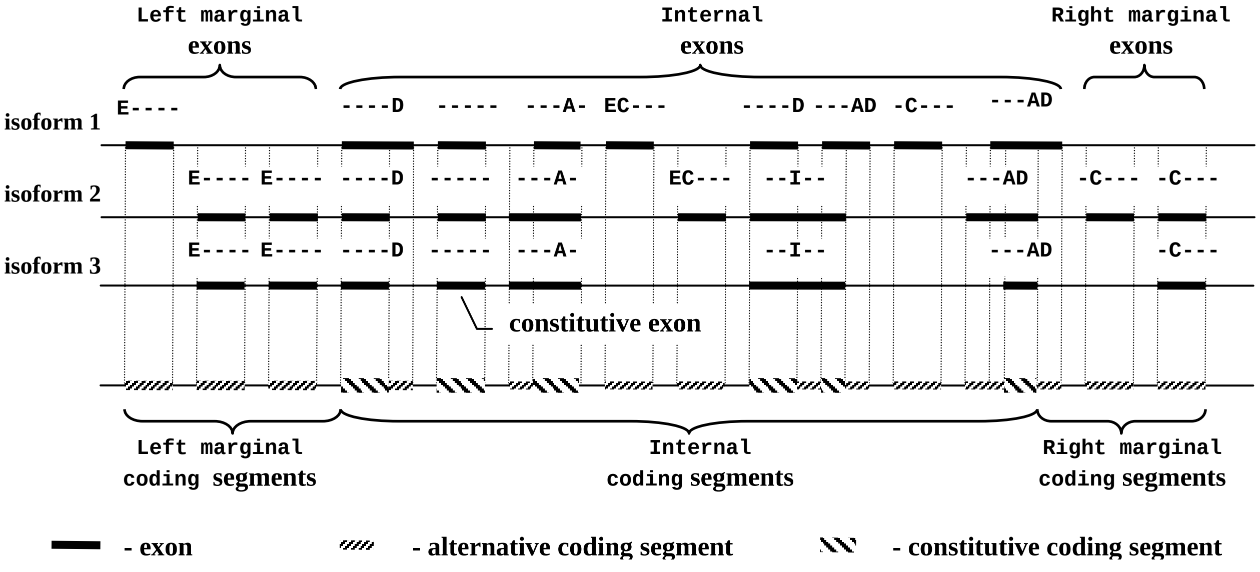
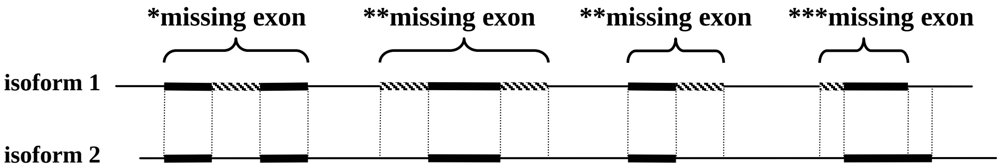
Supplementary Figure S1. Classification of exons and coding segments.
Top: pseudocode definitions of elementary alternatives.
Bottom: a schematic representation of an example (a gene with three isoforms). Shading types for different types of coding segments are listed under the scheme. Vertical lines correspond to splicing sites. Five-component strings (`ECIAD`) representing types of elementary alternatives are shown above each exon, e.g., cassette exon having two variants with different donor sites is described as (`-C--D`), two adjacent exons included independently were both cassette and mutually exclusive (`EC---`), whereas a constitutive exon was described by the empty string (`-----`).

```
Consider two isoforms.
Consider an exon in one isoform.
if common segments with two or more exons from the other isoform
   then retained-intron exon (I)
if common segments with one exon from the other isoform then
   if alternative region (in either isoform) 5′ of the common region
      then alternative-acceptor exon (A)
   if alternative region (in either isoform) 3′ of the common region
      then alternative-donor exon (D)
if no common segments with the other isoform then
   if common regions on both sides then consider the closest pair
      if no other complete exons between the common regions
         then cassette exon (C)
      else (at least one exon in the other isoform completely between
      the common regions)
         then mutually exclusive, or alternative, exon (E)
   if common regions only at 3′-side then consider the closest one
      if no other complete exons 5′ of the common region
         then cassette exon (C)
      else (at least one exon in the other isoform completely 5′ of the
      common region)
         then mutually exclusive, or alternative, exon (E)
   if common regions only at 5′-side then consider the closest one
      if no other complete exons 3′ of the common region
         then cassette exon (C)
      else (at least one exon in either isoform completely 3′ of the
      common region)
         then mutually exclusive, or alternative, exon (E)
```



Supplementary Figure S2. Definition of conserved and missing exons.
Top: formal definitions as the pseudocode.
Bottom: schematic representations of cases when exons that contain both missing and conserved coding segments are considered missing. The isoform 1 exons differ from the isoform 2 exons by missing segments.

```
Consider an exon
if there is at least one missing coding segment in the exon then
   if the exon does not contain a conserved coding segment specific for
   this exon then
      if the exon is a retained-intron exon
         then the exon is considered missing *
      else
         if there exists a shorter exon containing the same set
         of conserved segments
            then the exon is considered missing **
         else
            if there exists a (not retained-intron) exon containing the
            same set of conserved segments plus other conserved segments
               then the exon is considered missing ***
            else the exon is considered conserved
   else (the exon contains a coding segment specific for this exon)
      the exon is considered conserved
else (all coding segments are conserved)
   the exon is considered conserved
```

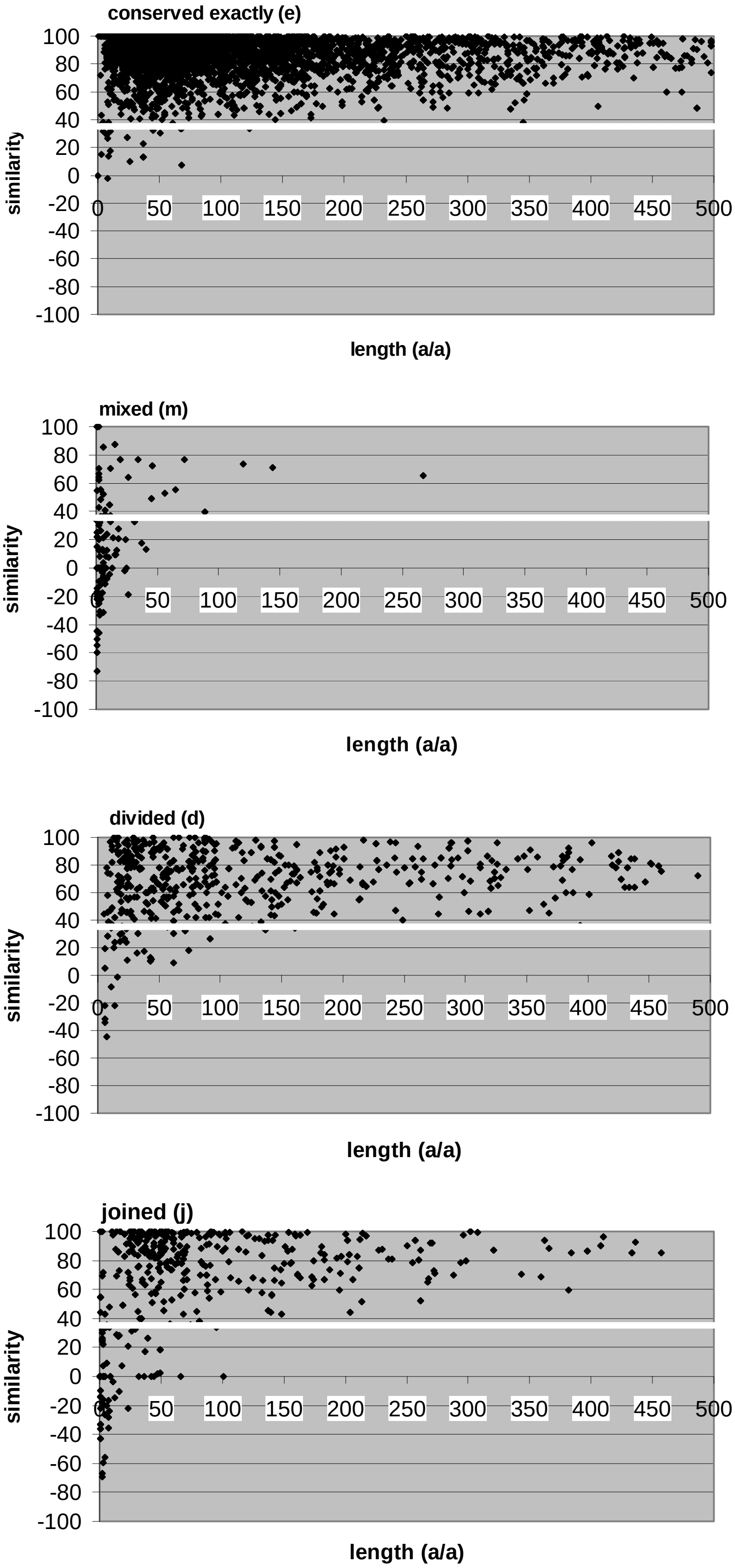


▨ **missing segment: similarity in alignments of all isoforms including this segment was less than 35%**

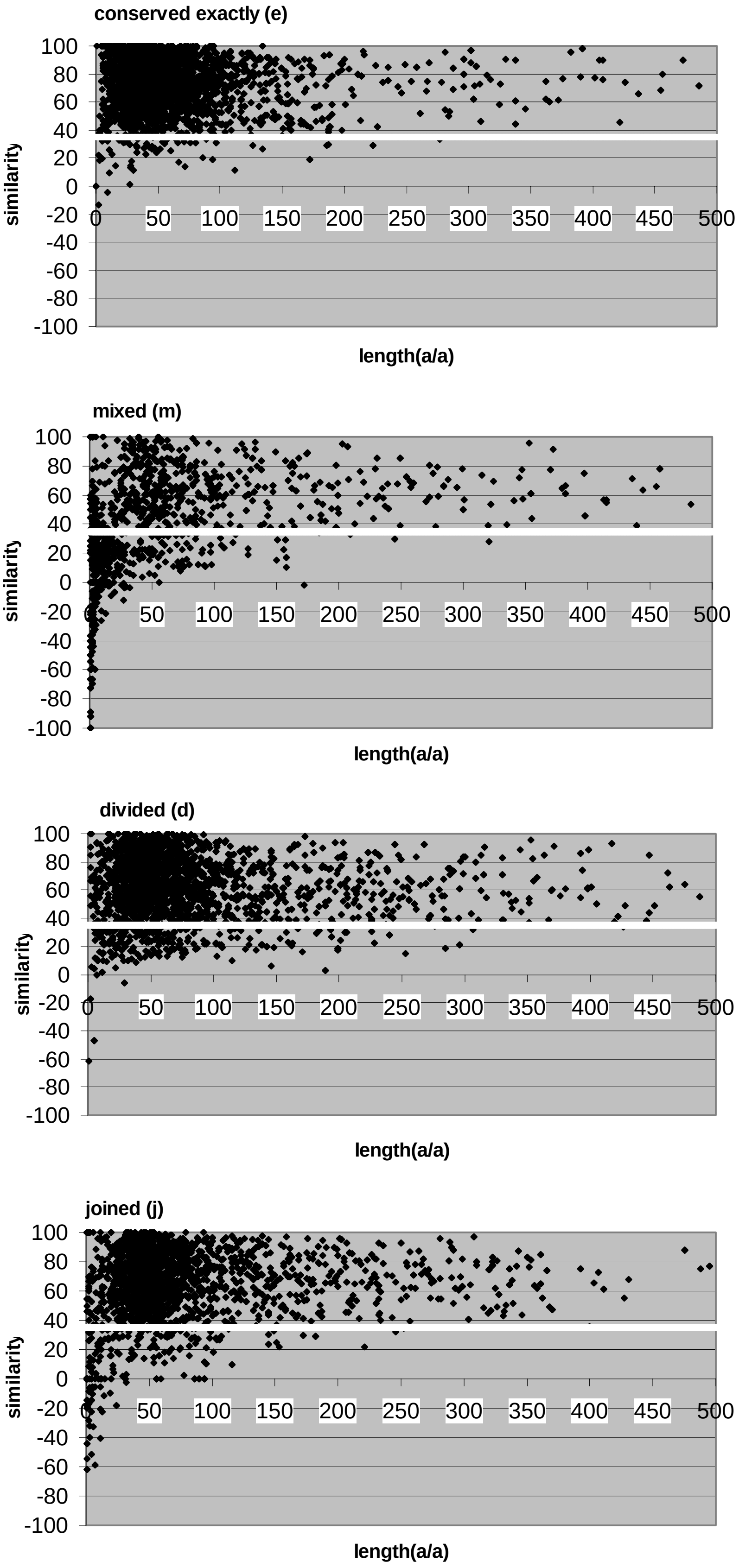
▬ **conserved segment: similarity in the alignment of at least one isoform including this segment was greater than 35%**

Supplementary Figure S3. Dependence of the slice similarity score on its length for *D.melanogaster-D.pseudoobscura* pairs.

Slices are defined as coding regions between two adjacent intron shadows (irrespective of the origin of these shadows in Dme or Dps). The 35% cutoff is shown by a straight line. Slices in different types of exons (see definitions in the text) are considered separately. Conserved exactly slices coincide with exons. Negative and low positive similarity scores arise from non-conserved slices whose alignment is constrained by the alignment of adjacent conserved regions.

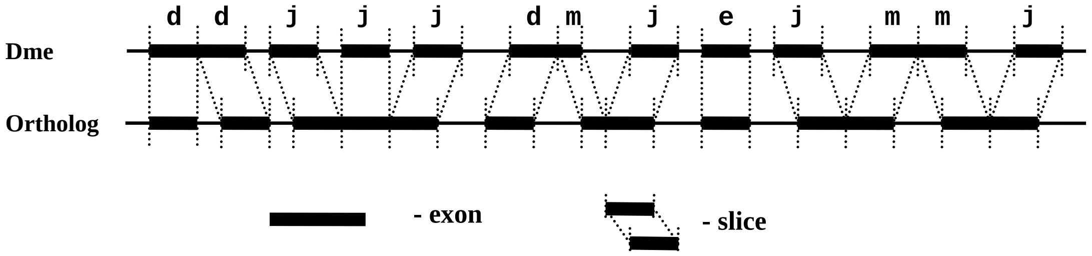


Supplementary Figure S4. The dependence of the slice similarity score on its length for *D.melanogaster-A.gambiae* pairs.
For definitions see the legend to Fig. S3. Existence of a large number of sub-cutoff slices shows that the alignment at exon termini is not very reliable.



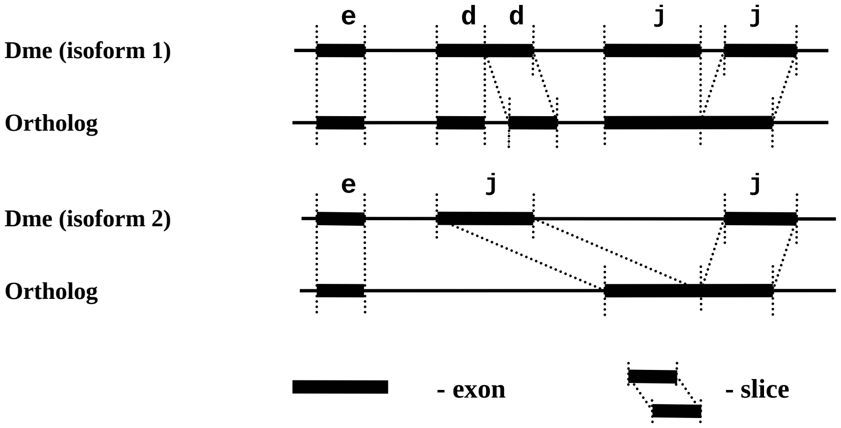
Supplementary Figure S5. Classification of slice events for the *D.melanogaster* exons.
Definition of *slices* see in the legend to Suppl. Fig. 3.

d - divided exon; **j** - joined exon;
e - exactly conserved exon; **m –** mixed;

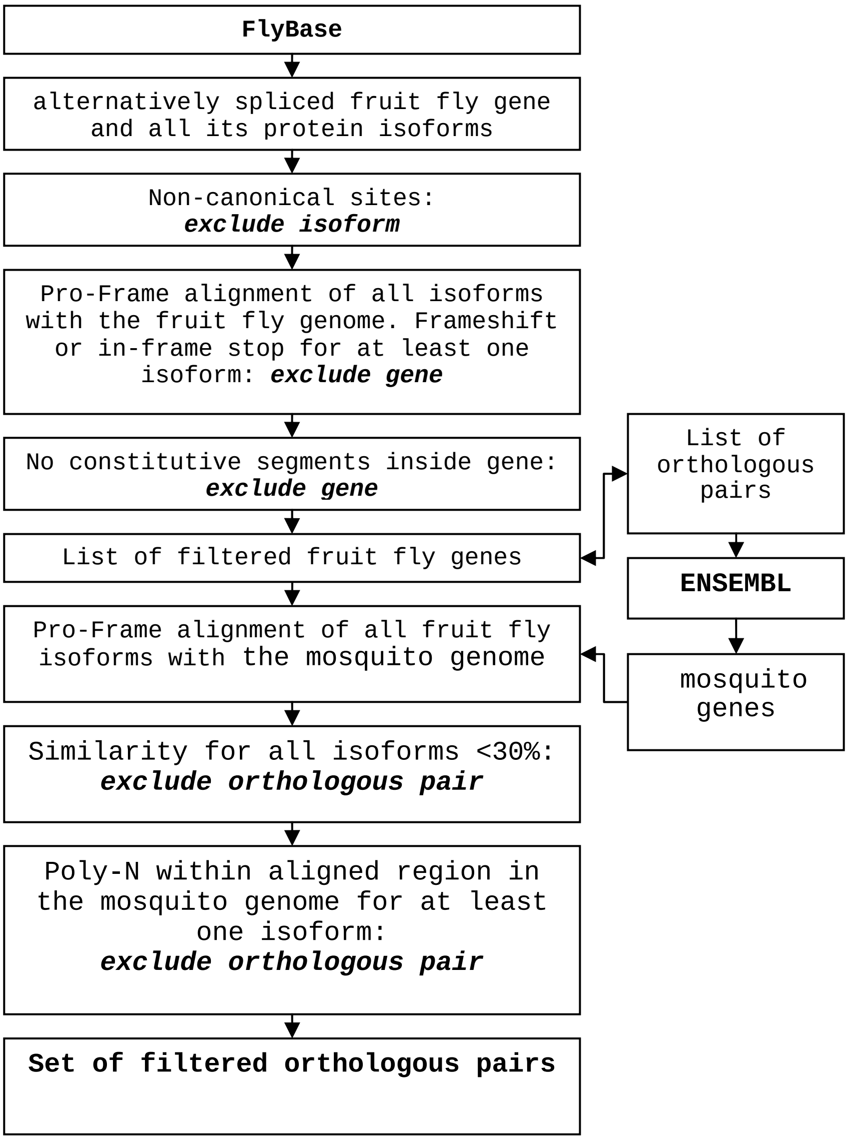


Supplementary Figure S6. Different types of events for the same exon dependent on an isoform.

e - exactly conserved exon; **d** - exon divided event; **j** - exon joined event.



Supplementary Figure S7. Filtering of the dataset.



Supplementary Table S1. Types of elementary alternatives and conservation (valid alignment) of *D. melonogaster* exons in the *D. pseudoobscura* and *A. gambiae* genomes. Top-down: Dme-Dps, small (curated) sample; Dme-Dps, large (non-curated) sample; Dme-Aga, small (curated) sample; Dme-Aga, large (non-curated) sample.

Exon Type		**Missing**	**Conserved: slice composition (event type)**					**Total**
			`--j-`	`-d--`	`-d-m`	`e---`	`e-j-`	
left marginal	`-C---`	1		3		9		13
	`E----`	7				15		22
	`EC---`	2						2
internal	`-----`	6	4	8	1	230	2	251
	`----D`	4				25		29
	`---A-`	2			2	34		38
	`--I--`	1				2		3
	`-C---`	3				7		10
	`E----`					8		8
	`EC---`					2		2
right marginal	`-C---`	1		1		13		15
	`E----`	6		1		7		14
	`E---D`	1	1					2
	`EC---`						1	1
Total								**410**

Exon Type		**Missing**	**Conserved: slice composition (event type)**								**Total**
			`---m`	`--j-`	`-d--`	`-d-m`	`e---`	`e-j-`	`ed--`	`ed-m`	
left marginal	`-C---`	68		18	5	3	365			1	460
	`-C--D`	2									2
	`-C-A-`				1		18				19
	`-C-AD`						1				1
	`E----`	87		3	9		167				266
	`E---D`						2				2
	`E--A-`	5					6				11
	`EC---`	34		1	2		80				117
	`EC--D`	1									1
	`EC-A-`	1					7				8
	`EC-AD`	1					3				4
internal	`-----`	67	1	53	39	10	2203	10	2	1	2386
	`----D`	69		10	5	3	214				301
	`---A-`	77		7	20	2	567				673
	`---AD`	14			4		40				58
	`--I--`	16		1	1	1	18				37
	`--I-D`						1				1
	`--IA-`	1					3				4
	`-C---`	14		1	4		73				92
	`-C--D`	1					1				2
	`-C-A-`						4				4
	`E----`	2					19				21
	`E---D`						4				4
	`E--A-`						2				2
	`EC---`						7				7
right marginal	`-C---`	44		1	5		184	1			235
	`-C--D`	2					6				8
	`E----`	41		12	3		139				195
	`E---D`	3					4	1			8
	`E--A-`	2									2
	`EC---`	10			3		42				55
Total											**4986**

Supplementary Table S1 continued

Exon Type		Missing	Conserved: slice composition (event type)							Total
			---m	--j-	-d--	-d-m	e---	e-j-	ed--	
left marginal	-C---	7		4		1	1			13
	E----	20		1		1				22
	EC---	2								2
internal	-----	51	1	38	17	13	126	4	1	251
	----D	18		1		2	8			29
	---A-	16	1	4	3	1	13			38
	--I--	1			2					3
	-C---	7		1			2			10
	E----	3					5			8
	EC---						2			2
right marginal	-C---	5		3	1	1	5			15
	E----	10		1		1	1	1		14
	E---D	2								2
	EC---	1								1
Total										**410**

Exon Type		Missing	Conserved: slice composition (event type)											Total
			---m	--j-	--jm	-d--	-d-m	-dj-	-djm	e---	e-j-	ed--	ed-m	
left marginal	-C---	217	4	80		12	9			138				460
	-C--D	2												2
	-C-A-	12				1				6				19
	-C-AD	1												1
	E----	179		20		8	4			54	1			266
	E---D	2												2
	E--A-	9		1						1				11
	EC---	80		6		1	2			28				117
	EC--D									1				1
	EC-A-	5				1				2				8
	EC-AD	3								1				4
internal	-----	561	16	543	1	166	148	1	6	919	17	3	5	2386
	----D	134	2	41		19	13			92				301
	---A-	256	7	109		75	34			191		1		673
	---AD	29		7		5	3			14				58
	--I--	16		3		7	5			6				37
	--I-D	1												1
	--IA-	1				1				2				4
	-C---	62		5			2			23				92
	-C--D	1								1				2
	-C-A-					2				2				4
	E----	6		1			1			13				21
	E---D	1								3				4
	E--A-	1								1				2
	EC---									7				7
right marginal	-C---	105		48		10	9			62	1			235
	-C--D	3		2						3				8
	E----	83	3	37		13	7			52				195
	E---D	5								3				8
	E--A-	2												2
	EC---	42				1				12				55
Total														**4986**