Supplementary material

Gene ID	Annotation
DDB0190413	Formin: *forE*
DDB0201787	Putative transport transmembrane protein
DDB0190415	Acetyltransferase (GNAT) family + protein kinase domain
DDB0190416	TatD related DNase domain
DDB0237678	-
DDB0237324*	rnu2b – spliceosomal ncRNA gene
DDB0190418	Protein kinase: *irlD*
DDB0216669	Protein kinase: *irlC*
DDB0190420*	-
DDB0190421	EF hand containing
DDB0190422	Histone-like transcription factor
DDB0190423*	Contains KOW motif
DDB0190424	Mak16l (involved in ribosome biosynthesis)

Table S1. Genes present in the Ax2 duplication. Genes marked with an asterisk (*) do not have probes on our array.

Gene ID	Annotation
DDB0219507	-
DDB0188659	RNA recognition motif
DDB0188660	-
DDB0188661	rps9 (40S ribosomal protein S9)
DDB0188662*	-
DDB0188663*	-
DDB0188664*	-
DDB0188665	*sigF*
DDB0188666*	-
DDB0188667	Cellulase domain
DDB0188669*	-
DDB0216146	-
DDB0188671	Pumilio-family RNA binding protein
DDB0215951*	-
DDB0188672*	-
DDB0188673	-
DDB0188674	Acyl-CoA oxidase
DDB0188675*	Contains FNIP repeat
DDB0188676*	-
DDB0188677	-
DDB0188678	-
DDB0219508*	-
DDB0219509*	-
DDB0188681*	-
DDB0188682*	*grxA*: glutaredoxin
DDB0215953*	-
DDB0188683*	DnaJ domain
DDB0188684*	-
DDB0188685*	-
DDB0188686	Sepiapterin reductase
DDB0188687	*gxcL* (RhoGEF domain)

Table S2. Genes present in the chromosome 5 duplication found in many strains derived from Ax3. Genes marked with an asterisk (*) do not have probes on our array.

Legends to supplementary figures

Figure S1 (Additional data file 1). The Ax3/4 duplication on chromosome 2. The known duplication on chromosome 2 of the sequenced strain Ax4(Ku) is clearly apparent in comparison with our reference strain Ax2(Ka) at the level of genomic DNA. All probes for chromosome 2 genes are ordered according to dictyBase assembly version 2.5, and log2 ratios indicated by vertical lines.

Figure S2 (Additional data file 2). Genealogy of NC4-derived strains, combining known relationships with those posited from our study. Strains beneath vertical lines alone arose by simple lineal descent of haploid lines, with or without selection and/or mutagenesis; horizontal lines divide multiple lines of simple descent from the same strain (at different times); strains beneath conjoining vertical and diagonal lines arose through parasexual crosses: both haploid parents are indicated, but the diploid intermediate is omitted for clarity. The dashed blue vertical line divides strains that possess the 2-gene duplication on chromosome 3 (left) from those that lack it (right). We assume that strain DdB was selected in the Sussman laboratory after the isolation of the brown mutant br-1, which was relatively early [70,71]. X22 descends both from br-1 and TS12, so we infer that it inherited its copy of chromosome 3 from the former (indicated by cyan lines). XP55 and its descendent XP99 descend in part from X22 [43] but lack the duplication. This is expected because they carry the *bsgA* mutation on that chromosome, inherited from NP194, which descends from the Newell laboratory stock of DdB [42,55]. This line of descent is shown in green. XP99 descends in part from Ax3, and possesses a duplication of chromosome 5 sequence common in derivatives of it. We can trace chromosome 5 of XP99 back to strain X2, because its offspring NP62 gained the *cobA* mutation on this chromosome [43]. We assume that X2 inherited chromosome 5 from Ax3 via NP2; this line to XP99 is marked in red. The relationship of NC4(B) with other strains is uncertain.

Figure S3 (Additional data file 3). NP81 and HU32 show multiple copy number variants. Chromosome 5 data are shown for both strains; all other chromosomes also show apparent losses relative to the reference strain, and duplications observed are

listed in Table 2. The single clear duplication on chromosome 5 shown here is the one also found in many Ax3 descendents.