data showing *Tox1* as a single genetic element are reconciled with those proving it is two loci by the fact that *Tox1* is inseparably linked to the breakpoints of a reciprocal translocation; the translocation results in a four-armed linkage group. In crosses where the translocation is heterozygous (*i.e.*, race T by race O), all markers linked to the four-armed intersection appear linked to each other; in crosses between induced $Tox1^-$ mutants, complications due to the translocation are eliminated and the two loci segregate independently.

TWO races of *Cochliobolus heterostrophus*, the cause of Southern Corn Leaf Blight, are known: race T produces a polyketide (T-toxin) that specifically affects corn containing Texas male sterile (T) cytoplasm and is highly virulent on T-cytoplasm corn, whereas race O does not produce T-toxin and lacks high virulence to T-cytoplasm corn (Yoder *et al.* 1997). When these two races are crossed to each other, only parental types appear in the progeny, usually in a 1:1 ratio (Yoder and Gracen 1975; Yoder 1976). Since *C. heterostrophus* is haploid, a 1:1 ratio is consistent with segregation of two alleles at a single locus, one specifying the race T phenotype and the other the race O phenotype. This genetically defined locus has been designated *Tox1* (Leach *et al.* 1982b). Although segregation ratios other than 1:1 have been observed in progeny of certain crosses between races O and T, all could be explained if *Tox1* were linked to an ascospore abortion factor encoded by a spore killer gene, as suggested earlier (Taga *et al.* 1985; Bronson *et al.* 1990). In a survey of the field population of *C. heterostrophus*, no loci other than *Tox1* were found to control the phenotypic difference between races O and T (Bronson *et al.* 1990).

Despite the simple inheritance pattern, certain observations have suggested that *Tox1* is not a typical Mendelian element. Comparison of nonviable ascospore frequencies in crosses homozygous *vs.* heterozygous at *Tox1* led to the proposal that races T and O differ by a reciprocal translocation (Figure 1A), the breakpoints of which are genetically inseparable from *Tox1* (Bronson 1988). Subsequently, in the course of constructing a genetic map of *C. heterostrophus*, a four-armed linkage group diagnostic of a reciprocal translocation was identified (Tzeng *et al.* 1992), with *Tox1* located at the intersection (Figure 1B). This linkage group consists of a pair (6 and 12) of race O chromosomes and a pair (6;12 and 12;6) of race T chromosomes that are reciprocally translocated with respect to the race O pair; the chromosomal nomenclature for *C. heterostrophus*, defined previously (Tzeng *et al.* 1992), is based on sizes of chromosomes, numbered sequentially (largest to smallest), in field isolate Hm540 (Figure 2). Significantly, the sizes of translocated chromosomes 6;12 and 12;6 in race T sum to ~1.2 Mb more DNA than the sizes of chromosomes 6 and 12 in near-isogenic race O (Figure 2), suggesting a large insertion associated with the translocation in race T (Yoder *et al.* 1994; Turgeon *et al.* 1995; Chang and Bronson 1996). Because the strains used for the analysis were highly inbred, but heterozygous at *Tox1*, the inserted DNA must be located at or near *Tox1*. Although the large insertion could not be traced in the field population of *C. heterostrophus* because of abundant

This report is dedicated to the memory of Dr. Ge Yang, our friend and colleague, whose creativity established the intellectual framework for this study.

Corresponding author: B. G. Turgeon, Department of Plant Pathology, 334 Plant Science Bldg., Cornell University, Ithaca, NY 14853. E-mail: bgt1@cornell.edu

[1] *Present address:* Laboratory of Plant Pathology, Faculty of Agriculture, Tottori University, 680 Tottori, Japan.

[2] *Present address:* Novartis Seeds, Seeds Biotechnology Research Unit, 3054 Cornwallis Rd., Research Triangle Park, Raleigh, NC 27709.

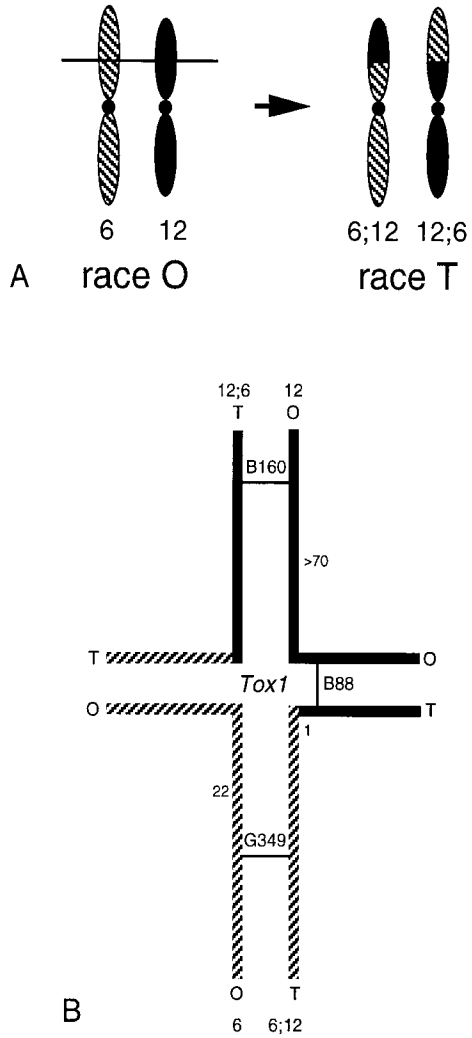

Figure 1.—(A) A reciprocal translocation involving chromosomes 6 and 12 of race O created a unique pair of chromosomes in race T, called 6;12 and 12;6 (the first digit indicates the race O chromosome contributing the larger portion of the translocated chromosome; Tzeng *et al.* 1992). (B) The four-armed linkage group resulting from the translocation described in A. Linkage data show that *Tox1* is inseparable from the translocation breakpoints, but do not reveal whether *Tox1* is positioned at one, the other, or both breakpoints. In crosses heterozygous at *Tox1* (represented here), all markers that are linked to the breakpoints on any of the four chromosomes will appear linked to each other. Positions of RFLP markers B160, B88, and G349 are shown; each uniquely identifies one translocated and one normal sequence chromosome. Numbers near lines indicate map distances in cM (Tzeng *et al.* 1992); the distance from B160 to the breakpoint has been recently recalculated (Tasma and Bronson 1998) and the distance from B88 to the breakpoint could be 8 cM (see note in Table 3, footnote *e*). T, race T; O, race O.

length polymorphisms involving chromosomes in addition to 6, 12, 6;12, and 12;6, it is clear that the reciprocal translocation associated with *Tox1* is a characteristic difference between races T and O in nature (Chang and Bronson 1996).

As an entrée into molecular analysis of *Tox1*, we generated a series of mutants deficient in production of T-toxin. Some of these were induced by chemical mutagenesis (Yang *et al.* 1994), whereas others were insertional mutants in which the *Tox1* locus was tagged with a gene (*hygB*) for resistance to hygromycin B (Lu *et al.* 1994; Rose *et al.* 1996). In this article we describe the genetic and physical mapping of several *Tox*$^-$ mutations, which has led us to the surprising conclusion that *Tox1* is really two loci on two different chromosomes.

MATERIALS AND METHODS

Fungal strains, growth conditions, and mating: *C. heterostrophus* strains used in this study are listed in Table 1. Fungal growth media included complete (CM), minimal (MM), and CMX, which is CM with xylose instead of glucose (Tzeng *et al.* 1992). Growth conditions and storage of *C. heterostrophus* have been described (Turgeon *et al.* 1985; Yoder 1988), as have procedures for mating (Leach *et al.* 1982a).

Preparation of chromosomal DNA: Protoplasts were prepared by growing and digesting mycelium as described earlier (Yoder 1988), except that harvested mycelium was washed with 50 mm EDTA pH 8.0 prior to protoplasting in enzyme-osmoticum (0.7 m NaCl, 10 mg Driselase/ml, 3 mg Novozyme/ml, 0.1 mg chitinase/ml). Protoplasts were washed three times in 1.2 m sorbitol in 50 mm EDTA and resuspended in the same solution at a final concentration of 2–8 $\times$ 10^8/ml. Low-melting-temperature agarose (1.6% w/v, Fisher Scientific, Pittsburgh) at 42–45° was mixed 1:1 with protoplasts (final concentration, 1–4 $\times$ 10^8/ml in 0.8% agarose) and transferred to plug molds (BioRad, Richmond, CA). Plugs (containing protoplasts) were incubated in 1% (w/v) N-lauroyl sarcosine (Sigma, St. Louis), 0.2% (w/v) proteinase K (Sigma), and 0.5 m EDTA in 10 mm Tris (pH 9.0) for 24 hr at 37° and then washed in 50 mm EDTA three times and stored in the same solution at 4°.

CHEF gel electrophoresis: Chromosomal DNAs were separated by contour-clamped homogeneous electric field (CHEF) electrophoresis (Chu *et al.* 1986) using a CBS apparatus with 0.8% agarose gels (pulsed-field certified agarose, Bio-Rad) in constantly circulating 0.5$\times$ Tris-borate EDTA (TBE) buffer (Sambrook *et al.* 1989) at 14°; buffer was replaced every 48 hr during extended runs. Five different running conditions (voltage, switching time, run time) were used: (1) 150 V, 1 min, 36 hr; (2) 150 V, 50 sec, 24 hr; (3) 100 V, 8 min, 18 hr; 7.5 min, 18 hr; 7 min, 28 hr; 6.5 min, 22 hr; 6 min, 22 hr; (4) 60 V, 30 min, 24 hr; 25 min, 24 hr; 20 min, 24 hr; 15 min, 24 hr; 10 min, 20 hr; 8 min, 20 hr; 6 min, 20 hr; 4 min, 10 hr; (5) 60 V, 30 min, 25 hr; 25 min, 30 hr; 20 min, 35 hr; 15 min, 30 hr; 10 min, 30 hr; 7 min, 15 hr. Conditions 1 and 2 were used to check chromosome integrity, condition 3 to separate chromosomes in the middle-sized range (2.0–2.6 Mb), and conditions 4 and 5 to separate the largest chromosomes (2.5–3.7 Mb). Gels were stained with 0.5 μg ethidium bromide/ml and photographed under UV light. Size standards were chromosome preparations (Bio-Rad) from *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Hansenula wingei*.

DNA-DNA hybridization: Capillary transfer of DNA from gels to Nytran Plus membranes (Schleicher and Schuell, Keene, NH) was done according to the manufacturer's instructions. DNA probes (Table 2) were labeled with α-[^{32}P]dCTP using standard random priming techniques (Feinberg and Vogelstein 1983; Sambrook *et al.* 1989). Hybridization conditions have been described (Turgeon *et al.* 1993).

Heterokaryon formation: Strains carrying the auxotrophic

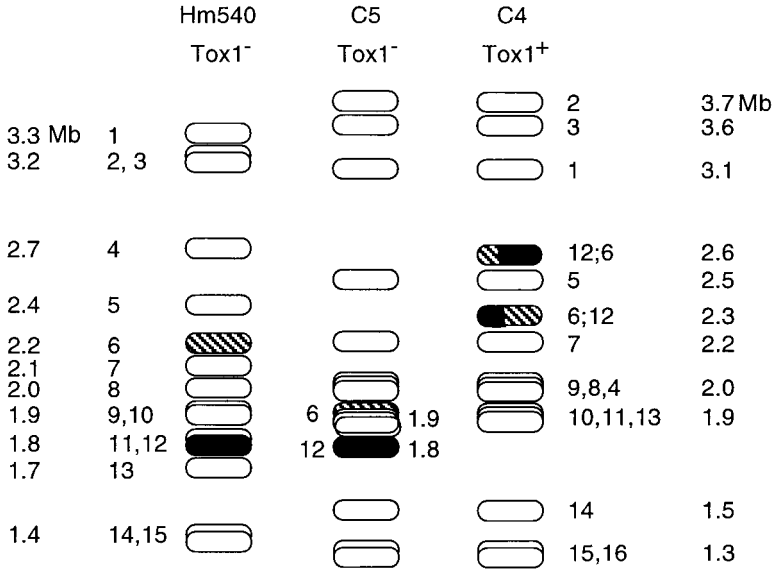

Figure 2.—Diagram of electrophoretically separated *C. heterostrophus* chromosomes, derived from published (Tzeng *et al.* 1992; Chang and Bronson 1996) observations and from data presented in this report. The karyotype of field isolate Hm540 was chosen (Tzeng *et al.* 1992) as the reference for numbering the chromosomes, the largest being chromosome 1. Hm540 displays many length polymorphisms with respect to laboratory strains C4 (race T) and C5 (race O), a pair that was bred for near-isogenicity except for heterozygosity at *Tox1* and *MAT* (mating type) and therefore has similar karyotypes; Hm540 was not among the progenitors of C4 and C5 (Leach *et al.* 1982a). Note that C5 chromosomes 6 (1.9 kb) and 12 (1.8 kb) are missing in C4, and that C4 chromosomes 12;6 (2.6 kb) and 6;12 (2.3 kb) are missing in C5 and are translocated with respect to their counterparts in C5. The sum of the sizes of chromosomes 12;6 and 6;12 is ~1.2 Mb more than that of chromosomes 6 and 12, indicating extra DNA in race T that appears, because of the near-isogenicity of the genomes, to map at *Tox1* (Chang and Bronson 1996). Chromosome 16 in the lab strains is not found in Hm540.

markers *ade1* and *met2*, denoting requirements for adenine and methionine, respectively, were crossed to each mutant and recombinant *Tox1*$^-$ auxotrophic progeny were used to form heterokaryons. Blocks of CM bearing mycelium and conidia of strains with different auxotrophic markers were paired on MM at 23° as described previously (Leach and Yoder 1982). Heterokaryons were transferred to fresh MM at 30° and tested for T-toxin production (Ciuffetti *et al.* 1992).

TABLE 1

***C. heterostrophus* strains used in this study**

Strain[a]	Genotype[b]	Characteristics	Reference
C4	*Tox1*$^+$;*MAT-2*	Wild-type race T	Leach *et al.* (1982a)
C5	*Tox1*$^-$;*MAT-1*	Wild-type race O	Leach *et al.* (1982a)
C9	*Tox1*$^+$;*MAT-1*	Wild-type race T	Lu *et al.* (1994)
ctm45[c]	*Tox1A*$^-$;*MAT-2*;*hygB*	Chemically induced mutation at *Tox1A*	Yang *et al.* (1994)
R.C4.186	*Tox1A*$^-$;*MAT-2*;*hygB*	Insertional mutation at *Tox1A*	Lu *et al.* (1994)
R.C4.350L	*Tox1A*$^-$;*MAT-2*;*hygB*	Insertional mutation at *Tox1A*	Lu *et al.* (1994)
C4.PKS.13	*Tox1B*$^-$;*MAT-2*;*hygB*	Insertional mutation at *Tox1B*	Rose *et al.* (1996)
C4.510B	*Tox1*$^+$;*MAT-2*;*hygB*	Insertion at B88, a *Tox1B*-linked marker	This study
1151-3-1	*Tox1A*$^-$;*MAT-1*	Progeny of ctm45 × C9	This study
420R14	*Tox1*$^+$;*met2*;*MAT-1*	Source of *met2*	Leach and Yoder (1982)
422R27	*Tox1*$^+$;*ade1*;*MAT-1*	Source of *ade1*	Leach and Yoder (1982)
421R17	*Tox1*$^-$;*met2*;*MAT-1*	Source of *met2*	Leach and Yoder (1982)
422R30	*Tox1*$^-$;*ade1*;*MAT-1*	Source of *ade1*	Leach and Yoder (1982)
1213R65	*Tox1B*$^-$;*MAT-1*;*hygB*	Progeny of C9 × C4.PKS.13	This study
1258R9	*Tox1A*$^-$;*met2*	Progeny of 420R14 × ctm45	This study
1259R12	*Tox1A*$^-$;*ade1*	Progeny of 422R27 × ctm45	This study
1260R2	*Tox1A*$^-$;*ade1*;*MAT-2*;*hygB*	Progeny of 422R27 × R.C4.186	This study
1260R6	*Tox1A*$^-$;*ade1*;*MAT-1*;*hygB*	Progeny of 422R27 × R.C4.186	This study
1262-1-1	*Tox1A*$^-$;*met2*;*hygB*	Progeny of 420R14 × R.C4.186	This study
1261-2-1	*Tox1B*$^-$;*ade1*;*hygB*	Progeny of 1260R2 × 1213R65	This study
1263R4	*Tox1B*$^-$;*met2*;*hygB*	Progeny of 420R14 × C4.PKS.13	This study
1264-1-1	*Tox1A*$^-$;*ade1*;*hygB*	Progeny of 422R27 × R.C4.350L	This study
1265R5	*Tox1A*$^-$;*met2*;*hygB*	Progeny of 420R14 × R.C4.350L	This study

[a] Progenitors of all auxotrophic mutants and *Tox1*$^-$ mutants were near-isogenic strains C2 (*Tox1*$^+$;*MAT-2*;*alb1*) and C4, respectively.

[b] *Tox1*, locus controlling race and T-toxin production; *MAT*, locus controlling mating type; *hygB*, *E. coli* gene for resistance to hygromycin B; *ade1*, mutation for adenine auxotrophy; *met2*, mutation for methionine auxotrophy.

[c] In ctm45, *hygB* is unlinked to *Tox1*; in all other insertional mutants, *hygB* maps at (or near in C4.510B) *Tox1*.

TABLE 2

Probes used in this study

Name	Characteristic	Reference
pUCATPH	REMI transformation vector	Lu *et al.* (1994)
pT15-1	Vector inserted at *Tox1B* in C4.PKS.13	Rose (1996)
B264	Chromosome 2-specific	Tzeng *et al.* (1992)
G172	Chromosome 3-specific	Tzeng *et al.* (1992)
G38	Chromosome 1-specific	Tzeng *et al.* (1992)
B160	Chromosome 12;6-specific,[a] Figure 1B	Tzeng *et al.* (1992)
G214	Chromosome 5-specific	Tzeng *et al.* (1992)
B88	Chromosome 6;12-specific,[a] Figure 1B	Tzeng *et al.* (1992)
G349	Chromosome 6;12-specific,[b] Figure 1B	Tzeng *et al.* (1992)
G144	Chromosome 7-specific	Tzeng *et al.* (1992)
0.7-kb fragment	0.7-kb *Bam*HI/*Hin*dIII fragment flanking the vector insertion point in R.C4.186; unique to chromosome 2 in wild-type Tox^+	Yang (1995)
1.3-kb fragment	1.3-kb *Bam*HI/*Hin*dIII fragment contiguous with the 0.7-kb fragment in wild-type Tox^+	Yang (1995)

[a] Chromosome 12-specific in race O (Figure 1B).
[b] Chromosome 6-specific in race O (Figure 1B).

T-toxin assay: For uniform cell overlay in the microbial bioassay for T-toxin (Ciuffetti *et al.* 1992; Yang *et al.* 1994), the toxin-sensitive bacterial suspension (~5 ml) was poured onto LB plates containing 50 μg ampicillin/ml, the plates were tilted to distribute the suspension uniformly over the entire agar surface, excess suspension was poured off, and the plates were air-dried in a laminar flow hood prior to inoculation with fungus.

RESULTS

***Tox1* is two loci: genetic analyses:** *Linkage among $Tox1^-$ mutations:* We reported previously that the restriction enzyme-mediated integration (REMI)-induced tagged mutations in Tox^- strains R.C4.186 and R.C4.350L both map at the *Tox1* locus (Lu *et al.* 1994) and that the chemically induced nontagged mutation in Tox^- strain ctm45 also maps at *Tox1* (Yang *et al.* 1994); all three of these mutations are inseparably linked to each other (Lu *et al.* 1994). Similar genetic analyses (Table 3) were performed with a recently acquired Tox^- mutant, C4.PKS.13, which also carries the *hygB* marker (in pT15-1, Table 2; Rose 1996). When this mutant was crossed to either race T strain C4 or race O strain C5, only parental types appeared in the progeny, indicating a single mutation that mapped at *Tox1* and was tagged with *hygB* (Table 3). However, when crossed with R.C4.186, R.C4.350L, or ctm45, ~25% of the progeny were Tox^+, implying that the Tox^- mutation in C4.PKS.13 is not linked to the other $Tox1^-$ mutations. Crosses to $Tox1^+$ strain C4.510B (Table 3) showed that the $Tox1^-$ mutation in C4.PKS.13 is linked to the B88 RFLP marker, which maps near *Tox1* on chromosome 6;12 in wild-type $Tox1^+$ strains (Tzeng *et al.* 1992).

Heterokaryon analysis: To further examine the nonallelic nature of mutations at *Tox1*, heterokaryons were formed between all possible pairs of auxotrophic T-toxin-deficient mutants (Table 1) and then examined for T-toxin production (Figure 3, Table 4). The amount of T-toxin produced by race T/race T heterokaryons was the same as that produced by race T/race O heterokaryons. Race O/race O heterokaryons did not produce T-toxin. All heterokaryons formed between race T and the four induced $Tox1^-$ mutants produced T-toxin, in amounts comparable to the race T/race T heterokaryons. None of the heterokaryons formed between race O and the four induced $Tox1^-$ mutants produced detectable T-toxin. This result makes it seem unlikely that the race O nucleus has functional genes necessary to complement mutations in the race T nucleus that cause a Tox^- phenotype.

Heterokaryons formed between two pairs of $Tox1^-$ mutants (ctm45/C4.PKS.13 and R.C4.350L/C4.PKS.13) produced halos about the same size as the halo of the control heterokaryon formed by two wild-type race T auxotrophs, demonstrating that the defects in ctm45 and R.C4.350L can be complemented by products of the C4.PKS13 nucleus and the defect in C4.PKS.13 can be complemented by products of either the ctm45 or R.C4.350L nucleus. This is consistent with the hypothesis that the C4.PKS.13 mutation is at a locus different from that of the ctm45 and R.C4.350L mutations. Heterokaryons formed between another pair of mutants (R.C4.186/C4.PKS.13) produced only a small amount of T-toxin, evident as a tiny halo in the *E. coli* assay (Figure 3, Table 4). Other heterokaryons formed between induced mutant pairs produced no detectable T-toxin. Results remained unchanged when auxotrophic markers were reversed in the strains used to make heterokaryons.

Lack of a difference in sizes of halos produced by race

TABLE 3

Genetic analysis reveals the *Tox*$^-$ mutation in C4.PKS.13 maps at *Tox1* but is not linked to other *Tox1*$^-$ mutations

	Progeny phenotype (no.)[a]				
	Tox$^-$		Tox$^+$		
Tester strain[b]	hygB^R	hygB^S	hygB^R	hygB^S	Interpretation: C4.PKS.13 mutation is
C5 (*Tox1*$^-$)[c]	49	43	0	0	At *Tox1*
C4 (*Tox1*$^+$)[c]	96	0	0	83	Tagged with *hygB*
R.C4.186 (*Tox1*$^-$)[d]	43	0	0	19	Unlinked to a known *Tox1* mutation
R.C4.350L (*Tox1*$^-$)[d]	100	0	0	23	Unlinked to a known *Tox1* mutation
ctm45 (*Tox1*$^-$)[d]	34	26	0	14	Unlinked to a known *Tox1* mutation
C4.510B (*Tox1*$^+$)[e]	35	0	37	6	Linked to a chromosome 6;12 marker near *Tox1* (Figure 1B)

[a] Progeny segregations are consistent with ratios of 1:1 (the first two crosses), 3:1 (the next two crosses), 2:1:1 (the next cross), and 1:1 with 8% recombination (the last cross).

[b] Each tester strain (Table 1) was crossed to mutant C4.PKS.13 (*Tox*$^-$;*hygB*^{R};*MAT-2*) or its ascospore progeny strain 1213R65 (*Tox*$^-$;*hygB*^{R};*MAT-1*).

[c] Near-isogenic wild-type testers, Table 1.

[d] Each of these testers (Table 1) lacks T-toxin-producing ability because of a single mutation shown previously to map at *Tox1*. The first two are *hygB*-tagged REMI mutants (Lu *et al.* 1994); the third, nontagged chemically induced mutant ctm45 (Yang *et al.* 1994), was represented by its ascospore progeny 1151-3-1 (*Tox1*$^-$;*MAT-1*).

[e] Strain C4 carrying *hygB* inserted at the RFLP marker B88 (Table 1), which maps near *Tox1* on translocated chromosome 6;12 (Tzeng *et al.* 1992), was crossed to 1213R65. When C4.510B (*Tox1*$^+$;*hygB*) was crossed to ctm45 (strain 1151-3-1, *Tox 1*$^-$), progeny segregated 74 Tox$^-$ (half were hygB^R): 75 Tox$^+$ (half were hygB^R), indicating no linkage between the *Tox1*$^-$ mutation in ctm45 and the *hygB*-tagged B88 marker in C4.510B. Note that the recombination frequency (8%) between B88 and *Tox1* appears greater than that observed (1%) by Tzeng *et al.* (1992). The difference is probably due to translocation heterozygosity (progeny were from a race O × race T cross), which could reduce recovery of recombinant progeny, in the former study *vs.* isosequential chromosomes (progeny were from a cross between two *Tox*$^-$ mutants that shared the C4 nucleus) in the cross shown here.

T/race O and race T/race T heterokaryons suggests that the race O nucleus does not encode enzymes for metabolism of T-toxin. However, the data in Table 4 and Figure 3 represent a single time point and do not rule out differences in T-toxin accumulation at other points during the production time course. Thus, T-toxin in culture was quantified over a 25-day incubation period (Figure 4). At most points up to the plateau of the curve, T-toxin production by heterokaryons was less than wild type, probably because heterokaryons grow on MM at a slightly slower rate than wild type. Differences in T-toxin production among heterokaryons could not be detected with this assay, even though race T/race O heterokaryons are expected (Leach *et al.* 1982b) to have only half as many nuclei carrying *Tox* genes as race T/race T heterokaryons.

***Tox1* is two loci: physical analysis:** Chromosomes of race T strain C4, race O strain C5, and three *Tox1*$^-$ mutants were separated by CHEF gel electrophoresis under conditions that resolve large-to-intermediate-size fragments. The karyotypes of C4 and the three mutants derived from C4 appeared identical, with two exceptions (Figure 5A): (1) Chromosome 6;12 (open arrowhead, lane 13) of mutant C4.PKS.13 migrated slightly faster than the corresponding chromosome in C4 (lane C4), suggesting a deletion, and (2) two novel chromosomes were evident in mutant R.C4.186 (lane 186, arrow and asterisk); moreover, the largest band, which contains C4 chromosomes 2 and 3 (Figure 2), was fainter in the mutant. No karyotype variability was observed between C4 (lane C4) and R.C4.350L (lane 350).

To identify the tagged chromosome in each *Tox1*$^-$ mutant and thus physically locate the *Tox1*$^-$ mutations, the gel was blotted and probed with labeled transformation vector. The hybridizing chromosome was a different size in each mutant (Figure 5B). To determine which chromosomes hybridized, two RFLP probes, B88 and B160 (Table 2; Tzeng *et al.* 1992), were used; B88 is specific to chromosome 12 in race O and 6;12 in race T and B160 is specific to chromosome 12 in race O and 12;6 in race T (Figure 1B). Probe B88 identified chromosome 12 in C5 (Figure 5C, lane C5) and chromosome 6;12 in C4 (Figure 5C, lane C4), and hybridized to a chromosome of the same size as 6;12 in R.C4.350L and R.C4.186 (Figure 5C, lanes 350, 186). The vector did not hybridize to chromosome 6;12 in these mutants. In C4.PKS.13, B88 hybridized to a slightly smaller chromosome (Figure 5C, lane 13, open arrowhead), which also hybridized to the vector probe (Figure 5B, lane 13, open arrowhead). Thus, the insertional mutation in

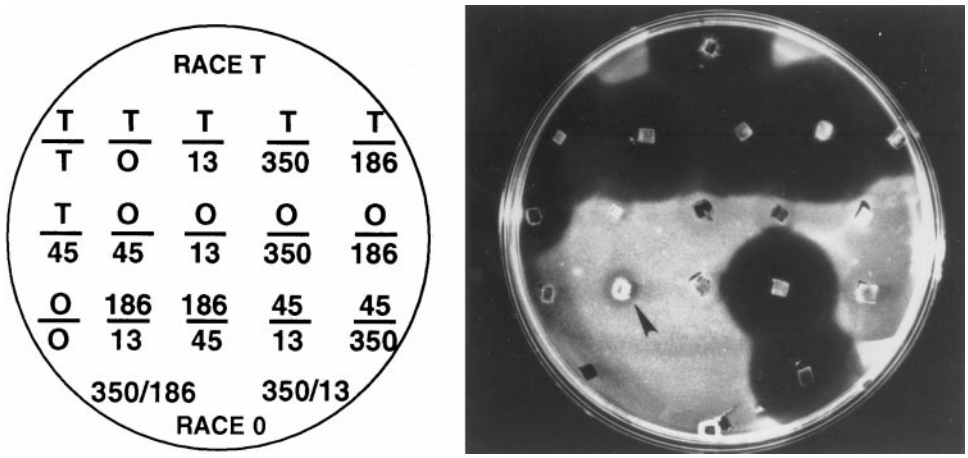

Figure 3.—T-toxin production by *C. heterostrophus* heterokaryons (for strain descriptions, see Tables 1 and 4). Left: diagram of the assay plate on the right, showing the identity of each heterokaryon, and the wild-type controls (race T, top; race O, bottom). T, wild-type race T progenitor strain C4 (*Tox1A*$^+$;*Tox1B*$^+$); O, wild-type race O strain C5 (*Tox1A*$^-$;*Tox1B*$^-$);13, C4.PKS.13 (*Tox1A*$^+$; *Tox1B*$^-$);350, R.C4.350L (*Tox1A*$^-$;*Tox1B*$^+$);186, R.C4.186 (*Tox1A*$^-$; *Tox1B*$^+$); 45, ctm45 (*Tox1A*$^-$;*Tox1B*$^+$). Right: assay plate distinguishing T-toxin production (inocula with halos) from no production (inocula without halos). Note that all heterokaryon halos are approximately the same size as the halo of the wild-type race T control, except for the R.C4.186/C4.PKS.13 heterokaryon, which produced, in repeated assays, a distinct but unusually small halo (arrowhead).

C4.PKS.13 is in chromosome 6;12; however, this chromosome appears to have sustained a deletion. Probe B160 identified chromosome 12 in C5 (Figure 5D, lane C5) and chromosome 12;6 in C4 (Figure 5D, lane C4) and hybridized to a chromosome of the same size in C4.PKS.13 and R.C4.350L (Figure 5D, lanes 13 and 350). In R.C4.350L, this chromosome also hybridized to the vector (Figure 5B, lane 350) indicating that the *Tox1*$^-$ mutation in R.C4.350L is in chromosome 12;6. In R.C4.186, the vector hybridized to the smaller of the two new bands (Figure 5B, lane 186, asterisk), indicating the location of the *Tox1*$^-$ mutation, while chromosome 12;6-specific probe B160 hybridized to the larger of the two new chromosomes, showing that chromosome 12;6 is out of place in this mutant and therefore likely rearranged (Figure 5D, arrow). These hybridization data, along with the genetic analyses described above, prove that *Tox1* is two loci on two different chromosomes: the locus on chromosome 12;6 is called *Tox1A* and the one on chromosome 6;12 is called *Tox1B*.

Chromosome rearrangements at *Tox1A* and *Tox1B*: Since chromosomes carrying *Tox1* in mutants C4.PKS.13 (*Tox1B*$^-$) and R.C4.186 (*Tox1A*$^-$) appear rearranged (Figure 5), they were examined further using additional chromosome-specific probes. Previous gels (Figure 5A) showed that the largest chromosomal band in R.C4.186 was faint, suggesting absence of a chromosome normally found in this band; RFLP probes B264 and G172, which are specific to chromosomes 2 and 3, respectively, were used to determine if either of these chromosomes was rearranged. The largest band in C4, C5, and C4.PKS.13 hybridized to chromosome 2-specific probe B264 (Fig-

TABLE 4

T-toxin production by heterokaryons

	ade1 strain					
met2 strain	race T (422R27)	race O (422R30)	ctm45 (1259R12)	R.C4.186 (1260R6)	R.C4.350L (1264-1-1)	C4.PKS.13 (1261-2-1)
race T (420R14)	+	+	+	+	+	+
race O (421R17)	+	−	−	−	−	−
ctm45 (1258R9)	+	−	−	−	−	+
R.C4.186 (1262-1-1)	+	−	−	−	−	+/−
R.C4.350L (1265R5)	+	−	−	−	−	+
C4.PKS.13 (1263R4)	+	−	+	+/−	+	−

Each *met2* strain was paired on MM with each *ade1* strain (parentheses indicate strain numbers used; Table 1). T-toxin production was tested with the microbial assay (Figure 3). +, T-toxin (halo, the size expected for wild-type race T); −, no T-toxin (no halo); +/−, a small amount of T-toxin, evident as a small halo (Figure 3).

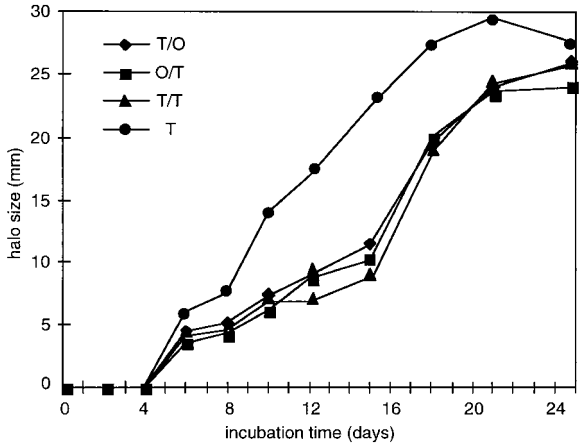

Figure 4.—Time course of T-toxin production by *C. heterostrophus* heterokaryons and wild-type race T. Strains were grown on MM agar for varying numbers of days (incubation time) and transferred (4-mm agar cylinders bearing mycelium) to a lawn of T-toxin-sensitive *E. coli* cells (Ciuffetti *et al.* 1992) and incubated at 30°. Diameters of halos (Figure 3) surrounding inocula (indicating T-toxin production) were measured after 24 hr; each point represents the mean of five replicate halo measurements. Heterokaryons are described in Table 4; T, race T; O, race O.

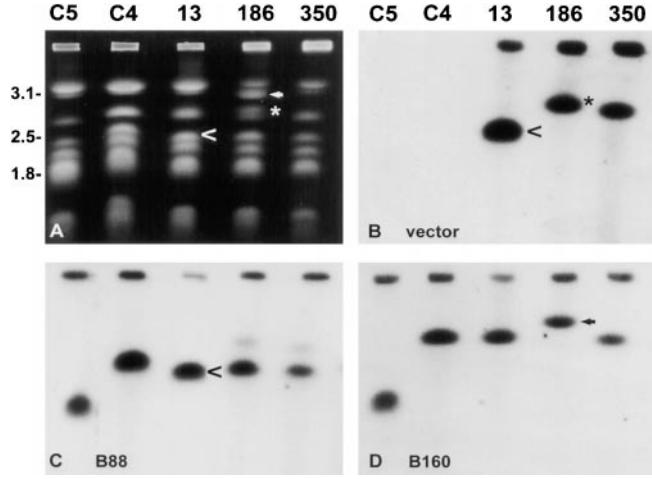

Figure 5.—Electrophoretic karyotypes and identification of wild-type and mutant *C. heterostrophus* chromosomes. In each panel, chromosomes in lanes are: C5, wild-type race O (Tox$^-$) strain C5; C4, wild-type race T (Tox$^+$) strain C4; 13, Tox$^-$ mutant strain C4.PKS.13; 186, Tox$^-$ mutant strain R.C4.186; 350, Tox$^-$ mutant strain R.C4.350L. Numbers at left of A indicate size standards in megabases. Bands at the top of each panel show positions of the wells. (A) Ethidium-bromide-stained gel showing separation of the larger *C. heterostrophus* chromosomes. The open arrowhead points to chromosome 6;12, which is smaller than normal in mutant C4.PKS.13 (lane 13); two novel chromosomes resulting from a translocation in mutant R.C4.186 are indicated by a solid arrow and an asterisk (lane 186). All chromosomes in mutant R.C4.350L (lane 350) appear identical to those in wild-type race T progenitor strain C4 (lane C4). Differences between the pair of translocated chromosomes (Figure 2) in races O (lane C5) and T (lane C4) are not apparent under the separation conditions used here. (B–D) Autoradiograms, after blotting, of the gel shown in A, probed with the transformation vector (B), chromosome 6;12-specific fragment B88 (C), or chromosome 12;6-specific fragment B160 (D). Conclusions are that the insertional *Tox*$^-$ mutation occurred in a different chromosome in C4.PKS.13 than in R.C4.186 or R.C4.350L (B), and that in R.C4.350L the vector (B, lane 350) and chromosome 12;6-specific probe B160 (D, lane 350) hybridized to the same chromosome; thus the mutation is in chromosome 12;6. In C4.PKS.13, the vector (B, lane 13) and chromosome 6;12-specific probe B88 (C, lane 13, open arrowhead) hybridized to the same chromosome; thus, the mutation is in chromosome 6;12. This chromosome, however, appears to run faster than its counterpart in wild-type strain C4 (lane C4) or in mutants R.C4.186 (lane 186) and R.C4.350L (lane 350), indicating it sustained a deletion. In R.C4.186, the vector hybridized to the smaller (B, lane 186, asterisk), and probe B160 hybridized to the larger (D, lane 186, arrow) of the two novel chromosomes. The size of chromosome 6;12 remained unchanged, as indicated by probe B88 (C, lane 186). Thus, the R.C4.186 mutation involves a rearranged form of chromosome 12;6.

ure 6, A and C); in R.C4.186 it hybridized to the larger of the two new chromosomes (Figure 6, A and C, lane 186, arrow). Chromosome 3-specific probe G172 hybridized to the largest band, which was the same size in all strains (Figure 6D). Combined use of these two probes indicates that chromosome 2, and not chromosome 3, is altered in R.C4.186.

Probes specific to chromosomes 5 (G214, Figure 6G), 1 (G38, Figure 6E) and 7 (G144, not shown) detected no polymorphisms, indicating that these chromosomes are normal in all the *Tox1*$^-$ mutants. Under these running conditions, the smaller size of chromosome 6;12 in C4.PKS.13, relative to C4 (Figure 5), was not obvious, as shown by the B88 probe (Figure 6H); however, under the separation conditions illustrated in Figure 5C (lanes C4 and 13) and Figure 7, A and B (lanes C4 and 13), the difference is clear.

Chromosome 12;6-specific probe B160 hybridized to the larger of the two new chromosomes in R.C4.186 (Figure 6, A and F, lane 186, arrow; see also Figure 5D, lane 186). Elsewhere, we determined that a 0.7-kb *Hin*dIII/*Bam*HI DNA fragment (unique to chromosome 2 in wild type) flanks the plasmid insertion point in R.C4.186 (Yang 1995); this fragment hybridized to the largest band in C4 (Figure 6I, lane C4) and in C4.PKS.13 (Figure 6I, lane 13) and to the smaller of the two new chromosomes in R.C4.186, which also hybridized to the vector (Figure 6, B and I, lane 186, asterisks). A 1.3-kb *Bam*HI/*Hin*dIII fragment contiguous with the 0.7-kb fragment on chromosome 2 in wild-type C4 hybridized to the same chromosome as the 0.7-kb fragment in C4 and C4.PKS.13 (Figure 6, I and J) but to the larger of the new chromosomes in R.C4.186 (Figure 6J, lane 186, arrow). Thus, two lines of evidence indicate a reciprocal translocation between chromosomes 12;6 and 2 in R.C4.186. First, probes adjacent to

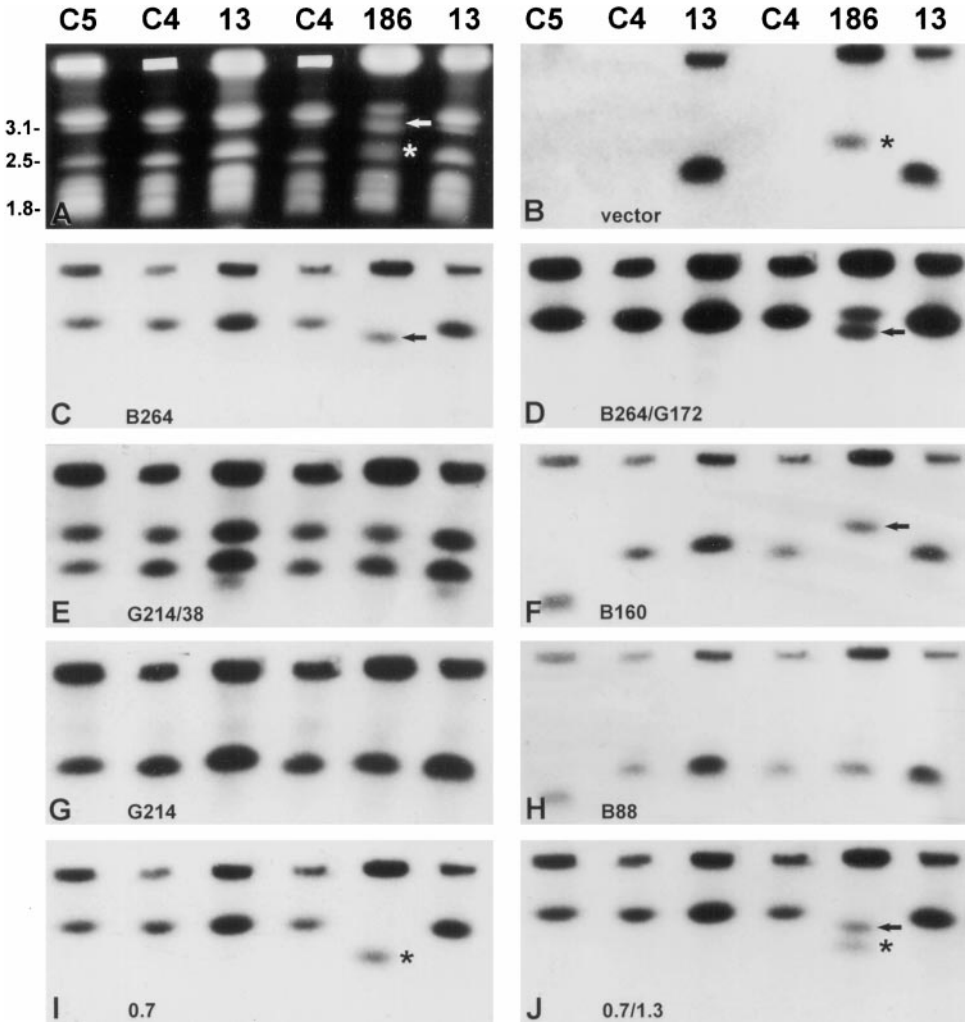

Figure 6.—Electrophoretic karyotype and analysis of rearranged chromosomes in *Tox1A*$^-$ mutant R.C4.186. Contents of lanes: C5, wild-type race O strain C5; C4, wild-type race T strain C4; 13, mutant C4.PKS.13; 186, mutant R.C4.186. Bands at the top of each panel show positions of the wells. (A) Ethidium-bromide-stained gel showing separation of intermediate to large size chromosomes. After blotting, the gel shown in panel A was probed with the transformation vector (panel B) or fragments specific to chromosomes 2 (B264, panel C), 3 (G172, panel D), 1 (G38, panel E), 12;6 (B160, panel F), 5 (G216, panel G), 6;12 (B88, panel H), 2 (0.7-kb fragment immediately flanking the R.C4.186 insertion point, panel I), and 2 (1.3-kb fragment contiguous in wild type with the 0.7-kb fragment, panel J). In D, E, and J, multiple bands appear in certain (or all) lanes because the blot was probed without first stripping the previous probe: in D, probe G172 was preceded by probe B264; in E, probe G38 was preceded by probe G214; in J, the 1.3-kb probe was preceded by the 0.7-kb probe. Probes specific for chromosomes 1, 3, and 5 show these chromosomes unchanged in all mutants. Chromosome 2-specific probe B264 indicates that this chromosome in R.C4.186 sustained a rearrangement (C, lane 186 arrow); chromosome 12;6-specific probe B160 hybridized to the same chromosome (F, lane 186 arrow) as B264, indicating a translocation between chromosomes 2 and 12;6. This is supported by the observation that the 0.7-kb probe (I, lane 186 asterisk) and the 1.3-kb probe (J, lane 186 arrow), which are adjacent to each other on chromosome 2 in wild-type strain C4, each hybridized to a different novel chromosome in R.C4.186, indicating that chromosome 2 broke between these two fragments. The vector probe (B, lane 186 asterisk) showed that the vector inserted in the smaller of the two novel (translocated) chromosomes in R.C4.186.

each other in wild type (the 0.7-kb and 1.3-kb fragments) are on different chromosomes (both novel) in R.C4.186. Second, probes on different chromosomes in wild type (B160 on chromosome 12;6 and B264 on chromosome 2) are on the same (novel) chromosome in R.C4.186 (Figure 6, C and F, lane 186, arrow).

C4.PKS.13 deletion size estimated by chromosome polymorphism: To investigate the difference in size of chromosome 6;12 in C4.PKS.13 compared with other strains, chromosomes of C5, C4, C4.PKS.13, and R.C4.186 were separated using running conditions appropriate for intermediate-sized fragments, and the gel was blotted and probed with the chromosome 6;12-specific marker B88. Hybridization with the B88 probe revealed, as noted in Figure 5, that chromosome 6;12 of C4.PKS.13 was smaller than its wild-type C4 counterpart (Figure 7A, lane 13, arrow). Probing with a second chromosome 6;12-specific probe, G349 (Figure 7B), located on the opposite side of the translocation breakpoint (Figure 1B), indicated the same difference in size. Comparisons with chromosome size standards (not shown) indicated a difference of ~100 kb. Figure 8 presents a graphic summary of electrophoretic karyotypes of wild-type and mutant *C. heterostrophus* strains.

DISCUSSION

Early genetic analyses established that the essential difference between races T and O of *C. heterostrophus* is controlled by *Tox1* (Leach *et al.* 1982b), an apparent single locus defined by 1:1 segregation of parental types in progeny of crosses between races T and O (Yoder and Gracen 1975; Yoder 1976). In this article we provide firm evidence, based on both genetic and physical

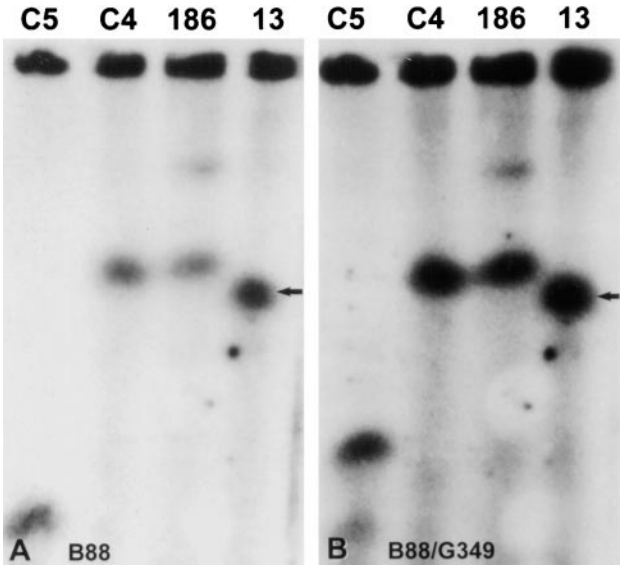

Figure 7.—Deletion size in mutant C4.PKS.13 estimated by chromosome polymorphism. A gel was run under conditions favoring separation of intermediate sized chromosomes, blotted, and probed, first with chromosome 6;12-specific RFLP marker B88 (A), followed (without stripping) by marker G349 (B); these markers are on opposite sides of the translocation breakpoint (Figure 1B). Because both are present in C4.PKS.13, the deletion occurred between them. By comparison with size standards, both probes showed that chromosome 6;12 in mutant C4.PKS.13 (lane 13, arrow) was ~100 kb smaller than in mutant R.C4.186 (lane 186) or in wild-type progenitor strain C4 (lane C4). Probe B88 detected chromosome 12 (A), and probe G349 detected chromosome 6 (B), in race O strain C5 (lane C5); both chromosomes can be seen in B (lane C5) because the blot was not stripped prior to probing with G349.

analyses, that *Tox1* is really two loci, unlinked to each other (on two different chromosomes), which we now define as *Tox1A* on race T chromosome 12;6 and *Tox1B* on race T chromosome 6;12. How could *Tox1* at first appear to be a single genetic element when it is in fact two unlinked loci? The answer lies in the association of *Tox1* with the breakpoints of a reciprocal translocation (Tzeng *et al.* 1992; Chang and Bronson 1996) and the peculiar genetics of translocations. As stated by Fincham *et al.* (1979), "in a cross heterozygous for a reciprocal translocation, genes which normally belong to two different linkage groups will behave as if tied together in a single four-armed linkage group, the arms meeting at the interchange point" (p. 154). This phenomenon is illustrated here (Figure 1) for the *C. heterostrophus Tox1* linkage group. In any cross between races T and O, the translocated chromosomes are heterozygous for the translocation and all markers, including *Tox1A* and *Tox1B*, linked to the breakpoints will segregate as if linked to each other. Prior to obtaining induced *Tox*$^-$ mutations in a race T genetic background, the only possible cross heterozygous at *Tox1* was between naturally occurring races T and O, and therefore all available genetic evidence indicated that *Tox1* was a single locus, because *Tox1A* and *Tox1B* always cosegregated due to the four-armed linkage group. When it became possible to cross pairs of induced *Tox*$^-$ mutants, one member of each pair defective at *Tox1A* (*e.g.*, R.C4.350L, R.C4.186, or ctm45) and the other defective at *Tox1B* (*e.g.*, C4.PKS.13), complications due to the translocation were eliminated and genes at the two loci segregated independently, thus revealing the lack of linkage between them. Note that there is as yet no physical evidence associating the translocation breakpoints directly with *Tox1* or with genes mapping at *Tox1*. However, the ~1.2 Mb of extra DNA in race T that is missing in race O is likely to reside at *Tox1* (Figure 8), because the extra DNA is evident even between members of a pair of strains, one race T and the other race O, that were bred for isogenicity except for heterozygosity at *Tox1* (Chang and Bronson 1996). The extra DNA is likely at both *Tox1A* and *Tox1B*, because genes at these two loci are unique to the genome of race T (Rose 1996).

Tagged mutations at *Tox1A* and *Tox1B* have led to the cloning of genes involved in T-toxin biosynthesis: *PKS1*, encoding a polyketide synthase (Yang *et al.* 1996), and a second gene with similarity to decarboxylases (Rose *et al.* 1996). Probing of electrophoretically separated chromosomes with either of these cloned genes indicates that *PKS1* is at *Tox1A* and the decarboxylase encoding gene is at *Tox1B* (Rose 1996). Targeted inactivation of either gene results in loss of ability to produce T-toxin and reduced virulence on T-cytoplasm corn. *PKS1* and *DEC1*, and a reductase-encoding gene (*RED1*) linked to *DEC1* but with no apparent role in T-toxin biosynthesis, are all found in the field population of race T and all are missing in the field population of race O, as determined by gel blot analysis of genomic DNAs from a world-wide collection of field isolates (Rose 1996). Since these genes are found only in the genome of race T, they are probably located in the "extra" DNA of race T.

Results of heterokaryon tests are consistent with those of progeny segregation analyses and with the observations of gel-separated chromosomes, *i.e*, mutants defective at *Tox1A* will complement the T-toxin deficiency in a *Tox1B* mutant and vice versa, whereas mutants defective at the same *Tox1* locus do not complement for T-toxin production. The outcome of these experiments is precisely what would be predicted if *Tox1* were two different loci. An unexpected result is the poor complementation between R.C4.186 (defective at *Tox1A*) and C4.PKS.13 (defective at *Tox1B*). Normal complementation was expected because *Tox1A* in C4.PKS.13 is fully functional, *i.e.*, C4.PKS.13 complements R.C4.350L and ctm45 to produce wild-type halos, and *Tox1B* in R.C4.186 is fully functional, *i.e.*, 25% of the progeny from the cross R.C4.186 × C4.PKS.13 are Tox$^+$ (Table 3). Thus, it is difficult to explain the R.C4.186/C4.PKS.13 result, because between them this pair of mutants contributes fully functional *Tox1A* and *Tox1B* loci

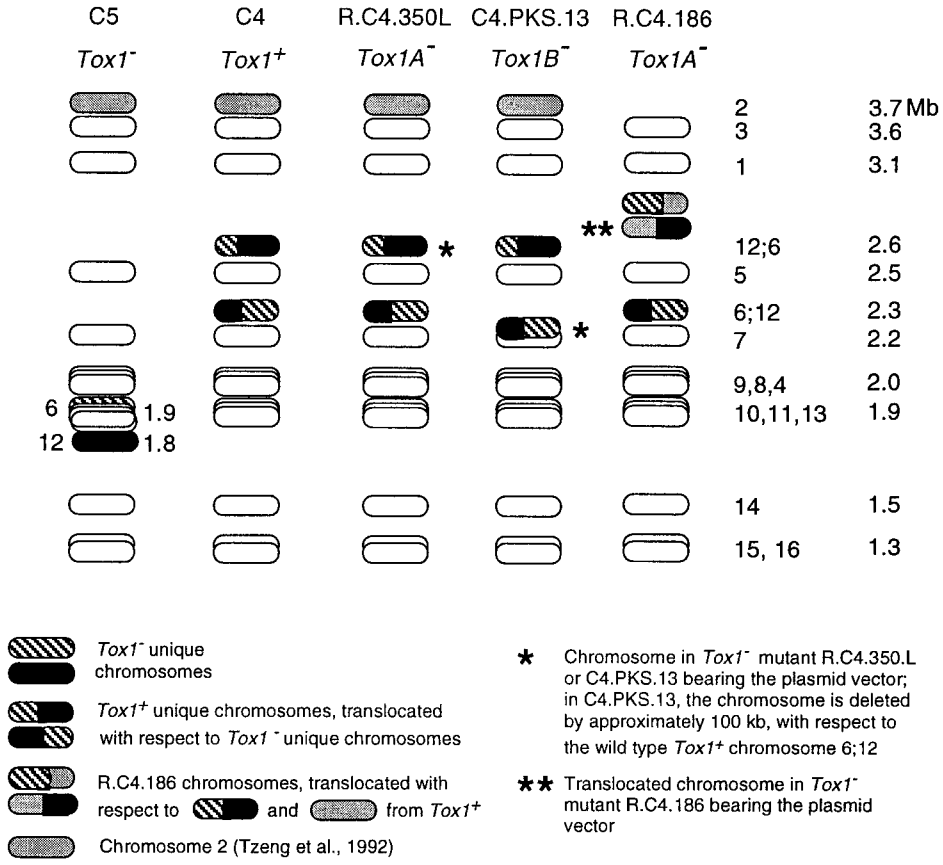

Figure 8.—Diagrammatic representation of chromosome polymorphisms in *Tox1*⁻ mutants *vs.* *Tox1*⁺ wild type. The karyotype of R.C4.350L appears normal because it sustained a simple insertion of the transformation vector (5.1 kb) in chromosome 12;6. In C4.PKS.13, one chromosome (6;12) carries the vector and is deleted for ~100 kb. In R.C4.186, three mutational events, all at the same locus, are evident: the vector inserted, a reciprocal translocation occurred between chromosomes 12;6 and 2 (causing loss of these two chromosomes and appearance of two new ones), and one (or both) of the translocated chromosomes suffered a deletion (Yang 1995). The amount of deleted DNA can be approximated by subtracting the sum of the sizes of the two novel chromosomes (~2.7 Mb + ~2.9 Mb = ~5.6 Mb) from the sum of the sizes of chromosomes 2 and 12;6 (3.7 Mb + 2.6 Mb = 6.3 Mb), *i.e.*, ~700 kb.

to the heterokaryon. One possibility is that the DNA deleted at *Tox1A* in R.C4.186 contains a gene required for regulation of genes at *Tox1B*. This hypothetical regulatory gene would be intact in C4.PKS.13, but in R.C4.186/C4.PKS.13 heterokaryons its product (a transcription factor?) would need to move to the R.C4.186 nucleus so that *Tox1B* genes could be induced; if movement were inefficient, expression of genes at *Tox1B* would be suboptimal and this could explain why little T-toxin is synthesized. In heterokaryons between C4.PKS.13 and either ctm45 or R.C4.350L (neither of which appears to be deleted at *Tox1A*), both component nuclei would encode the putative transcription factor, obviating the need for it to move from one nucleus to the other. Although the existence of a regulatory gene is speculative at this point, the idea is consistent with our observation that a full-length functional clone of *PKS1* does not complement T-toxin deficiency when transformed into R.C4.186, indicating additional genes at *Tox1A* required for T-toxin production (X. Zhu, B. G. Turgeon and O. C. Yoder, unpublished results). A host-specific toxin regulatory gene (*TOXE*) encoding a protein with a bZIP basic DNA-binding domain and four ankyrin repeats has been identified in *C. carbonum* race 1; the protein is required for toxin biosynthesis (Walton 1997).

Earlier heterokaryon analyses suggested that genes determining race T are dominant to their counterparts in race O (Leach *et al.* 1982b), an argument subject to several uncertainties, leaving open the possibility that, conversely, race O genes are dominant (Bronson 1991). At that time, the issue of dominance seemed important with respect to hypotheses regarding the T-toxin biosynthetic pathway, *i.e.*, dominance of genes for race O would be consistent with the idea that T-toxin is an intermediate in a biosynthetic pathway common to both race O and race T; in race T the pathway would be blocked so that T-toxin accumulates, whereas in race O the entire pathway would be functional and T-toxin would be metabolized to nontoxic products (Yoder 1980). Dominance relationships are now irrelevant to hypotheses regarding T-toxin biosynthesis, because *Tox* genes are unique to race T and have no alleles in race O (Rose 1996; Yang *et al.* 1996). However, the possibility remains that races T and O have genes in common encoding enzymes for metabolism of T-toxin. For example, *Bipolaris* (*Helminthosporium*) *sacchari*, which produces the host-specific HS-toxin, also produces low levels of an enzyme (β-galactofuranosidase) that in culture slowly degrades HS-toxin to nontoxic products (Livingston and Scheffer 1983). The toxin metabolism hypothesis for *C. heterostrophus*, however, is not supported by results of quantitative assays for T-toxin production by heterokaryons over time in culture (Figure 4), since no differences among heterokaryons were observed.

Evidence that the *Tox1* locus is complex, both geneti-

cally and physically, extends beyond the facts that (1) it is located on two different chromosomes and (2) the 1.2 Mb of "extra" DNA in race T maps at *Tox1*. The *Tox1* region appears to have an unusual amount of repetitive DNA, since half the probes mapping within 4 cM of *Tox1* are repetitive, in contrast to only ~4% repetitive probes in the remainder of the genome (Tzeng *et al.* 1992); moreover, DNA immediately flanking both *PKS1* and the decarboxylase-encoding gene (each of which is single copy in the genome) is repetitive and A+T-rich (Yang *et al.* 1996; M. S. Rose, O. C. Yoder and B. G. Turgeon, unpublished results). There is also a high density of RFLPs near *Tox1*; in construction of the *C. heterostrophus* map, it was found that the average distance between RFLPs over the entire genome was 75 cM, whereas near *Tox1* the distance was 1.5 cM (Tzeng *et al.* 1992). These features of *C. heterostrophus Tox1* are reminiscent of the *C. carbonum Tox2* locus, which contains a group of linked genes encoding proteins required for biosynthesis of the cyclic peptide pathogenicity factor HC-toxin; *Tox2* also carries a high density of RFLPs (Panaccione *et al.* 1992) and repetitive DNAs (Panaccione *et al.* 1996), but differs from *C. heterostrophus Tox1* in that genes at *Tox2* are multicopy (Walton 1996), while those found to date at *Tox1* are single copy (Rose 1996; Yang *et al.* 1996).

An additional level of complexity at *C. heterostrophus Tox1* is that both *Tox1A* and *Tox1B* appear to be gene clusters. This deduction is based on the observation (described above) that a full-length functional clone of *PKS1* will not complement T-toxin deficiency when transformed into mutant R.C4.186, which sustained a large *Tox1A* deletion (Figure 8), and the decarboxylase-encoding gene will not complement T-toxin deficiency when transformed into *Tox1B*-deletion mutant C4.PKS.13 (X. Zhu, B. G. Turgeon and O. C. Yoder, unpublished results). These results suggest that there are additional genes, as yet undiscovered, at both *Tox1A* and *Tox1B* that are necessary for T-toxin biosynthesis. The number of genes in each cluster and their functions remain to be determined.

This work was supported by the U.S. Department of Agriculture National Research Initiative (NRI) Program (O.C.Y. and B.G.T.) and the Ministry of Education, Science and Culture of Japan (M.K.).

LITERATURE CITED

Bronson, C. R., 1988 Ascospore abortion in crosses of *Cochliobolus heterostrophus* heterozygous for the virulence locus *Tox1*. Genome **30:** 12–18.

Bronson, C. R., 1991 The genetics of phytotoxin production by plant pathogenic fungi. Experientia **47:** 771–776.

Bronson, C. R., M. Taga and O. C. Yoder, 1990 Genetic control and distorted segregation of T-toxin production in field isolates of *Cochliobolus heterostrophus*. Phytopathology **80:** 819–823.

Chang, H. R., and C. R. Bronson, 1996 A reciprocal translocation and possible insertion(s) tightly associated with host-specific virulence in *Cochliobolus heterostrophus*. Genome **39:** 549–557.

Chu, G., D. Vollrath and R. W. Davis, 1986 Separation of large DNA molecules by contour clamped homogeneous electric fields. Science **234:** 1582–1585.

Ciuffetti, L. M., O. C. Yoder and B. G. Turgeon, 1992 A microbiological assay for host-specific fungal polyketide toxins. Fungal Genet. Newsl. **39:** 18–19.

Feinberg, A. P., and B. Vogelstein, 1983 A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132:** 6–13.

Fincham, J. R. S., P. R. Day and A. Radford, 1979 *Fungal Genetics*. Blackwell Scientific Publications, Oxford.

Leach, J., and O. C. Yoder, 1982 Heterokaryosis in *Cochliobolus heterostrophus*. Exp. Mycol. **6:** 364–374.

Leach, J., B. R. Lang and O. C. Yoder, 1982a Methods for selection of mutants and *in vitro* culture of *Cochliobolus heterostrophus*. J. Gen. Microbiol. **128:** 1719–1729.

Leach, J., K. J. Tegtmeier, J. M. Daly and O. C. Yoder, 1982b Dominance at the *Tox1* locus controlling T-toxin production by *Cochliobolus heterostrophus*. Physiol. Plant Pathol. **21:** 327–333.

Livingston, R. S., and R. P. Scheffer, 1983 Conversion of *Helminthosporium sacchari* toxin to toxoids by β-galactofuranosidase from *Helminthosporium*. Plant Physiol. **72:** 530–534.

Lu, S. W., L. Lyngholm, G. Yang, C. Bronson, O. C. Yoder *et al.*, 1994 Tagged mutations at the *Tox1* locus of *Cochliobolus heterostrophus* using restriction enzyme-mediated integration. Proc. Natl. Acad. Sci. USA **91:** 12649–12653.

Panaccione, D. G., J. S. Scott-Craig, J. A. Pocard and J. D. Walton, 1992 A cyclic peptide synthetase gene required for pathogenicity of the fungus *Cochliobolus carbonum* on maize. Proc. Natl. Acad. Sci. USA **89:** 6590–6594.

Panaccione, D. G., J. W. Pitkin, J. D. Walton and S. L. Annis, 1996 Transposon-like sequences at the *TOX2* locus of the plant-pathogenic fungus *Cochliobolus carbonum*. Gene **176:** 103–109.

Rose, M. S., 1996 Molecular genetics of polyketide toxin production in *Cochliobolus heterostrophus*. Ph.D. Thesis, Cornell University, Ithaca, NY.

Rose, M. S., O. C. Yoder and B. G. Turgeon, 1996 A decarboxylase required for polyketide toxin production and high virulence by *Cochliobolus heterostrophus*. Proceedings of the 8th Int. Symp. Mol. Plant-Microbe Int., Knoxville, TN, pp. J–49.

Sambrook, J., E. F. Fritsch and T. Maniatis, 1989 *Molecular Cloning: A Laboratory Manual*, Ed. 2. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Taga, M., C. R. Bronson and O. C. Yoder, 1985 Nonrandom abortion of ascospores containing alternate alleles at the *Tox1* locus of the fungal plant pathogen *Cochliobolus heterostrophus*. Can. J. Genet. Cytol. **27:** 450–456.

Tasma, I. M., and C. R. Bronson, 1998 Genetic mapping of telomeric DNA sequences in the maize pathogen *Cochliobolus heterostrophus*. Curr. Genet. **34:** 227–233.

Turgeon, B. G., R. C. Garber and O. C. Yoder, 1985 Transformation of the fungal maize pathogen *Cochliobolus heterostrophus* using the *Aspergillus nidulans amdS* gene. Mol. Gen. Genet. **201:** 450–453.

Turgeon, B. G., H. Bohlmann, L. M. Ciuffetti, S. K. Christiansen, G. Yang *et al.*, 1993 Cloning and analysis of the mating type genes from *Cochliobolus heterostrophus*. Mol. Gen. Genet. **238:** 270–284.

Turgeon, B. G., M. Kodama, G. Yang, M. S. Rose, S. W. Lu *et al.*, 1995 Function and chromosomal location of the *Cochliobolus heterostrophus Tox1* locus. Can. J. Bot. **73:** S1071–S1076.

Tzeng, T. H., L. K. Lyngholm, C. F. Ford and C. R. Bronson, 1992 A restriction fragment length polymorphism map and electrophoretic karyotype of the fungal maize pathogen *Cochliobolus heterostrophus*. Genetics **130:** 81–96.

Walton, J. D., 1996 Host-selective toxins: agents of compatibility. Plant Cell **8:** 1723–1733.

Walton, J. D., 1997 Enzymology, molecular genetics, regulation, and mode of action of the host-selective toxin HC-toxin, pp. 25–34 in *Molecular Genetics of Host-Specific Toxins in Plant Disease*, edited by K. Kohmoto and O. C. Yoder. Kluwer, Dordrecht, The Netherlands.

Yang, G., 1995 The molecular genetics of T-toxin biosynthesis by *Cochliobolus heterostrophus*. Ph.D. Thesis, Cornell University, Ithaca, NY.

Yang, G., B. G. Turgeon and O. C. Yoder, 1994 Toxin-deficient mutants from a toxin-sensitive transformant of *Cochliobolus heterostrophus*. Genetics **137:** 751–757.

Yang, G., M. S. Rose, B. G. Turgeon and O. C. Yoder, 1996 A polyketide synthase is required for fungal virulence and production of the polyketide T-toxin. Plant Cell **8:** 2139–2150.

Yoder, O. C., 1976 Evaluation of the role of *Helminthosporium maydis* race T toxin in Southern Corn Leaf Blight, pp. 16–24 in *Biochemistry and Cytology of Plant Parasite Interaction*, edited by K. Tomiyama, J. M. Daly, I. Uritani, H. Oku and S. Ouchi. Elsevier, New York.

Yoder, O. C., 1980 Toxins in pathogenesis. Annu. Rev. Phytopathol. **18:** 103–129.

Yoder, O. C., 1988 *Cochliobolus heterostrophus*, cause of Southern Corn Leaf Blight, pp. 93–112 in *Genetics of Plant Pathogenic Fungi*, edited by G. S. Sidhu. Academic Press, San Diego.

Yoder, O. C., and V. E. Gracen, 1975 Segregation of pathogenicity types and host-specific toxin production in progenies of crosses between races T and O of *Helminthosporium maydis* (*Cochliobolus heterostrophus*). Phytopathology **65:** 273–276.

Yoder, O. C., G. Yang, M. S. Rose, S. W. Lu and B. G. Turgeon, 1994 Complex genetic control of polyketide toxin production by *Cochliobolus heterostrophus*, pp. 223–230 in *Advances in the Molecular Genetics of Plant-Microbe Interactions*, edited by M. J. Daniels, J. A. Downie and A. E. Osbourn. Kluwer, Dordrecht, The Netherlands.

Yoder, O. C., V. Macko, T. J. Wolpert and B. G. Turgeon, 1997 *Cochliobolus* spp. and their host-specific toxins, pp. 145–166 in *The Mycota: Plant Relationships*, Vol. 5, Part A, edited by G. Carroll and P. Tudzynski. Springer-Verlag, Berlin.

Communicating editor: R. H. Davis