

1 **Whole-chromosome fusions in the karyotype evolution of *Sceloporus* (Iguania, Reptilia)**
2 **are more intense in sex chromosomes than autosomes**

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15 generation sequencing

16

17 **Abstract**

18 There is a growing body of evidence that the common ancestor of vertebrates had a bimodal
19 karyotype, i.e. consisting of large macrochromosomes and small microchromosomes. This
20 type of karyotype organization is preserved in most reptiles. However, certain species
21 independently experience microchromosome fusions. The evolutionary forces behind this are
22 unclear. We investigated the karyotype of the green spiny lizard, *Sceloporus malachiticus*, an
23 iguana species which has $2n=22$, whereas the ancestral karyotype of iguanas had $2n=36$. We
24 obtained and sequenced flow-sorted chromosome-specific DNA samples and found that most
25 of the microchromosome fusions in this species involved sex chromosomes. We found that
26 certain ancestral squamate chromosomes, such as the homologue of the *Anolis carolinensis*
27 chromosome 11, are repeatedly involved in sex chromosome formation in different species.
28 To test the hypothesis that the karyotypic shift could be associated with changes in
29 recombination patterns, and to study sex chromosome synapsis and recombination in meiosis,
30 we performed synaptonemal complex analysis in this species and in *S. variabilis*, a related
31 species with $2n=34$. We found that in the species studied the recombination patterns correlate
32 more with phylogeny than with the structure of the karyotype. The sex chromosomes had two
33 distal pseudoautosomal regions and a medial differentiated region.

34

35 **Introduction**

36 There are two main types of karyotype organization in vertebrates: unimodal and bimodal [1].
37 In bimodal karyotypes there is a clear distinction between normal-sized macrochromosomes
38 and small “dot-like” microchromosomes. This is a characteristic feature for some amphibians
39 and fishes (mostly basal clades), birds, and a majority of reptiles [2]. Unimodal karyotypes
40 have chromosomes of gradually decreasing size. They are characteristic for many amphibians
41 (derived clades), teleost fishes, mammals, and some reptiles [3]. The bimodal karyotype
42 organization is thought to be ancestral for vertebrates, since the microchromosomes of

43 different lineages share high homology [4,5]. The unimodal organization originated
44 independently in different lineages by fusion of microchromosomes with each other and with
45 the macrochromosomes [6,7].

46 This parallel process is interesting in the context of the search for general patterns of
47 karyotype and genome evolution in animals. Fixation of chromosomal rearrangements is
48 often presumed to be neutral and random, but the repetition of similar rearrangements points
49 to their possible biological significance, as suggested by Morescalchi [1,8]. According to this
50 hypothesis, decreasing the overall recombination rate via lowering the chromosome number
51 under a selection for decreased recombination could be a possible biological mechanism for
52 shifts to unimodality. In this case, it could be expected that other forces, such as changes in
53 crossover rates, would also act to decrease recombination, and the species with unimodal
54 karyotypes would have lower crossing over rates than related species with bimodal
55 karyotypes. The molecular cytogenetic and genomic methods offer new possibilities to study
56 the parallel shifts to unimodality.

57 To address the question of possible adaptive significance of microchromosome fusions, it is
58 important to determine whether different microchromosomes fuse in random combinations,
59 or if certain chromosomes tend to fuse with each other repeatedly in different lineages. This
60 could happen if the linkage between the alleles of certain loci is favored by selection [1,9].
61 The fusions involving sex chromosomes are particularly interesting, since it has been
62 suggested that certain chromosome pairs are more often involved in sex chromosome
63 formation in the vertebrate evolution than others, both by becoming sex chromosomes *de*
64 *novo* and by fusing with already existing sex chromosomes [10,11]. Mammals and teleost
65 fishes experienced loss of microchromosomes early in their evolution, and subsequent
66 chromosomal and genomic rearrangements obscured the initial steps of the
67 microchromosome fusion [5]. Squamate reptiles, on the contrary, contain species with both
68 bimodal and unimodal karyotypes, and some clades with unimodal karyotypes have formed
69 recently and have close relatives with bimodal karyotypes.

70 The genus *Sceloporus* (Iguania, Pleurodonta, Phrynosomatidae) displays higher chromosomal
71 variability than reptiles on average: $2n$ varies in this genus from 22 to 46. All these
72 karyotypes can be derived from the ancestral phrynosomatid karyotype ($2n=34$) via
73 chromosomal fusions and fissions [12,13]. These lizards have sex chromosomes of the
74 XX/XY type, which are homologous to the sex chromosomes of most other pleurodont
75 iguanians, and are involved in fusions in the species with low diploid numbers [12,14].

76 In this study we investigated the karyotype of *S. malachiticus*, which represents the clade
77 with $2n=22$. We obtained flow-sorted chromosome samples, which were used for FISH and
78 low coverage sequencing to determine their homology with the chromosomes of *Anolis*
79 *carolinensis*, a species which retains the ancestral karyotype of Iguania [15].

80 To check the hypothesis on the connection between chromosome fusion and evolution of
81 recombination patterns, we applied an immunocytological approach to detect meiotic
82 crossing over in synaptonemal complex (SC) spreads of *S. malachiticus* and *S. variabilis*, a
83 related species with the unaltered ancestral karyotype ($2n=34$), and analyzed the
84 recombination patterns using the *r* parameter [16]. This parameter is designed to measure
85 recombination rate in meiosis by taking account of chromosome number, crossover number
86 and crossover location. Its physical sense is the probability of two randomly selected loci to

87 recombine in a meiotic act. It has two components: the interchromosomal, which reflects
88 recombination via independent chromosome segregation, and the intrachromosomal, which
89 reflects crossover recombination. We also studied sex chromosome synapsis and
90 recombination to validate the FISH and sequencing data on their structure.

91 **Materials and methods**

92 The male specimens of *S. malachiticus* and *S. variabilis* were obtained from the pet trade. To
93 confirm the species identification, the 5'-fragment of the mitochondrial COI gene was
94 sequenced using protocols and primers described previously [17].

95 The primary fibroblast cell cultures of *S. malachiticus* were obtained in the Laboratory of
96 Animal Cytogenetics, the Institute of Molecular and Cellular Biology, Russia, using the
97 protocols described previously [18,19]. All cell lines were deposited in the IMCB SB RAS
98 cell bank ("The general collection of cell cultures", 0310-2016-0002). Metaphase
99 chromosome spreads were prepared from chromosome suspensions obtained from early
100 passages of primary fibroblast cultures as described previously [20–22].

101 C-like DAPI staining was performed in the following way. The slides were incubated in 0.2
102 M HCl for 20 min at room temperature. Then they were kept in Ba(OH)₂ solution at 55° C for
103 4 min, and then incubated in 2xSSC at 60° C for 60 min. Then they were washed in distilled
104 water at room temperature, and stained with DAPI using the Vectashield mounting medium
105 with DAPI (Vector Laboratories).

106 The SC spreads of *S. malachiticus* and *S. variabilis* were prepared and immunostained as
107 described previously, using the antibodies to SYCP3, the protein of the lateral element of the
108 SC; to MLH1, the mismatch-repair protein which marks mature recombination nodules; and
109 to CENP, the centromere proteins [23].

110 The flow-sorted chromosome samples of *S. malachiticus* were obtained using the Mo-Flo®
111 (Beckman Coulter) high-speed cell sorter at the Cambridge Resource Centre for Comparative
112 Genomics, Department of Veterinary Medicine, University of Cambridge, Cambridge, UK,
113 as described previously [24]. The painting probes were generated from the DOP-PCR
114 amplified samples by a secondary DOP-PCR incorporation of biotin-dUTP and digoxigenin-
115 dUTP (Sigma) [25]. FISH was performed with standard techniques [26].

116 The preparations were analyzed with an Axioplan 2 Imaging microscope (Carl Zeiss)
117 equipped with a CCD camera (CV M300, JAI), CHROMA filter sets, and the ISIS4 image
118 processing package (MetaSystems GmbH). The brightness and contrast of all images were
119 enhanced using Corel PaintShop Photo Pro X6 (Corel Corp).

120 For the preparation of the sorted chromosomes of *S. malachiticus* for sequencing, we used
121 TruSeq Nano DNA Low Throughput Library Prep Kit (Illumina). Paired-end sequencing was
122 performed on Illumina MiSeq using Reagent Kits v2, 600-cycles. The NGS data were
123 deposited in NCBI SRA database (PRJNA616430). Sequencing data were processed using
124 the "DOPseq_analyzer" pipeline [27, 28]. The following parameters were used: for read
125 trimming "ampl" was set to "dop", Illumina adapter trimming was enabled, additional
126 cutadapt options "--trim-n --minimum-length 20" were specified. Reads were aligned to *A.*
127 *carolinensis* genome (AnoCar2.0) downloaded from Ensembl (www.ensembl.org) using the
128 BWA MEM algorithm with default parameters. Additional filters were minimum MAPQ =
129 20 and minimum alignment length = 20. For target region identification, scaffolds with

130 chromosome assignments and scaffolds over 50 kb in size were used. The key parameter in
131 the “DOPseq_analyzer” output to determine the target scaffolds and chromosomes is
132 *pd_mean*, which is mean distance between the positions of the scaffold which are covered by
133 the sequencing reads. The target scaffolds are characterized by lower *pd_mean* and
134 contaminant scaffolds are characterized by higher *pd_mean*. The position of the scaffolds on
135 the chromosomes of *A. carolinensis* was determined using the data on their synteny to the
136 chicken chromosomes, obtained from UCSC Genome Browser (www.genome.ucsc.edu), and
137 the previously obtained data on the synteny between the *Anolis* and chicken chromosomes
138 [28,29].

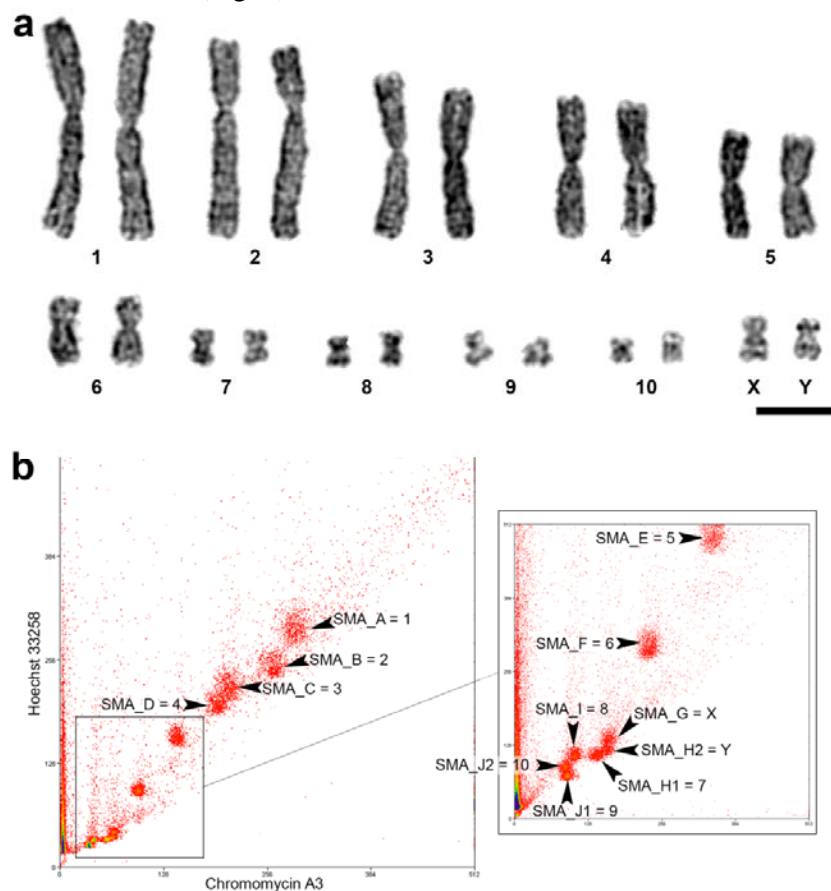
139

140 Results

141 *Species identification and the mitotic karyotype of S. malachiticus*

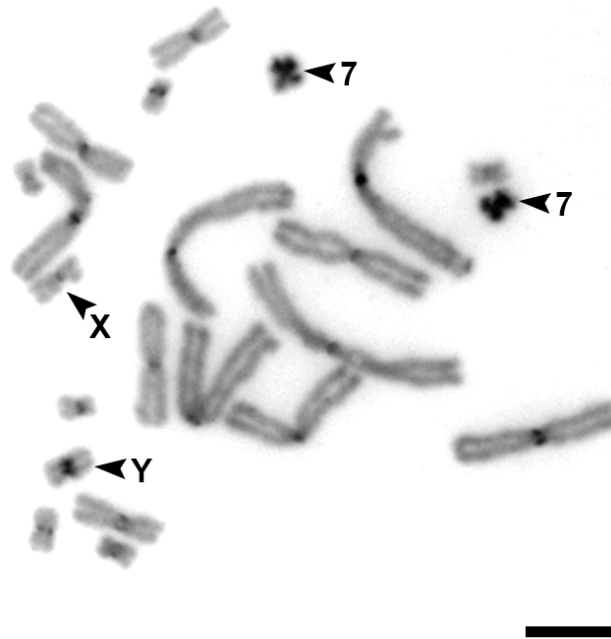
142 The COI gene sequence confirmed that the specimens studied belong to *S. malachiticus*
143 (GenBank MT140115) and *S. variabilis* (GenBank MT131783). The mitotic karyotype of *S.*
144 *malachiticus* was typical for the $2n=22$ clade of *Sceloporus* [12], and included six pairs of
145 large metacentric chromosomes, medium-sized X and Y chromosomes, and four pairs of
146 small metacentric chromosomes (Fig. 1a). The flow-sorted karyotype consisted of 12 peaks
147 (Fig. 1b).

148 C-like DAPI staining showed DAPI-positive bands in the centromeres of each chromosome.
149 The Y chromosome had more prominent band than the X chromosome. The chromosome 7
150 was totally heterochromatic (Fig. 2).



151

152 Fig. 1. Karyotype of *S. malachiticus*. a: G-banded karyotype. Scale bar: 10 μ m. b: flow-
153 sorted karyotype. Peak IDs and contents are indicated by the arrowheads. X and Y axes:
154 fluorescence intensity for each fluorochrome.



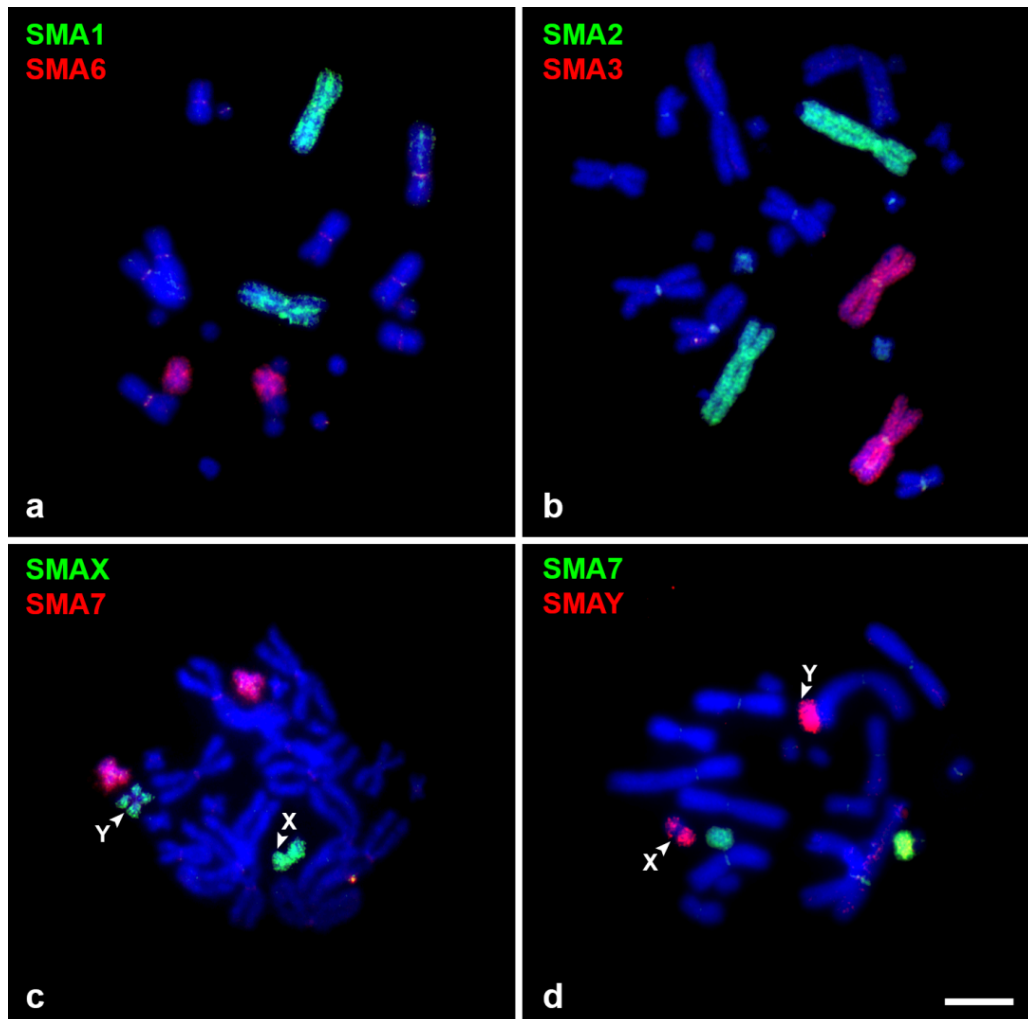
155
156 Fig. 2. C-like DAPI staining of the *S. malachiticus* metaphase plate. Arrowheads indicate the
157 heterochromatic chromosome 7 and the sex chromosomes. Scale bar: 10 μ m.

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159 *FISH with the flow-sorted chromosome-specific probes*

160 FISH with the labelled probes derived from each peak was performed on the metaphase
161 plates of the same specimen. The probes derived from most peaks hybridized with single
162 chromosome pairs (Fig. 3). Two peaks, SMA_G and SMA_H2, both hybridized with the sex
163 chromosomes, and were concluded to correspond to the chromosomes X and Y, respectively.
164 The peak SMA_H2 (mostly consisting of the Y chromosome), in addition, showed weak
165 hybridization with the similar-sized chromosome 7 (mostly contained in the peak SMA_H1)
166 probably due to contamination during flow sorting (Fig. 3d).

167 Both sex chromosome probes hybridized with the respective chromosomes along the whole
168 length, but showed weaker or no hybridization in the medial, centromere-adjacent region of
169 the homologous chromosome (Fig. 3c, d). This pattern corresponds to the presence of two
170 homologous pseudoautosomal regions in the distal parts of the sex chromosomes, and the
171 differentiated non-recombining regions in the middle.



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173 Fig. 3. Examples of FISH with the flow-sorted chromosome-specific probes of the male *S.*
174 *malachiticus* on its metaphase plates. Note that the sex chromosome-specific probes label the
175 respective chromosomes across the whole length, but label the sex chromosomes of the
176 opposite type (e.g., the SMA_X probe on the Y chromosome) only in the distal
177 pseudoautosomal regions. Scale bar: 10 μ m.

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179 *Sequencing of the flow-sorted chromosome-specific probes*

180 The NGS data analysis showed that the six largest macrochromosomes of *S. malachiticus*
181 (SMA1-SMA6) correspond to the macrochromosomes of *A. carolinensis* (ACA1-ACA6),
182 with two notable exceptions (Table 1, Supplementary file 1).

183 In addition to high homology to ACA2, SMA2 shows homology with the segment located
184 between the positions 204854915-205800612 of ACA1 (AnoCar2.0). In ACA1, this fragment
185 represents an isolated segment of homology with the chicken chromosome 12 (GGA12),
186 surrounded by the segments homologous to GGA3. It is notable that the segments
187 homologous to GGA12 are mostly located on ACA2.

188 Similarly, SMA6, along with homology to ACA6, shows homology with the segment located
189 between the positions 192604161-196661610 of ACA1 (AnoCar2.0). In ACA1, this genomic

190 block is homologous to a segment of GGA2, and is also surrounded by the segments
191 homologous to GGA3. The adjacent segments of GGA2 are homologous to ACA6.
192 The sex chromosomes of *S. malachiticus* contain the genomic blocks which are homologous
193 to the chromosomes ACAX, ACA11, ACA16, ACA17, and ACA18. In the Y chromosome-
194 specific DNA sample, 3 of 47 (6%) identified homologous ACA scaffolds belonged to
195 ACAX, in contrast with 7 of 53 (13%) in the X chromosome. These scaffolds showed higher
196 *pd_mean* than the scaffolds corresponding to other ACA chromosomes. They probably
197 represent a contamination of the Y-sample by the X chromosome due to similar size.
198 The chromosomes SMA8, SMA9, and SMA10 correspond to the chromosomes
199 ACA7+ACA10, ACA9+ACA14, and ACA8+ACA12, respectively. In the chromosome
200 SMA7, only the genomic blocks homologous to ACA15 were found.
201 The data on the homology between the chromosomes of *S. malachiticus* and *A. carolinensis*
202 are summarized in the Table 1 and Supplementary File 1.

203

204 Table 1. The homology between the chromosomes of *S. malachiticus* and *A. carolinensis*,
205 inferred from the NGS data.

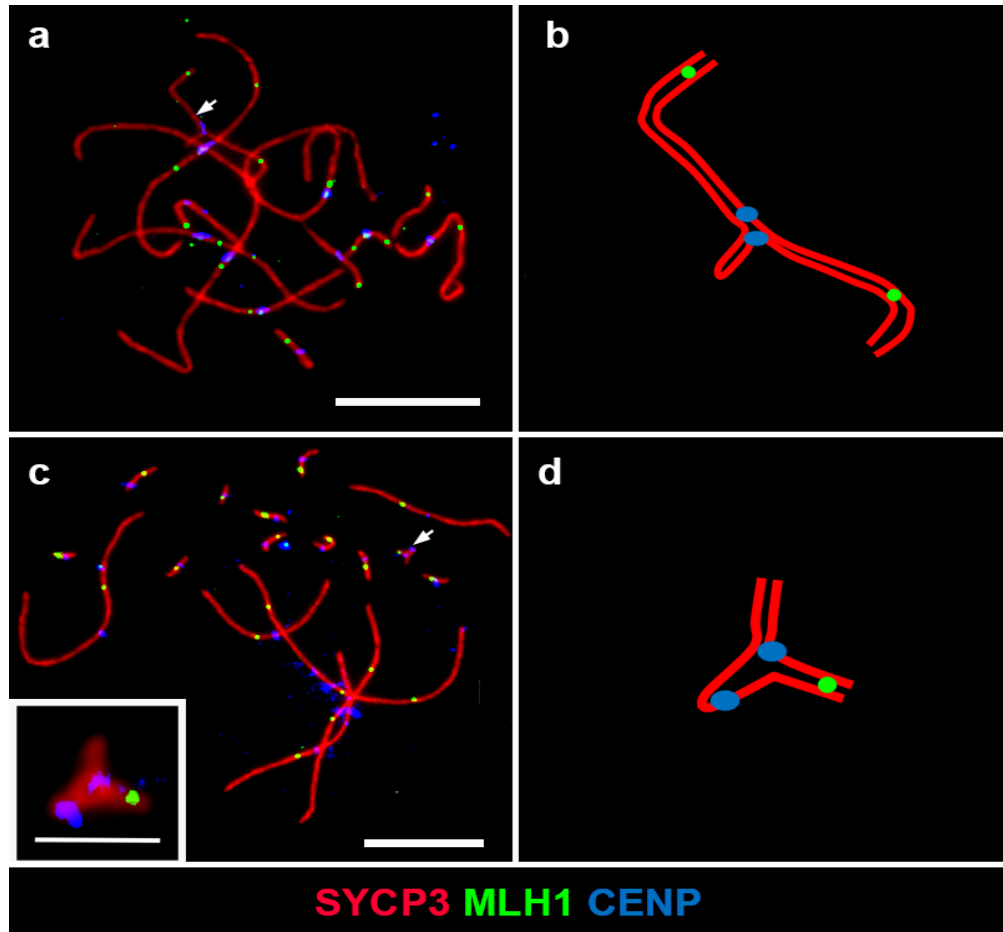
<i>S. malachiticus</i> chromosomes	<i>A. carolinensis</i> chromosomes
1	1
2	2, 1
3	3
4	4
5	5
6	6, 1
X	X, 11, 16, 17, 18
Y	11, 16, 17, 18
7	15
8	7, 10
9	9, 14
10	8, 12

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207 *Synaptonemal complex analysis*

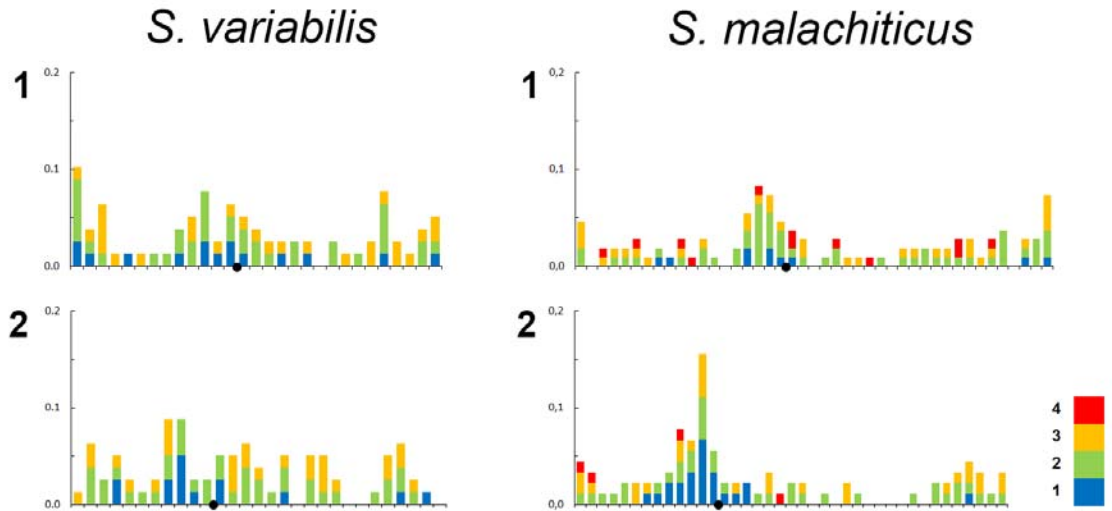
208 The SC karyotype of *S. malachiticus* consisted of 11 metacentric bivalents, including the XY
209 bivalent with misaligned centromeres and a lateral buckle on one of the homologues (Fig.
210 4a). This corresponds to the previously reported SC karyotype of a closely related species *S.*
211 *undulatus* (Reed et al., 1990), and to the mitotic karyotype of *S. malachiticus* obtained in the

212 present work. The recombination nodules, marked with the MLH1 protein, were located in
213 the distal parts of the sex bivalent (Fig. 4b). The SC karyotype of *S. variabilis* consisted of 17
214 bivalents: 6 metacentric macrochromosomal bivalents and 11 microchromosomal bivalents
215 (Fig. 4c). This corresponds to its previously known mitotic karyotype [30]. One of the micro-
216 bivalents consisted of homologues of unequal lengths, with the larger homologue forming a
217 lateral buckle (Fig. 4c,d). It was concluded to be the sex bivalent, basing on its similarity with
218 the previously studied micro-sex bivalents in *Anolis* [31].



219 Fig. 4. Synaptonemal complex analysis of *S. malachiticus* (a,b) and *S. variabilis* (c, d). a,c:
220 immunofluorescent staining of SC spreads. The sex bivalents are indicated by arrows. Scale
221 bars: 10 μ m. Insert: the micro-sex bivalent of *S. variabilis*, scale bar: 2 μ m. b,d: schematic
222 drawings of the sex bivalents.
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225 The average crossover numbers were 15.6 ± 2.3 per spread in *S. malachiticus* and 20.9 ± 1.7 per
226 spread in *S. variabilis*. However, the r parameter shows that the lower number of crossovers
227 in *S. malachiticus* reflects only its lower chromosome number and not a change in crossing
228 over patterns: the intra-chromosomal components of r are 0.032 ± 0.005 in *S. malachiticus* and
229 0.033 ± 0.006 in *S. variabilis*. It is notable that both species have elevated crossover numbers
230 near the centromeres of macro-autosomes, as shown previously by the analyses of meiotic
231 metaphase chromosomes [12,14] (Fig. 5).



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Fig. 5. Numbers and distributions of the MLH1 foci on two largest chromosomes of *S. variabilis* and *S. malachiticus*. The x-axis shows the positions of MLH1 foci along the SCs in relation to the centromere (black circle). One scale division represents a segment of the average length of each SC equal to 1 μ m. The y-axis shows the proportion of MLH1 foci in each interval. Different colors show bivalents with different MLH1 numbers, from 1 to 4.

Discussion

The results of FISH and whole chromosome-specific DNA sequencing show that the macrochromosomes were stable during karyotypic evolution of *S. malachiticus*, in contrast to the microchromosomes. The segments of ACA1 which show homology to SMA2 and SMA6 are homologous to the segments of the chicken chromosomes GGA12 and GGA2. Most parts of these chicken chromosomes have homology with SMA2/ACA2 and SMA6/ACA6, respectively. This indicates that these segments of ACA1 most probably represent relatively recent translocations from proto-ACA2 and proto-ACA6, which occurred in the *Anolis* lineage and are not shared by *Sceloporus*. Alternatively, this might represent an assembly error in the *A. carolinensis* genome.

SMA7 is larger than other small autosomes, which contain homologues of two *A. carolinensis* microchromosomes each (Fig. 1b, Table 1). However, its sequence shows homology only with ACA15. It is possible that SMA7 also contains genomic segments which are not covered or not assembled in the current version of the *A. carolinensis* genome, or is enlarged due to repeat accumulation. The latter explanation is supported by the high heterochromatinization of SMA7 (Fig. 2).

There are much data on the homology between the macrochromosomes of *A. carolinensis* and chromosomes of other squamates [6,7,32]. However, data for the microchromosomes are much less available. The established homologies between the microchromosomes of *A. carolinensis* and chromosomes of other squamates mainly concern the chromosomes which are involved in sex chromosome formation in different species.

The sex chromosome-autosome fusions can become fixed in different ways. According to the most widespread model of sex chromosomes evolution, the translocations of loci with sexual antagonistic alleles on the sex chromosomes are positively selected, and such loci accumulate

263 in the sex chromosomes [33,34]. Another model shows that such fusions could be favored in
264 cases of selection for heterozygosity [35]. There is also a model which implies that the
265 fusions are deleterious and are fixed by drift [36]. Thus, the predisposition of fusions between
266 certain autosomes and sex chromosomes, irrespectively of the genomic origin of the sex
267 chromosomes, to become fixed, may arise if sex linkage or high heterozygosity are
268 particularly beneficial for certain loci, or if these fusions are the least deleterious.

269 The sex chromosomes in *S. malachiticus* experienced more fusions than the autosomes. Both
270 FISH and crossover mapping data show the presence of two distal pseudoautosomal regions,
271 and a lack of homology between the X and Y chromosomes in the median parts. This
272 indicates that the autosomal fragments translocated onto the sex chromosomes from both
273 sides. However, our methods do not allow determination of the relative positions of each
274 syntenic block inside the sex chromosomes.

275 Of the fusions which we identified in the sex chromosomes of *S. malachiticus*, several appear
276 to be homoplasies, as they appear independently in different squamate lineages. Namely, the
277 combination of the homologues of ACAX+ACA11 occur independently in *Ctenonotus*
278 (Dactyloidae, Pleurodonta) [37,38] and in the Z chromosome of *Paroedura* (Gekkonidae)
279 [39]; the combination of the homologues of ACAX+ACA18 occur in *Norops* (Dactyloidae,
280 Gekkota) [28]; and the combination of the homologues of ACA11+ACA16 occur in the Z
281 chromosome of Lacertidae [40]. It is also notable that both in *Norops* and *Sceloporus* the sex
282 chromosomes stand out by undergoing more fusions than autosomes. However, the
283 homologues of ACA9 and ACA12, which constitute the sex chromosomes of *Norops* along
284 with ACAX and ACA18, are not fused with the sex chromosomes and with each other in *S.*
285 *malachiticus*. The fusion of the homologues of ACA15 and ACA16, which created the
286 chromosome 12 of *Norops* [28], did not take place in *S. malachiticus*.

287 Thus, although we detected some repeated microchromosome fusions, the paucity of the
288 available comparative data prevents us from determining whether certain combinations of
289 microchromosomes fuse significantly more frequently than others. The repeated involvement
290 of the homologues of ACA11 in the formation of sex chromosomes requires special attention,
291 since this chromosome is homologous to the sex chromosome system of therian mammals.
292 More comprehensive understanding of our data and more detailed comparative analysis will
293 be available with the emergence of more syntenic maps of squamates with fused
294 microchromosomes. In some of them, the existing syntenic maps cover only the homologues
295 of the *A. carolinensis* macrochromosomes, as in lacertids [6] and geckos [7] or have too small
296 numbers of markers, as in tuatara [41]. For others, *i.e.* skinks, syntenic maps do not exist at
297 all [42].

298 We did not confirm the hypothesis that *S. malachiticus* could have lower crossing over rates
299 than the related bimodal species. On the contrary, both species of *Sceloporus* show similar
300 intra-chromosomal components of r , which are higher than in two previously studied bimodal
301 *Anolis* species [23]. Thus, at least in the studied species of Pleurodonta, the crossover patterns
302 depend on the phylogenetic position of the species, and not on the structure of its karyotype.
303 The high r values in *Sceloporus* are due to the centromeric peaks of crossover distribution, in
304 contrast with *Anolis*, which demonstrate mostly distally located crossovers. As shown by
305 Veller et al., [16], median crossovers contribute much more to the effective recombination
306 rate than the distal ones. It is highly unusual for vertebrates to have crossover peaks near the

307 centromeres: the more common pattern is a reduction of crossover rate in the centromeric
308 regions, which is called the “centromere effect” [43]. The physiological mechanisms and
309 possible biological significance of the altered crossover patterns in *Sceloporus* deserve
310 further study.

311

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321 **References**

- 322 1. Morescalchi A. 1977 Phylogenetic Aspects of Karyological Evidence. In *Major*
323 *Patterns in Vertebrate Evolution*, pp. 149–167. Springer US. (doi:10.1007/978-1-
324 4684-8851-7_7)
- 325 2. Olmo E, Signorino G. 2005 Chromorep: a reptiles chromosomes database.
326 www.chromorep.univpm.it
- 327 3. Morescalchi A. 1980 Evolution and karyology of the amphibians. *Bolletino di Zool.*
328 **47**, 113–126. (doi:10.1080/11250008009438709)
- 329 4. Braasch I *et al.* 2016 The spotted gar genome illuminates vertebrate evolution and
330 facilitates human-teleost comparisons. *Nat. Genet. Vol. 48*, 427–437.
331 (doi:10.1038/ng.3526)
- 332 5. Uno Y, Nishida C, Tarui H, Ishishita S, Takagi C. 2012 Inference of the
333 Protokaryotypes of Amniotes and Tetrapods and the Evolutionary Processes of
334 Microchromosomes from Comparative Gene Mapping. *PLoS One 7*.
335 (doi:10.1371/journal.pone.0053027)
- 336 6. Srikulnath K, Matsubara K, Uno Y, Nishida C, Olsson M, Matsuda Y. 2014
337 Identification of the linkage group of the Z sex chromosomes of the sand lizard
338 (*Lacerta agilis*, Lacertidae) and elucidation of karyotype evolution in lacertid lizards.
339 *Chromosoma 123*, 563–575. (doi:10.1007/s00412-014-0467-8)
- 340 7. Srikulnath K, Uno Y, Nishida C, Ota H, Matsuda Y. 2015 Karyotype reorganization in
341 the Hokou Gecko (*Gekko hokouensis*, Gekkonidae): The process of microchromosome
342 disappearance in Gekkota. *PLoS One 10*. (doi:10.1371/journal.pone.0134829)
- 343 8. Morescalchi A. 1977 Adaptation and Karyotype in Amphibia. *Ital. J. Zool. 44*, 287–
344 294. (doi:10.1080/11250007709430182)
- 345 9. Charlesworth D. 2016 The status of supergenes in the 21st century: recombination
346 suppression in Batesian mimicry and sex chromosomes and other complex adaptations.
347 *Evol. Appl. 9*, 74–90. (doi:10.1111/eva.12291)
- 348 10. Deakin JE, Ezaz T. 2019 Understanding the Evolution of Reptile Chromosomes
349 through Applications of Combined Cytogenetics and Genomics Approaches.
350 *Cytogenet Genome Res 157*, 7–20. (doi:10.1159/000495974)
- 351 11. Sigeman H, Ponnikas S, Chauhan P, Dierickx E, De Brooke M, Hansson B. 2019
352 Repeated sex chromosome evolution in vertebrates supported by expanded avian sex
353 chromosomes. *Proc. R. Soc. B Biol. Sci. 286*, 20192051. (doi:10.1098/rspb.2019.2051)
- 354 12. Hall WP. 2009 Chromosome variation, genomics, speciation and evolution in

- 355 Sceloporus lizards. *Cytogenet. Genome Res.* **127**, 143–165. (doi:10.1159/000304050)
- 356 13. Leaché AD, Banbury BL, Linkem CW, Nieto-Montes De Oca A. 2016 Phylogenomics
357 of a rapid radiation: is chromosomal evolution linked to increased diversification in
358 north american spiny lizards (Genus *Sceloporus*)? *BMC Evol. Biol.* **16**, 63.
359 (doi:10.1186/s12862-016-0628-x)
- 360 14. Reed KM, Sudman PD, Sites JW, Greenbaum IF. 1990 Synaptonemal Complex
361 Analysis of Sex Chromosomes in Two Species of *Sceloporus*. *Copeia* , 1122–1129.
362 (doi:10.2307/1446497)
- 363 15. Deakin JE *et al.* 2016 Anchoring genome sequence to chromosomes of the central
364 bearded dragon (*Pogona vitticeps*) enables reconstruction of ancestral squamate
365 macrochromosomes and identifies sequence content of the Z chromosome. *BMC*
366 *Genomics* **17**, 447. (doi:10.1186/s12864-016-2774-3)
- 367 16. Veller C, Kleckner N, Nowak MA. 2019 A rigorous measure of genome-wide genetic
368 shuffling that takes into account crossover positions and Mendel’s second law. *Proc.*
369 *Natl. Acad. Sci. U. S. A.* **116**, 1659–1668. (doi:10.1073/pnas.1817482116)
- 370 17. Nagy ZT, Sonet G, Glaw F, Vences M. 2012 First large-scale DNA barcoding
371 assessment of reptiles in the biodiversity hotspot of madagascar, based on newly
372 designed COI primers. *PLoS One* **7**. (doi:10.1371/journal.pone.0034506)
- 373 18. Stanyon R, Galleni L. 1991 Italian Journal of Zoology A rapid fibroblast culture
374 technique for high resolution karyotypes A rapid fibroblast culture technique for high
375 resolution karyotypes. *Ital. J. Zool.* **58**, 81–83. (doi:10.1080/11250009109355732)
- 376 19. Romanenko SA *et al.* 2015 Segmental paleotetraploidy revealed in sterlet (*Acipenser*
377 *ruthenus*) genome by chromosome painting. *Mol. Cytogenet.* **8**, 90.
378 (doi:10.1186/s13039-015-0194-8)
- 379 20. Yang F, O’Brien PCM, Milne BS, Graphodatsky AS, Solanky N, Trifonov V, Rens W,
380 Sargan D, Ferguson-Smith MA. 1999 A Complete Comparative Chromosome Map for
381 the Dog, Red Fox, and Human and Its Integration with Canine Genetic Maps.
382 *Genomics* **62**, 189–202. (doi:10.1006/geno.1999.5989)
- 383 21. Graphodatsky A *et al.* 2000 Comparative cytogenetics of hamsters of the genus
384 *Calomyscus*. *Cytogenet. Cell Genet.* **88**, 296–304. (doi:10.1159/000015513)
- 385 22. Graphodatsky AS, Yang F, O’Brien PCM, Perelman P. 2001 Phylogenetic implications
386 of the 38 putative ancestral chromosome segments for four canid species Create new
387 project ‘Ultrasonic energy transport proved using femtosecond laser pulses’ View
388 project Cytogenomics in Amazonian bats View project. *Cytogenet. Genome Res.* **92**,
389 243–247. (doi:10.1159/000056911)
- 390 23. Lisachov AP, Tishakova K V., Tsepilov YA, Borodin PM. 2019 Male Meiotic
391 Recombination in the Steppe Agama, *Trapelus sanguinolentus* (Agamidae, Iguania,
392 Reptilia). *Cytogenet. Genome Res.* **157**, 107–114. (doi:10.1159/000496078)
- 393 24. Yang F, Carter NP, Shiu L, Ferguson-Smith MA. 1995 A comparative study of
394 karyotypes of muntjacs by chromosome painting. *Chromosoma* **103**, 642–652.
395 (doi:10.1007/BF00357691)
- 396 25. Telenius H, Carter NP, Bebb CE, Nordenskjöld M, Ponder BAJ, Tunnacliffe A. 1992
397 Degenerate oligonucleotide-primed PCR: General amplification of target DNA by a
398 single degenerate primer. *Genomics* **13**, 718–725. (doi:10.1016/0888-7543(92)90147-
399 K)
- 400 26. Liehr T, Kreskowski K, Ziegler M, Piaszinski K, Rittscher K. 2016 The Standard FISH
401 Procedure. In Springer Protocols Handbooks. pp. 109–118. Springer Berlin
402 Heidelberg. (doi:10.1007/978-3-662-52959-1_9)
- 403 27. Makunin AI *et al.* 2016 Contrasting origin of B chromosomes in two cervids (Siberian
404 roe deer and grey brocket deer) unravelled by chromosome-specific DNA sequencing.

- 405 *BMC Genomics* **17**, 618. (doi:10.1186/s12864-016-2933-6)
- 406 28. Kichigin IG *et al.* 2016 Evolutionary dynamics of Anolis sex chromosomes revealed
407 by sequencing of flow sorting-derived microchromosome-specific DNA. *Mol. Genet.*
408 *Genomics* **291**, 1955–1966. (doi:10.1007/s00438-016-1230-z)
- 409 29. Alföldi J *et al.* 2011 The genome of the green anole lizard and a comparative analysis
410 with birds and mammals. *Nature* **477**, 587–591. (doi:10.1038/nature10390)
- 411 30. Porter CA, Haiduk MW, Queiroz K DE. 1994 Evolution and Phylogenetic
412 Significance of Ribosomal Gene Location in Chromosomes of Squamate Reptiles.
413 *Copeia* , 302–313. (doi:10.2307/1446980)
- 414 31. Lisachov AP, Trifonov VA, Giovannotti M, Ferguson-Smith MA, Borodin PM. 2017
415 Immunocytological analysis of meiotic recombination in two anole lizards (Squamata,
416 Dactyloidae). *Comp. Cytogenet.* **11**, 129–141.
417 (doi:10.3897/CompCytogen.v11i1.10916)
- 418 32. Srikulnath K, Uno Y, Nishida C, Matsuda Y. 2013 Karyotype evolution in monitor
419 lizards: Cross-species chromosome mapping of cDNA reveals highly conserved
420 synteny and gene order in the Toxicofera clade. *Chromosom. Res.* **21**, 805–819.
421 (doi:10.1007/s10577-013-9398-0)
- 422 33. Rice WR. 1987 The Accumulation of sexually Antagonistic genes as a selective agent
423 promoting the evolution of reduced recombination between primitive sex
424 chromosomes. *Evolution (N. Y.)*. **41**, 911–914. (doi:10.1111/j.1558-
425 5646.1987.tb05864.x)
- 426 34. Charlesworth D, Charlesworth B. 1980 Sex differences in fitness and selection for
427 centric fusions between sex-chromosomes and autosomes. *Genet. Res.* **35**, 205–214.
428 (doi:10.1017/S0016672300014051)
- 429 35. Charlesworth B, Wall JD. 1999 Inbreeding, heterozygote advantage and the evolution
430 of neo-X and neo-Y sex chromosomes. *Proc. R. Soc. London. Ser. B Biol. Sci.* **266**,
431 51–56. (doi:10.1098/rspb.1999.0603)
- 432 36. Pennell MW, Kirkpatrick M, Otto SP, Vamosi JC, Peichel CL, Valenzuela N, Kitano J.
433 2015 Y Fuse? Sex Chromosome Fusions in Fishes and Reptiles. *PLoS Genet.* **11**.
434 (doi:10.1371/journal.pgen.1005237)
- 435 37. Giovannotti M *et al.* 2017 New insights into sex chromosome evolution in anole
436 lizards (Reptilia, Dactyloidae). *Chromosoma* **126**, 245–260. (doi:10.1007/s00412-016-
437 0585-6)
- 438 38. Lisachov AP, Makunin AI, Giovannotti M, Pereira JC, Druzhkova AS, Caputo
439 Barucchi V, Ferguson-Smith MA, Trifonov VA. 2019 Genetic Content of the Neo-Sex
440 Chromosomes in Ctenonotus and Norops (Squamata, Dactyloidae) and Degeneration
441 of the Y Chromosome as Revealed by High-Throughput Sequencing of Individual
442 Chromosomes. *Cytogenet. Genome Res.* **157**, 115–122. (doi:10.1159/000497091)
- 443 39. Rovatsos M, Farkačová K, Altmanová M, Johnson Pokorná M, Kratochvíl L. 2019
444 The rise and fall of differentiated sex chromosomes in geckos. *Mol. Ecol.* **28**, 3042–
445 3052. (doi:10.1111/mec.15126)
- 446 40. Rovatsos M, Vukić J, Kratochvíl L. 2016 Mammalian X homolog acts as sex
447 chromosome in lacertid lizards. *Heredity* **117**(1), 8–13. (doi:10.1038/hdy.2016.18)
- 448 41. O’Meally D, Miller H, Patel HR, Marshall Graves JA, Ezaz T. 2010 The first
449 cytogenetic map of the tuatara, *sphenodon punctatus*. *Cytogenet. Genome Res.* **127**,
450 213–223. (doi:10.1159/000300099)
- 451 42. Lisachov AP, Poyarkov N, Pawangkhanant P, Borodin P, Srikulnath K. 2018 New
452 karyotype of *Lygosoma bowringii* (Günther, 1864) suggests cryptic diversity.
453 *Herpetol. Notes* **11**, 1083–1088.
- 454 43. Nambiar M, Smith GR. 2016 Repression of harmful meiotic recombination in

455 centromeric regions. *Semin Cell Dev Biol* **54**, 188-197. (doi:
456 10.1016/j.semcdb.2016.01.042)
457