

1 **INVERSION POLYMORPHISM IN TWO SERBIAN NATURAL**  
2 **POPULATIONS OF *Drosophila subobscura*: ANALYSIS OF**  
3 **LONG-TERM CHANGES**

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5 Goran Zivanovic<sup>1</sup>, Conxita Arenas<sup>2</sup> and Francesc Mestres<sup>3</sup>

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7 <sup>1</sup>Department of Genetics, Institute for Biological Research "Sinisa Stankovic"  
8 University of Belgrade, Serbia.

9 <sup>2</sup>Departament d'Estadística, Universitat de Barcelona, Barcelona, Spain.

10 <sup>3</sup>Departament de Genètica, Universitat de Barcelona, Barcelona, Spain.

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12 Corresponding author:

13 Goran Zivanovic

14 Department of Genetics, Institute for Biological Research "Sinisa Stankovic",  
15 University of Belgrade, Bulevar Despota Stefana 142, 11000 Belgrade, Serbia

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17 Phone: (38111) 2764422

18 FAX: (38111) 2761433

19 E-mail: [goranziv@ibiss.bg.ac.rs](mailto:goranziv@ibiss.bg.ac.rs)

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21 **Running title:** Long-term changes in karyotypes

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23 To study whether inversions (or arrangements) by themselves or karyotypes are the  
24 global warming adaptive target of natural selection, two *Drosophila subobscura* Serbian  
25 populations (Apatin and Petnica) were re-analyzed using different statistical approaches.  
26 Both populations were sampled in an approximately 15 years period: Apatin in 1994  
27 and 2008+2009 and Petnica in 1995 and 2010. For all chromosomes, the four  
28 collections studied were in Hardy-Weinberg equilibrium. Thus, it seemed that  
29 inversions (or arrangements) combined at random to constitute populations' karyotypes.  
30 However, there were differences in karyotypic frequencies along the years, although  
31 they were significant only for Apatin population. Thus, inversions (or arrangements) are  
32 likely the target of natural selection, because they presented long-term changes, but  
33 combine at random to generate the corresponding karyotypic combinations.

34 In *Drosophila* genus, the chromosomal inversion polymorphism seems to be  
35 adaptive and it is subject to strong selection, because their frequencies change in time.  
36 For instance, short- (seasonal variation) and long-term changes (according to  
37 environmental variations) were reported in different species of this genus [1-5]. In this  
38 context, studies in the model species *Drosophila subobscura*, due to its rich  
39 chromosomal polymorphism for inversions, gave new insights on this adaptive process  
40 and the role of natural selection. Seasonal variation in chromosomal polymorphism  
41 frequencies was observed and interpreted as an adaptive process [6-9]. Furthermore,  
42 variations in the chromosomal polymorphism in time (long-term changes) were  
43 considered a key element to monitor the global climate change (for a review see [10].  
44 The role of natural selection was also observed in the latitudinal clinal variation of the  
45 inversion frequencies, both in Palearctic and American colonizing populations [11-14].  
46 Finally, in American populations of *D. subobscura*, the effect of natural selection on  
47 several inversions ( $O_5$  and  $O_{3+4+7}$ ) could be measured [15].

48 However, although short- and long-term changes in the composition and  
49 frequencies for chromosomal inversions or arrangements (overlapped inversions) have  
50 been intensively analyzed, this is not the case with regard to inversion karyotypes. Few  
51 studies have been carried out and limited information has been obtained [9, 16-20].  
52 Inversions on one chromosome could not act independently, because the genome is an  
53 integrated functional system. The genetic information carried by both homologous  
54 chromosomes could have an important effect on the adaptive capacity. For instance,  
55 some inversions (or arrangements) in one homologous chromosome combined with  
56 those of the other homologous of the pair could provide a better adaptation to certain  
57 environmental or climatic conditions. For this reason, the information provided by  
58 karyotypes could generate new insights in the adaptive changes along time. Our aim has

59 been to re-analyze -using different statistical approaches- data on chromosomal  
60 karyotypes from two Serbian populations, which were sampled two times each one in a  
61 15 years period and to study the variation in their karyotypic frequencies.

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## MATERIAL AND METHODS

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We have re-analyzed data from a couple of Serbian populations: Apatin (sampled in 1994 and 2008+2009) and Petnica (collected in 1995 and 2010). Detailed information regarding both populations can be found in [19] and [20]. Samples of different years were strictly collected in the same place, month and equivalent day. Males, and in some collections sons of wild females to increase the sample size, were crossed individually with virgin females of the Kussnacht strain that were homokaryotypic for standard chromosomal arrangements in all five chromosomes (A (X), E, J, U and O). Once dissected from third instar larvae, polytene chromosomes were stained and squashed in aceto-orcein solution. At least eight larvae from the progeny of each cross were examined in order to know the inversion pattern of both homologous chromosomes with a probability higher than 0.99. The chromosomal map of Kunze-Mühl and Müller [21] and Krimbas [22] was used for cytological analysis of the chromosomal inversions and arrangements and their nomenclature that of Kunze-Mühl and Sperlich [23]. Departure of chromosomal karyotypes from Hardy óWeinberg equilibrium and comparisons between samples were analyzed using Fisher's exact test (statistically significant  $p$ -value  $< 0.05$ ), as it is considered the best procedure in the case of multiple alleles per locus [24], in our case, different inversions (or arrangements) per chromosome. The corresponding  $p$ -values were obtained using the bootstrap procedure (100000 runs). These computations were carried out with R

83 packages (<http://CRAN.R-project.org>). Confidence intervals (CI) of karyotypic  
84 frequencies were estimated according to the binomial distribution.

85 .

## 86 RESULTS

87 The observed and expected frequencies of chromosomal karyotypes are  
88 presented in Table 1 (Apatin) and Table 2 (Petnica). With regard to the Apatin  
89 population (1994), all chromosomes were in H-W equilibrium: J (*p-value* = 0.8956), U  
90 (*p-value* = 0.8892), E (*p-value* = 0.4909) and O (*p-value* = 0.6626). For the same  
91 population, but analyzing the 2008 + 2009 sample, for all chromosomes not significant  
92 deviations from H-W equilibrium were detected: J (*p-value* = 0.8294), U (*p-value* =  
93 0.9558), E (*p-value* = 0.9059) and O (*p-value* = 0.9288). In the case of Petnica  
94 population, for the sample of 1995 all chromosomes were in H-W equilibrium: J (*p-*  
95 *value* = 0.8973), U (*p-value* = 0.9311), E (*p-value* = 0.9967) and O (*p-value* = 0.8980).  
96 Finally, for the same population, but sampled in 2010, H-W equilibrium was observed  
97 for all chromosomes: J (*p-value* = 1), U (*p-value* = 1), E (*p-value* = 0.9337) and O (*p-*  
98 *value* = 0.6089).

99 The comparisons between the karyotypic frequencies of both samples of Apatin  
100 (1994 and 2008+2009) are presented in Fig. 1 (1A, J chromosome; 1B, U chromosome;  
101 1C, E chromosome; 1D, O chromosome). With the exception of J chromosome, in all  
102 chromosomes there were significant differences for karyotypic frequencies: J (*p-value* =  
103 0.6376), U (*p-value* = 0.0026), E (*p-value* = 0.0148) and O (*p-value* = 0.0007). In  
104 2008+2009 sample, karyotypes containing the arrangements  $U_{1+2}$  and  $U_{1+8+2}$  increased  
105 in frequencies, whereas karyotypes with  $U_{st}$  tended to decrease, except  $U_{st}/U_{1+2}$  and  $U_{st}/$   
106  $U_{1+8+2}$  combinations (Table 1 and Fig. 1B). For the E chromosome (Table 1 and Fig.  
107 1C), the  $E_{st}/E_{st}$  karyotype decreased, but it was an increase of  $E_{st}/E_{1+2+9}$ ,  $E_{st}/E_8$  and

108  $E_{st}/E_{1+2+9+12}$  (not present in 1994). Interestingly, it was a dramatic decrease of  $O_{st}/O_{st}$   
109 karyotype and a substantial increase of  $O_{st}/O_{3+4}$  (Table 1 and Fig. 1D). In both cases, CI  
110 were almost non-overlapped. Many karyotypes presented in low frequencies in 1994  
111 were missing in 2008+2009 ( $O_{st}/O_6$ ,  $O_{st}/O_{3+4+2}$ ,  $O_6/O_{3+4}$ ,  $O_6/O_{3+4+1}$ ,  $O_{3+4}/O_{3+4+1}$  and  
112  $O_{3+4+1}/O_{3+4+1}$ ), whereas others were detected for the first time ( $O_{st}/O_{22}$ ,  $O_{st}/O_{3+4+22}$  and  
113  $O_{3+4+1}/O_{3+4+22}$ ). The  $O_{3+4}$  inversion increased in frequency (from 0.23 to 0.40), but not  
114 the frequency of  $O_{3+4}/O_{3+4}$  karyotype, which decreased.

115         The karyotypic frequencies of Petnica samples (1995 and 2010) are graphically  
116 shown in Fig. 2 (2A, J chromosome; 2B, U chromosome; 2C, E chromosome; 2D, O  
117 chromosome). In this case, there were not significant frequency differences for any  
118 chromosome: J (*p-value* = 0.3218), U (*p-value* = 0.0682), E (*p-value* = 0.0879) and O  
119 (*p-value* = 0.0624). However, the differences for the U and O chromosomes were rather  
120 close to significance. In 2010 collection,  $J_1/J_1$  increased and  $J_{st}/J_{st}$  disappeared (Table 2  
121 and Fig. 2A). With regard to the U chromosome (Table 2 and Fig. 2B), a small increase  
122 in the  $U_{1+2}/U_{1+2}$  was observed, and karyotypes  $U_{1+2}/U_{1+8+2}$  and  $U_{1+2+6}/U_{1+8+2}$  were not  
123 present in 1995. On the contrary, the frequency of homokaryotype  $U_{1+2+6}/U_{1+2+6}$   
124 homokaryotype decreased. The frequency of  $E_{st}/E_{st}$  karyotype decreased (Table 2 and  
125 Fig. 2C), but  $E_{st}/E_{1+2+9}$  and  $E_{st}/E_8$  also decreased in frequency (contrary to that found in  
126 Apatin). As in Apatin,  $E_{st}/E_{1+2+9+12}$  appeared for the first time in the 2010 collection.  
127 Several karyotypes found in 1995 were not found later ( $E_{1+2}/E_{1+2+9}$  and  $E_{1+2}/E_8$ ),  
128 whereas  $E_8/E_{1+2+9+12}$  appeared in the second collection. Also as in Apatin populations,  
129 the frequency of  $O_{st}/O_{st}$  karyotype decreased in time (Table 2 and Fig. 2D). However,  
130  $O_{3+4}/O_{3+4}$  also decreased in frequency and that of  $O_{st}/O_{3+4}$  remained without variation.  
131 Many karyotypic combinations disappeared in 2010 sample ( $O_{st}/O_{22}$ ,  $O_6/O_{3+4+1}$ ,  
132  $O_{3+4}/O_{3+4+2}$ ,  $O_{3+4+1}/O_{3+4+2}$  and  $O_{3+4+22}/O_{3+4+22}$ ), but it was possible to find for the first

133 time  $O_{3+4}/O_{3+4+8}$ . Finally, other karyotypes increased their frequencies ( $O_{3+4}/O_{3+4+22}$  and  
134  $O_{3+4+1}/O_{3+4+22}$ ).

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## 136 DISCUSSION

137 If a genetic marker is in H-W equilibrium, a random combination of alleles to  
138 constitute the next generation genotypes has to be assumed. In this situation, no relevant  
139 effect of selection (and other evolutionary forces) seems to act on the studied genetic  
140 marker. In our case, we focused in combinations of karyotypes, and for this reason to  
141 find H-W equilibrium implies that gene inversions or arrangements combine at random  
142 (or almost at random) to form the corresponding karyotypes. In Apatin population and  
143 for 1994 and 2008+2009 samples, all chromosomes were in H-W equilibrium. Thus, it  
144 seems that there is not a detectable effect of selection in Apatin. A similar situation was  
145 found in Petnica: all chromosomes in both samples (1995 and 2010) were also in H-W  
146 equilibrium. As in the population of Apatin, the effect of selection at this level seems  
147 very low. In general, other researchers reported similar results in distinct *D. subobscura*  
148 populations [16, 27-31]. However, several authors found several deviations from H-W  
149 equilibrium for particular chromosomes [28, 29, 32].

150 The situation is different when the same population is compared using samples  
151 collected in different years. In Apatin, significant changes in the frequencies of  
152 karyotypes have been found for the U, E and O chromosomes. The most interesting  
153 result is that inversion and karyotype frequencies have been changed in this 15 years  
154 period, but inversions (or arrangements) combine at random to produce the karyotypes  
155 (no H-W deviations). Thus, it seems that inversions *per se* are the target of selection,  
156 not the karyotypic combinations produced. In *D. subobscura*, long-term changes of  
157 inversions according to global warming expectations have been documented in both,

158 autochthonous and colonizing populations [10, 17-20, 25, 26, 33-35]. A similar result  
159 was obtained for Petnica population. However, the differences in karyotypic frequencies  
160 were not significant. Thus, the interactions of inversions located in the pair of  
161 homologous chromosomes seem not to be the target of selection. Inversions or their  
162 combinations (arrangements) are important by themselves and are directly under the  
163 effect of natural selection. For this reason, it is correct to use the terminology *öwarmö*  
164 and *öcoldö* adapted inversions. For instance,  $O_{st}$ ,  $E_{st}$ ,  $J_{st}$  or  $U_{st}$  can be considered *öcoldö*  
165 adapted due their genetic content, with independence of other inversions in their  
166 homologous chromosomes. However, in *D. subobscura* several examples of heterotic  
167 effect of inversions have been reported. For instance, in American colonizing  
168 populations the  $O_5$  inversions presents a heterotic effect [15], but it is due to its  
169 complete association with a lethal gene [36-38]. For this reason, the karyotype  $O_5/O_5$   
170 does not exist in American populations. A heterotic effect for an  $O_{\underline{3+4+7}}$  arrangement  
171 associated to a lethal gene was also described in the Californian population of Gilroy  
172 [15]. This arrangement also presented a heterotic effect in crosses carried out in  
173 laboratory conditions [39]. However, although it is a species with a rich chromosomal  
174 polymorphism, *D. subobscura* is considered not a system of balanced strain.  
175 Furthermore, it is accepted in general that homozygotes for gene arrangements are  
176 found in the expected panmictic frequencies [22].

177         However, our study presents a number of limitations. To properly study the  
178 karyotypic frequencies a large sample size is needed, because many inversion (or  
179 arrangement) combinations exist producing different kind of karyotypes. In most  
180 populations, many karyotypes will be present in low frequencies, thus non-negligible  
181 sample sizes are needed to obtain accurate estimates of their frequencies. However, to  
182 obtain karyotypic frequencies implies much more laboratory work than to only analyze



183 chromosomal polymorphism frequencies. Moreover, if the aim is to study the long-term  
184 changes in karyotype composition it must collect exactly at the same site, month and  
185 day (a correction is needed, because, according to Menzel et al. [40], spring/summer has  
186 advanced 2.5 days per decade in Europe). Thus, the number of flies obtained in the  
187 second period collection will depend on the particular conditions of the trapping day  
188 [19]. Another limitation is that only combinations of inversions (or arrangements) from  
189 the same pair of homologous chromosomes have been analyzed. Likely, the interactions  
190 of combinations between inversions located in non-homologous chromosomes would be  
191 an interesting topic, but an enormous sample size and laboratory work would be needed  
192 due to the large number of possible combinations between different inversions of  
193 distinct chromosomes.

194 In summary, our four samples analyzed (Apatin 1994, Apatin 2008+2009,  
195 Petnica 1995 and Petnica 2010) were in H-W equilibrium. Thus, inversions (or  
196 arrangements) seem to combine at random to form karyotypes. However, karyotypes of  
197 both populations have changed along time (significant in Apatin and non-significant in  
198 Petnica). Likely, inversions (or arrangements) have been under selection for being  
199 adaptive to climatic changes, but they combine at random to constitute the karyotypes.  
200 From our data, inversions (or arrangements) appear as the key elements being under  
201 selection in the global warming environment.

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363 FIGURE LEGENDS:

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365 **Fig. 1.** Graphical representation of karyotypic frequencies obtained in Apatin, in 1994  
 366 (white) and 2008+2009 (grey). CI for each frequency is also presented. **A)** J

367 chromosome. **B)** U chromosome. **C)** E chromosome. **D)** O chromosome, where, a:

368  $O_{st}/O_{st}$ ; b:  $O_{st}/O_6$ ; c:  $O_{st}/O_{22}$ ; d:  $O_{st}/O_{3+4}$ ; e:  $O_{st}/O_{3+4+1}$ ; f:  $O_{st}/O_{3+4+2}$ ; g:  $O_{st}/O_{3+4+22}$ ; h:

369  $O_6/O_{3+4}$ ; i:  $O_6/O_{3+4+1}$ ; j:  $O_{3+4}/O_{3+4}$ ; k:  $O_{3+4}/O_{3+4+1}$ ; l:  $O_{3+4}/O_{3+4+22}$ ; m:  $O_{3+4+1}/O_{3+4+1}$ ; n:

370  $O_{3+4+1}/O_{3+4+22}$ .

371

372 **Fig. 2.** Graphical representation of karyotypic frequencies obtained in Petnica, in 1995  
 373 (white) and 2010 (grey). CI for each frequency is also presented. **A)** J chromosome. **B)**

374 U chromosome. **C)** E chromosome. **D)** O chromosome, where, a:  $O_{st}/O_{st}$ ; b:  $O_{st}/O_{22}$ ; c:

375  $O_{st}/O_{3+4}$ ; d:  $O_{st}/O_{3+4+1}$ ; e:  $O_{st}/O_{3+4+2}$ ; f:  $O_6/O_{3+4+1}$ ; g:  $O_{3+4}/O_{3+4}$ ; h:  $O_{3+4}/O_{3+4+1}$ ; i:

376  $O_{3+4}/O_{3+4+2}$ ; j:  $O_{3+4}/O_{3+4+8}$ ; k:  $O_{3+4}/O_{3+4+22}$ ; l:  $O_{3+4+1}/O_{3+4+1}$ ; m:  $O_{3+4+1}/O_{3+4+2}$ ; n:

377  $O_{3+4+1}/O_{3+4+22}$ ; o:  $O_{3+4+22}/O_{3+4+22}$ .

378

379

380 **Table 1.** Frequencies (in percentage) of the observed (Obs.) and expected (Exp.)

381 karyotypes in both collections (1994 and 2008+2009) from Apatin population.

	1994			2008+2009	
	OBS.	EXP.		OBS.	EXP.
Karyotype	%	%		%	%
$J_{st}/J_{st}$	18.0	15.21		10.0	14.06
$J_{st}/J_1$	42.0	47.58		55.0	46.88
$J_1/J_1$	40.0	37.21		35.0	39.06
n	50	50		20	20
$U_{st}/U_{st}$	34.0	27.04		/	7.65
$U_{st}/U_{1+2}$	20.0	23.92		35.0	26.13
$U_{st}/U_{1+2+6}$	16.0	24.96		10.0	9.63



$U_{st}/U_{1+8+2}$	/	/		10.0	4.13
$U_1/U_{1+2}$	2.0	0.46		/	/
$U_{1+2}/U_{1+2}$	6.0	5.29		20.0	22.56
$U_{1+2}/U_{1+2+6}$	12.0	11.04		15.0	16.63
$U_{1+2}/U_{1+8+2}$	/	/		5.0	7.13
$U_{1+2+6}/U_{1+2+6}$	10.0	5.76		5.0	3.06
Other		1.53			3.08
n	50	50		20	20
$E_{st}/E_{st}$	58.0	49.0		30.0	39.06
$E_{st}/E_{1+2}$	2.0	2.8		/	/
$E_{st}/E_{1+2+9}$	16.0	23.8		30.0	18.75
$E_{st}/E_{1+2+9+12}$	/	/		10.0	6.25
$E_{st}/E_8$	6.0	15.4		25.0	22.88
$E_{1+2+9}/E_{1+2+9}$	4.0	2.89		/	2.25
$E_8/E_8$	2.0	1.21		5.0	3.06
$E_8/E_{1+2}$	2.0	0.44		/	/
$E_8/E_{1+2+9}$	10.0	3.74		/	5.25
Other		0.72			2.5
n	50	50		20	20
$O_{st}/O_{st}$	44.0	37.41		10.0	16.0
$O_{st}/O_6$	8.0	7.32		/	/
$O_{st}/O_{22}$	/	/		5.0	2.0
$O_{st}/O_{3+4}$	14.0	28.06		45.0	32.0
$O_{st}/O_{3+4+1}$	8.0	9.76		5.0	4.0

$O_{st}/O_{\underline{3+4+2}}$	4.0	2.44		/	/
$O_{st}/O_{\underline{3+4+22}}$	/	/		5.0	10.0
$O_6/O_{\underline{3+4}}$	2.0	2.76		/	/
$O_6/O_{\underline{3+4+1}}$	2.0	0.96		/	/
$O_{\underline{3+4}}/O_{\underline{3+4}}$	14.0	5.29		10.0	16.0
$O_{\underline{3+4}}/O_{\underline{3+4+1}}$	2.0	3.68		/	4.0
$O_{\underline{3+4}}/O_{\underline{3+4+22}}$	/	/		15.0	10.0
$O_{\underline{3+4+1}}/O_{\underline{3+4+1}}$	2.0	0.64		/	0.25
$O_{\underline{3+4+1}}/O_{\underline{3+4+22}}$	/	/		5.0	1.25
Other		1.86			4.5
n	50	50		20	20

382

383 Note: n = total number of karyotypes. "Other" stands for other karyotypic combinations

384 which were infrequent and were not found in the samples.

385

386 **Table 2.** Frequencies (in percentage) of the observed (Obs.) and expected (Exp.)

387 karyotypes in both collections (1995 and 2010) from Petnica population.

	1995			2010	
	OBS.	EXP.		OBS.	EXP.
Karyotype	%	%		%	%
$J_{st}/J_{st}$	5.7	7.51		/	2.56
$J_{st}/J_1$	43.4	39.78		32.0	26.88
$J_1/J_1$	50.9	52.71		68.0	70.56
n	53	53		25	25

$U_{st}/U_{st}$	/	1.69		/	1.44
$U_{st}/U_{1+2}$	15.1	9.80		16.0	11.52
$U_{st}/U_{1+2+6}$	11.3	12.77		8.0	7.68
$U_{st}/U_{1+8+2}$	/	/		/	1.92
$U_{1+2}/U_{1+2}$	13.2	14.21		20.0	23.04
$U_{1+2}/U_{1+2+6}$	34.0	37.02		32.0	30.72
$U_{1+2}/U_{1+8+2}$	/	/		8.0	7.68
$U_{1+2+6}/U_{1+2+6}$	26.4	24.11		8.0	10.24
$U_{1+2+6}/U_{1+8+2}$	/	/		8.0	5.12
$U_{1+8+2}/U_{1+8+2}$	/	/		/	0.64
Other		0.4			/
n	53	53		25	25
$E_{st}/E_{st}$	17.0	16.48		4.0	9.0
$E_{st}/E_{1+2}$	3.8	3.09		12.0	3.6
$E_{st}/E_{1+2+9}$	30.1	31.42		24.0	20.4
$E_{st}/E_{1+2+9+12}$	/	/		8.0	3.6
$E_{st}/E_8$	13.2	13.80		8.0	14.4
$E_{1+2}/E_{1+2+9}$	1.9	2.94		/	4.08
$E_{1+2}/E_8$	1.9	1.29		/	2.88
$E_{1+2+9}/E_{1+2+9}$	17.0	14.98		8.0	11.56
$E_8/E_8$	3.8	2.89		4.0	5.76
$E_8/E_{1+2+9}$	11.3	13.16		28.0	16.32
$E_8/E_{1+2+9+12}$	/	/		4.0	2.88
Other		/			5.52

n	53	53		25	25
$O_{st}/O_{st}$	15.1	12.82		3.7	4.93
$O_{st}/O_6$	/	0.64		/	/
$O_{st}/O_{22}$	1.9	0.64		/	/
$O_{st}/O_{\underline{3+4}}$	30.1	28.35		29.7	18.91
$O_{st}/O_{\underline{3+4+1}}$	7.5	10.17		3.7	4.93
$O_{st}/O_{\underline{3+4+2}}$	1.9	2.0		3.7	0.84
$O_{st}/O_{\underline{3+4+8}}$	/	/		/	3.39
$O_{st}/O_{\underline{3+4+22}}$	/	4.08		/	6.57
$O_6/O_{\underline{3+4+1}}$	1.9	0.26		/	/
$O_{22}/O_{\underline{3+4}}$	/	0.71		/	/
$O_{\underline{3+4}}/O_{\underline{3+4}}$	18.8	15.68		7.4	18.18
$O_{\underline{3+4}}/O_{\underline{3+4+1}}$	3.8	11.25		3.7	9.46
$O_{\underline{3+4}}/O_{\underline{3+4+2}}$	1.9	2.22		/	1.62
$O_{\underline{3+4}}/O_{\underline{3+4+8}}$	/	/		14.8	6.3
$O_{\underline{3+4}}/O_{\underline{3+4+22}}$	5.7	4.51		22.2	12.61
$O_{\underline{3+4+1}}/O_{\underline{3+4+1}}$	5.7	2.02		3.7	1.23
$O_{\underline{3+4+1}}/O_{\underline{3+4+2}}$	1.9	0.8		/	0.42
$O_{\underline{3+4+1}}/O_{\underline{3+4+8}}$	/	/		/	1.64
$O_{\underline{3+4+1}}/O_{\underline{3+4+22}}$	1.9	1.62		7.4	3.29
$O_{\underline{3+4+2}}/O_{\underline{3+4+2}}$	/	0.08		/	/
$O_{\underline{3+4+22}}/O_{\underline{3+4+22}}$	1.9	0.32		/	2.19
Other		1.83			3.49
n	53	53		27	27

389 Note: n = total number of karyotypes. "Other" stands for other karyotypic combinations  
390 which were infrequent and were not found in the samples.

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