Chromosome Research (2010) 18:371–382
DOI 10.1007/s10577-010-9121-3

Probing the meiotic mechanism of intergenomic exchanges by genomic in situ hybridization on lampbrush chromosomes of unisexual *Ambystoma* (Amphibia: Caudata)

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Received: 14 January 2010 / Revised: 16 February 2010 / Accepted: 18 February 2010 / Published online: 1 April 2010

Abstract The meiotic mechanism of unisexual salamanders in the genus *Ambystoma* was previously explained by observing lampbrush chromosomes (LBCs). In polyploid unisexual females, a pre-meiotic endomitotic event doubles the chromosome number so that, after meiotic reduction, the mature eggs have the same ploidy as the female. It was assumed that synapses during meiotic I prophase, which result in observed bivalents, join duplicated sister chromosomes. Previous studies also found LBC quadrivalents in some oocytes that could be explained by occasional synapses between homologs. The discovery of widespread intergenomic exchanges among unisexual populations has prompted new speculations on this meiotic mechanism. Synapses that involve homeologous chromosomes may be frequent during meiosis and could be responsible for intergenomic exchanges and the high embryonic mortality of unisexuals. Furthermore, LBC quadrivalents may be established by associations between homeologous rather than homologous chromosomes. The present study investigated these two important aspects pertaining to the mechanism of intergenomic exchanges: the frequency of homeologous synapses and the relationship between homeologous associations and meiotic quadrivalents. We applied genomic in situ hybridization (GISH) on LBCs from oocytes of 14 triploid and two tetraploid unisexual females. Homeologous bivalents were not observed, and all 13 LBC quadrivalents that we found were the result of homologous synapses and were not associated with any homeologous or exchanged LBCs. Intergenomic exchanges were used as markers to compare the same chromosomes at meiotic diplotene and mitotic metaphase stages. We conclude that contemporary intergenomic exchanges are very rare, and no direct link exists between intergenomic exchanges and high embryonic mortality. The actual mechanisms and evolutionary implications of intergenomic exchanges appear to be complicated and difficult to assess. The application of GISH-type molecular cytogenetic techniques will help to improve our understanding of the role that intergenomic interactions play in the persistence of unisexual *Ambystoma* and other unisexual vertebrates.

Responsible Editor: Herbert Macgregor.

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Keywords GISH · intergenomic interactions · LBCs · pre-meiotic endomitosis · quadrivalents · unisexual salamanders

Acronyms and Abbreviations

FITC	Fluorescein isothiocyanate

GISH	Genomic in situ hybridization
J	*Ambystoma jeffersonianum* chromosome
L	*Ambystoma laterale* chromosome
L1q (as an example for exchanged chromosomes)	The exchanged portion of the q arm of L1 from J in a unisexual individual
LBC	Lampbrush chromosome
LJJ	*Ambystoma laterale-2 jeffersonianum.* Unisexual (female) triploid salamander possessing one chromosome set of *A. laterale* and two chromosome sets of *A. jeffersonianum*
LLJ	*Ambystoma* 2 *laterale-jeffersonianum.* Unisexual (female) triploid salamander possessing two chromosome sets of *A. laterale* and one chromosome set of *A. jeffersonianum*
MC	Metaphase chromosome
MS222	Tricaine methanesulfonate
PI	Propidium iodide
SDS	Sodium dodecyl sulfate
SSC	Sodium chloride sodium citrate

Introduction

All female unisexual salamanders in the genus *Ambystoma* are the most ancient unisexual vertebrates known to exist (Hedges et al. 1992; Spolsky et al. 1992; Bogart et al. 2007). Via kleptogenesis, a flexible form of sperm-dependent reproduction (Bogart et al. 2007; Mable 2007; Avise 2008), unisexual individuals co-evolve with five distinct sexual congeners (*A. laterale*, *A. jeffersonianum*, *A. tigrinum*, *A. texanum*, and *A. barbouri*) throughout the Great Lake areas in North America (Uzzell 1963, 1964; Downs 1978; Morris 1985; Petranka 1998; Bogart and Klemens 2008; Bogart et al. 2009). Unisexual individuals persist as a "parasitic entity" by stealing sperm and incorporating nuclear genomes from sympatric sexual *Ambystoma*. So far, more than 30 diploid and polyploid biotypes have been discovered (Bogart et al. 2007; Bi et al. 2008a; Bogart et al. 2009).

Unisexual females usually undergo a cryptic chromosome duplication event, which likely takes place in an oogonial mitotic division before meiosis I. This process is termed a "pre-meiotic endomitosis" (Fig. 1) and was suggested and predicted by Macgregor and Uzzell (1964) through the cytogenetic examination of the lampbrush chromosomes (LBCs) in a few triploid females of unisexual *Ambystoma*. LBCs are known to occur at the early diplotene stage of meiosis I and appear as greatly enlarged transitory structures in many animals with the exception of mammals and some insects (Callan 1986; Macgregor 1993; http://projects.exeter.ac.uk/lampbrush/). In sexual organisms, LBCs form homologous bivalents which represent the haploid chromosome complement. All sexual ambystomatids have $2n=28$ chromosomes (King 1990; Sessions 2008), a haploid number of 14, so the number of LBC bivalents is 14 (Kezer et al. 1980; Callan 1986). Macgregor and Uzzell (1964) observed that most diplotenic oocytes of unisexual triploids ($3n=42$)

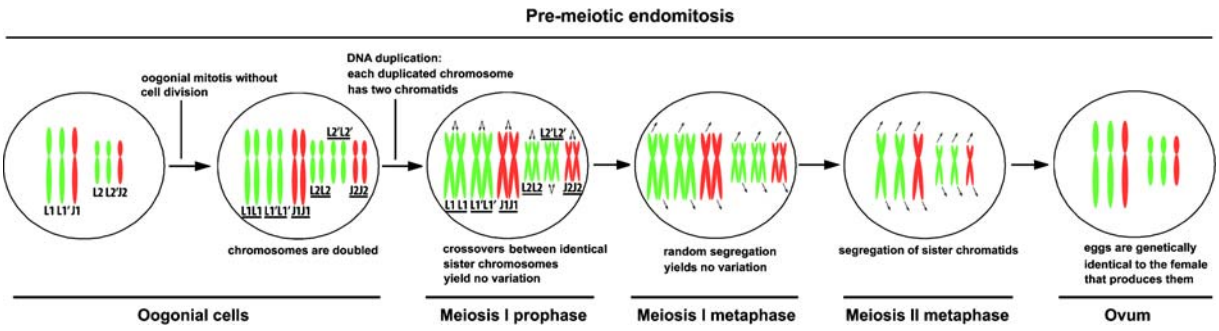

Fig. 1 A possible cytological mechanism of pre-meiotic endomitosis in unisexual *Ambystoma* (Macgregor and Uzzell 1964). The ideogram is modified from that described by Dawley (1989) with a triploid LLJ oocyte as an example. *Ambystoma laterale* chromosomes are shown in *green* and *A. jeffersonianum* chromosomes are shown in *red*. The release of polar bodies during meiosis is omitted from the ideogram. L and L′ are homologs. L (L′) and J are homeologs. The exact timing and mechanism of pre-meiotic endomitosis in the oocytes of unisexual *Ambystoma* is unknown

contained 84 LBCs forming 42 paired bivalents (hexaploid). This striking finding suggests that unisexual females embrace a pre-meiotic whole genome duplication process that doubles the chromosome number and, after meiotic reduction, mature eggs would have the same ploidy level (unreduced) as the females that produce them (Bogart 2003).

Unlike normal meiotic bivalents, synapses in unisexuals' LBCs are expected to only take place between the pre-meiotic duplicated sister chromosomes creating pseudo-bivalents where recombination would not result in any genetic variation (Macgregor and Uzzell 1964). Nevertheless, Macgregor and Uzzell (1964) found rare LBC quadrivalents in a few oocytes. They hypothesized that these chromosome configurations arise as a consequence of sufficient segregation of duplicated sister chromosomes (Fig. 2a) and predicted that such events would allow occasional gene exchange to occur between homologs. Bogart (2003) examined LBCs from additional unisexual females including diploid, triploid, and tetraploid biotypes. His data support the existence of pre-meiotic endomitosis. While most LBCs formed bivalents, rare non-sister homologous associations and quadrivalents were also observed. Reduced ploidy was discovered in a small portion of oocytes, which might provide a necessary foundation for genome replacement and intergenomic interactions (Bogart 2003; Bi et al. 2007a, 2008a).

The utilization of genomic in situ hybridization (GISH) enables us to distinguish homeologous chromosomes from their homologous complements in hybrid genomes (Schwarzacher et al. 1989; Raina and Rani 2001). The discovery of intergenomic recombinations and translocations in unisexual *Ambystoma* by GISH (Bi and Bogart 2006; Bi et al. 2007a, b, 2008b, 2009) rejects the long-held prediction that homeologous genomes evolve independently (Uzzell 1970) and provides evidence that meiotic crossovers must also occur between homeologous chromosomes. Intergenomic exchanges rely on associations between homeologous chromosomes and such processes are expected to more likely take place in reduced oocytes that bypass the pre-meiotic endomitosis (Bi et al. 2007a), although a case of possible exchanges after duplications was also reported (Bi et al. 2007b). The frequency of homeologous association during meiosis remains unknown, but the prevalence and continuous detection of various patterns of intergenomic exchanges in somatic cells from unisexuals suggest that it may be a common event. Furthermore, frequent and rapid chromosomal restructurings might be responsible for the high embryonic mortality that is a common feature for various unisexual populations (Bogart and Licht 1986; Bogart et al. 1987). Bi et al. (2007a) also suggested that the intergenomic exchanges could be linked with LBC quadrivalents and hypothesized that meiotic quadrivalents are associated with synapses between homeologous chromosomes (Fig. 2b) or are initiated by synapses of homosequential regions of exchanged homeologous chromosomes (Fig. 2c). In order to better understand the origin and mechanism of intergenomic exchanges, we employed GISH on LBCs.

The purposes of our study were: 1) to identify the composition of LBC bivalents and examine the fre-

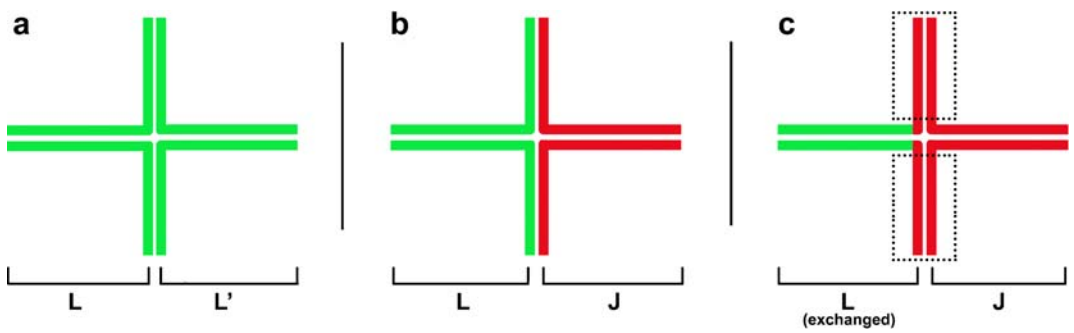

Fig. 2 The hypothetical origins of LBC quadrivalents. *Ambystoma laterale* LBCs are shown as *green* and *A. jeffersonianum* LBCs are shown as *red*. L and L′ are homologs, and L and J are homeologs. L (exchanged) represents an example for exchanged chromosomes. Each halve-quadrivalent represents a bivalent that contains four identical sister chromatids. **a** A quadrivalent initiated via synapsis between homologous chromosomes; **b** a quadrivalent initiated via synapsis between homeologous chromosomes that may result in intergenomic exchanges; **c** a quadrivalent initiated via partial homologous paring (*dashed boxes*) between homeologous chromosomes. The exchanged L contains a chromosome segment from J

quency of homeologous synapses; and 2) to test the alternative hypotheses by Macgregor and Uzzell (1964) and Bi et al. (2007a), whether the LBC quadrivalents result from association between homologous or homeologous chromosomes. Our predictions were that if ongoing intergenomic exchanges were present in meiosis I, homeologous bivalents and intergenomic crossovers would be identified via GISH because each halve-bivalent would have a different fluorescence. Likewise, if LBC quadrivalents embrace homeologs or exchanged chromosomes, such configurations would be visualized by GISH and be shown as having mixed fluorescence (Fig. 2b, c). We chose two dominant triploid biotypes (Lowcock et al. 1987) of unisexual *Ambystoma*: *A. laterale*-2 *jeffersonianum* (LJJ) and *A.* 2 *laterale-jeffersonianum* (LLJ) and their tetraploid derivatives (LJJJ and LLLJ).

Materials and methods

Samples

Salamanders were collected from the field or chosen from those that were maintained in the University of Guelph's animal holding facility. Some individuals were raised for 4 years from eggs hatched in the laboratory and whose patterns of intergenomic exchanges were known from chromosome samples taken during larval development. Chosen females had well-developed ovaries as judged by abdomens that were swollen just anterior to their cloacal regions. Sixteen unisexual females, collected as adults or raised from eggs, represented eight isolated populations in Ontario, Quebec, and Indiana (Table 1). It was a prolonged study because suitable unisexual females for LBC preparations are not easily obtained. The biotypes of the females were identified using microsatellite deoxyribonucleic acid (DNA) analyses or GISH examination of their metaphase chromosomes (Bi and Bogart 2006; Bogart et al. 2007). In total, 102 oocytes were examined.

LBC preparation

The procedure for LBC preparation has been described by Callan (1986), Sessions (1996) and is explained in detail on the website http://projects.exeter.ac.uk/lampbrush/. We used a 10× stock phosphate buffer (35 mM KH_2PO_4+35 mM Na_2HPO_4) that was diluted with double deionized water to 1× and used as water for oocyte isolation saline and LBC dispersing saline. The

Table 1 GISH examination of LBCs from unisexual females

JPB catalog number and biotype	Population sampled	Sampling site (county, state/province)	"Fall" or "spring" preparation	No. of oocytes examined	No. of oocytes that contained quadrivalents	Inherited exchanged LBCs present?
39018 LJJJ	Ancaster	Hamilton-Wentworth, Ontario	Spring	4	0	Yes
39757 LJJ	Ancaster	Hamilton-Wentworth, Ontario	Fall	3	0	Yes
40380 LJJ	Ancaster	Hamilton-Wentworth, Ontario	Fall	4	0	Yes
40381 LJJ	Ancaster	Hamilton-Wentworth, Ontario	Fall	5	0	Yes
36659 LLJ	Burlington	Halton, Ontario	Fall	2	0	Yes
40194 LJJ	Kitchener	Waterloo, Ontario	Spring and fall	6 and 8	1(1), 1(4)	Yes
38873 LLJ	La Pêche	La Vallée-de-la-Gatineau, Quebec	Fall	20	1(2), 1(2), 1(1)	Yes
38857 LLLJ	McPherson Tract	Waterloo, Ontario	Fall	7	1(1), 1(1)	No
38792 LLJ	Wakefield	La Vallée-de-la-Gatineau, Quebec	Fall	4	0	No
38798 LLJ	Wakefield	La Vallée-de-la-Gatineau, Quebec	Fall	9	1(1)	No
38801 LLJ	Wakefield	La Vallée-de-la-Gatineau, Quebec	Fall	3	0	No
36660 LJJ	Waterdown	Hamilton-Wentworth, Ontario	Fall	11	0	Yes
36798 LJJ	Waterdown	Hamilton-Wentworth, Ontario	Fall	6	0	Yes
39004 LJJ	Waterdown	Hamilton-Wentworth, Ontario	Spring	5	0	Yes
38842 LLJ	West Pond	St. Joseph, Indiana	Fall	2	0	No
38844 LLJ	West Pond	St. Joseph, Indiana	Fall	3	0	No

isolation saline was 0.1 M KCl. The dispersing saline used three parts isolation saline, seven parts 1× phosphate buffer, and two drops of concentrated formalin for 100 ml of dispersing saline. Observation chambers were constructed of 3-mm Plexiglas circles having a diameter of 24 mm and a central 7-mm hole. The floor of the chamber was an 18-mm square #2 cover slip that covered the hole and was sealed to the Plexiglas circle with paraffin. Salamanders were killed by prolonged anesthesia in a 7% solution of buffered (pH7.0) tricaine methanesulfonate (MS222, Sigma, USA). One or both ovaries were removed and were placed in embryo cups containing isolation saline. Embryo cups were sealed with Parafilm® (Fisher, USA) and kept at 4°C in a refrigerator or on ice while the eggs were being used. Dispersion of the LBCs was monitored using an inverted microscope and phase contrast optics. The observation chambers were placed in a humid chamber and the LBCs were allowed to disperse overnight at 4°C. Melted Vaseline was used to seal the edges of the round cover slip. The observation chambers were inserted in 3×10 cm centrifuge tubes and centrifuged in a swinging bucket at 5,000 rps for 5 min, followed by 12,000 rps for 7 min. The observation chamber was removed from the centrifuge tube and submerged in 70% EtOH, where the round cover slip was slid off the top of the observation chamber. The 18-mm cover slip was pried from the bottom with a small scalpel and placed in 70% EtOH in a small coplin jar overnight and then allowed to dry. Paraffin wax was removed from the 18-mm cover slip with a small scalpel, and the cover slip, with the LBCs on top, was mounted on a microscope slide using Permount® (Fisher). The preparation was kept at room temperature for 7 days and then frozen at −20°C.

Genomic in situ hybridization

Total genomic DNA from *A. laterale* and blocking DNA from *A. jeffersonianum* was extracted using a standard phenol/chloroform/isoamyl alcohol method (Sambrook and Russell 2001). *Ambystoma laterale* genomic DNA was labeled with digoxigenin using digoxigenin-nick translation kits (Roche, Switzerland). The hybridization mix consisted of 5 ng/μl *A. laterale* genomic probes, approximately 50-fold concentration of *A. jeffersonianum* blocking DNA, 2× sodium chloride sodium citrate (VWR, USA), 50% deionized formamide (Fisher), 10% dextran sulphate salt (Sigma), 1× Denhardt's (Sigma), 0.1% sodium dodecyl sulfate (SDS) (Sigma), and 500 ng/μl salmon testes DNA (Promega, USA). GISH was conducted on LBC preparations following the procedure described by Bi and Bogart (2006). The post hybridization washing procedure was similar to that outlined by Bi and Bogart (2006) and Bi et al. (2007a). Digoxigeninated *A. laterale* whole genome probes were visualized using anti-digoxigenin antibodies conjugated with fluorescein isothiocyanate (FITC) (Roche), and LBCs were counterstained with propidium iodide (PI) (Vector, UK). After in situ hybridization, LBCs were observed using a Leica fluorescent microscope equipped with the appropriate filter sets for FITC and PI. Green and red digital images were recorded using a charge-coupled device camera and analyzed with the software Openlab 3.5.0.

Results and discussion

GISH on lampbrush bivalents

The best LBC preparations were obtained using oocytes from mature unisexual females sacrificed in October and November (Fig. 3a), but accurate counting of LBC bivalents in these "fall" preparations was a difficult task owing to their extremely extended and overlapped structures. Multivalents and chromosome loss during the nuclei isolation process may also cause miscounting of the LBCs. Fewer than one fifth of the "fall" LBC preparations were countable. The majority of countable LBC preparations from triploid animals was found to have fewer than 42 but more than 28 bivalents, which still was an indication of chromosome doubling. Some females had some oocytes, in which LBC bivalents were fewer than 28, which might be an indication of reduced ploidy as suggested by Bogart (2003). Chromosome preparations from oocytes in early spring (March and April) contained less stretched to highly compact chromosomes, so they were easier to count (Fig. 3b). Lateral chromatin loops are one of the major features of LBCs that are associated with intensive ribonucleic acid transcription (Macgregor 1993; Galkina et al. 2006). "Spring" oocytes are close to ovulation and have compact bivalents without loops because of the shutdown of transcriptional activity (León and Kezer 1990), so chromosomes in "spring" preparations were actually no longer LBCs. Because the existence of

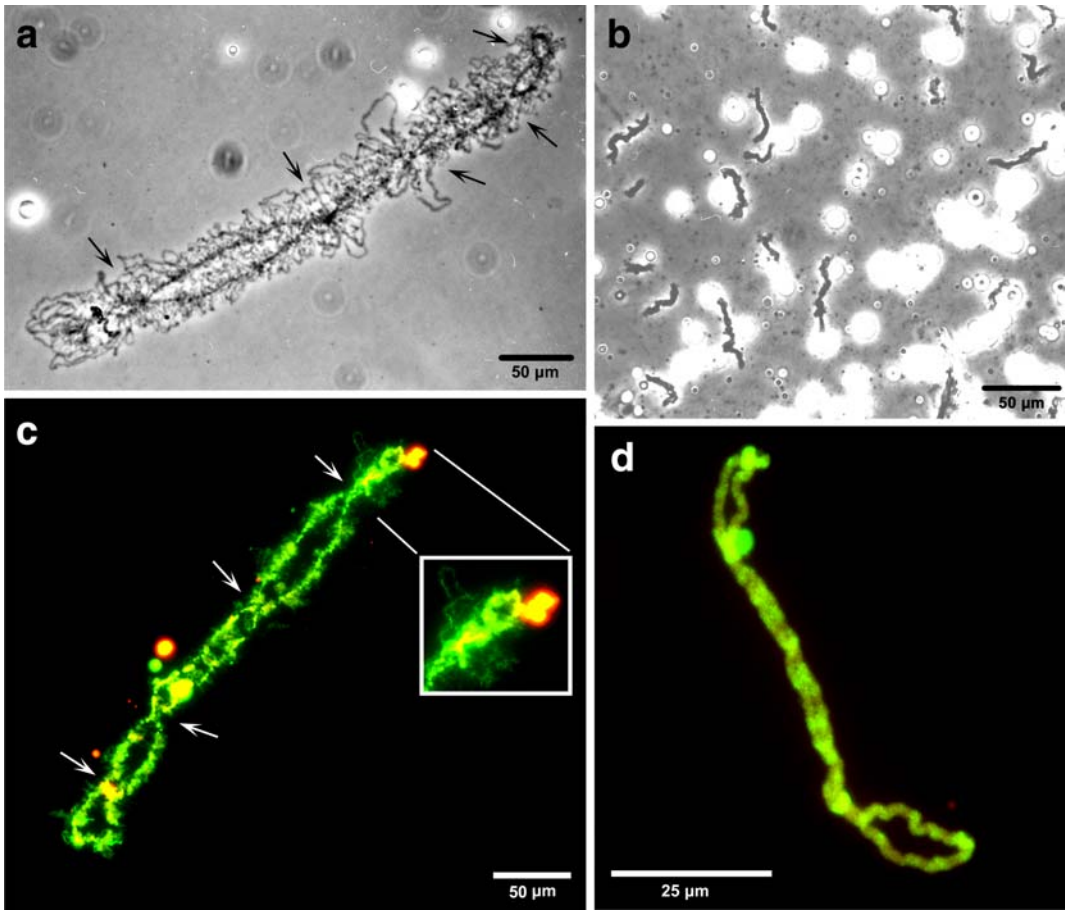

Fig. 3 Examples of LBC and meiotic metaphase bivalents by phase contrast (**a**, **b**) and fluorescent microscopy (**c**, **d**). The LBCs **(a**, **c)** were obtained from "fall" preparations, and the chromosomes (**b**, **d)** were obtained from "spring" preparations. *Ambystoma laterale* chromosomes were painted with green fluorescence by GISH (**c**, **d**). *Single arrows* **(a**, **c)** point to possible chiasmata. The *boxed* chromosome fragment (**c**) represents an enlarged section of the LBC and shows the stronger GISH signals on LBC main axis compared with the weaker GISH signals on chromatin loops. The *scale bars* represent 50 μm (**a**–**c)** and 25 μm (**d)**

pre-meiotic endomitosis, chromosome doubling, and "reduced" triploid oocytes have been empirically demonstrated in triploid unisexual *Ambystoma* (Macgregor and Uzzell 1964; Bogart 2003) and have also been observed in other unisexual vertebrates (e.g., Cuellar 1971; Itono et al. 2006), our focus for this study was not to accurately count the LBCs but to search for potential homeologous associations.

GISH revealed a clear distinction between LBCs of *A. laterale* (L) and *A. jeffersonianum* (J) in all preparations, but it could not discriminate real bivalents (homologous chromosomes) from pseudo-bivalents (duplicated sister chromosomes) because all L chromosomes hybridized with *A. laterale*-labeled genomic probes. Chiasmata were commonly observed in LBC bivalents in "fall" preparations by light microscopy and still easily identifiable after GISH (Fig. 3a, c). LBC main axes are composed of relatively higher condensed chromatin compared with the greatly uncompacted lateral loops (Callan 1986; Austin et al. 2009). As a result, although some loop structures were observable by GISH, their signals were generally much weaker compared to those along the main axes of the LBCs (Fig. 3c). An example of "spring" bivalents by GISH is shown in Fig. 3d, loops are not obvious in bivalents at this stage.

Homeologous LBC bivalent (L–J) would be confirmed if each halve-bivalent fluoresced with a

different color by GISH. We did not find homeologous bivalents in any of the 102 LBC preparations we examined, which included possible "reduced" oocytes. Rather, all the bivalents were exclusively formed by synapses of homologous or duplicated sister chromosomes. This zero detection of homeologous bivalents was unexpected, although such results could be biased by sampling issues such as the number of females used, the low percentage of oocytes sampled per female, and the relatively small number of populations sampled. Even so, the actual rate of contemporary intergenomic exchanges must be low and contradicts observations of the prevalence of intergenomic exchanges in somatic cells of unisexual salamanders across various populations. Through an extensive investigation of mitotic metaphase chromosomes of 154 *A. jeffersonianum* sperm-dependent (LJJ and LJJJ) and *A. laterale* sperm-dependent (LLJ and LLLJ) specimens collected from 30 populations, 85% of the *A. jeffersonianum*-dependent and 23% of the *A. laterale*-dependent populations were found to contain intergenomic exchanges (Bi et al. 2008b, 2009). These various intergenomic exchanges must have originated from meiotic crossovers or translocations between homeologs. Mitotic exchanges in somatic cells are unlikely because no mosaic cells from the individuals carrying different exchanges have been ever discovered and, where data are available, females contain the same exchanged pattern as found in their offspring. More importantly, mitotic intergenomic exchanges would be less likely to spread throughout the populations. Our LBC data show that contemporary homeologous exchanges are much less frequent than we expected. Nevertheless, a very low frequency of homeologous associations would help to explain why novel and unique intergenomic exchanges are rare in unisexual populations. Exchanged chromosomes are formed historically and are subsequentially shared by multiple individuals in various unisexual populations (Bi et al. 2008b).

High embryonic mortality is typically associated with unisexual salamanders (Piersol 1910; Clanton 1934). For example, hatching rate for unisexual populations is 19.5% in Kelleys Island, Ohio (Bogart et al. 1987), 21.4% in Pelee Island, Ontario (Bogart and Licht 1986), and 30.5% in a population in southern Ohio (Bogart et al. 2009). The hatching rate also varies among collected egg masses from the same populations (Bogart et al. 2009). If this phenomenon is attributed to extensive intergenomic reconstructions, as previously speculated (Bi et al. 2007a), then some ongoing "lethal" intergenomic interactions would be expected and observed by GISH during meiosis. The rationale is that many intergenomic exchanges would be lethal and would not be observed in mitotic metaphases of somatic cells from surviving larvae. If, however, as our study reveals, contemporary intergenomic exchanges are very rare, they cannot be considered as a serious contributor to the high mortality observed in unisexual populations.

Inherited exchanged LBCs in meiosis

Inherited intergenomic exchanges, which are derived historically and passed on by the progenitors, were observed in well-spread "spring" or "fall" LBC preparations of some unisexual individuals (Table 1, Figs. 4 and 5). Exchanged LBCs underwent premeiotic endomitosis and formed pseudo-bivalents, none of which was involved in any homeologous associations. Exchanged LBCs were observed in unisexual females from Waterdown and Ancaster, where intergenomic exchanges were discovered from mitotic metaphase preparations in previous studies (Bi and Bogart 2006; Bi et al. 2007a, 2008b). New intergenomic exchanges were detected in LBC preparations from females JPB38873 (LLJ, La Pêche) and JPB40194 (LJJ, Kitchener). Intergenomic exchanges are effective cytogenetic markers for chromosome identification and were used to construct a genealogy of unisexual populations in southern Ontario (Bi et al. 2008b). As shown in Fig. 4, L1q (an exchanged chromosome segment from J is located on the q arm of L chromosome 1) is one of the most common exchanged chromosomes in southern Ontario, and L6q and L14q are unique exchanges confined to the population at Waterdown. Figure 4 vividly demonstrates the giant size of diplotenic LBCs by comparison of the same exchanged chromosomes from mitotic metaphases. These exchange markers enable us to identify the same chromosomes with confidence at different developmental stages. By comparison, the LBCs were found to be, on average, more than 25 times longer than the corresponding mitotic metaphase chromosomes.

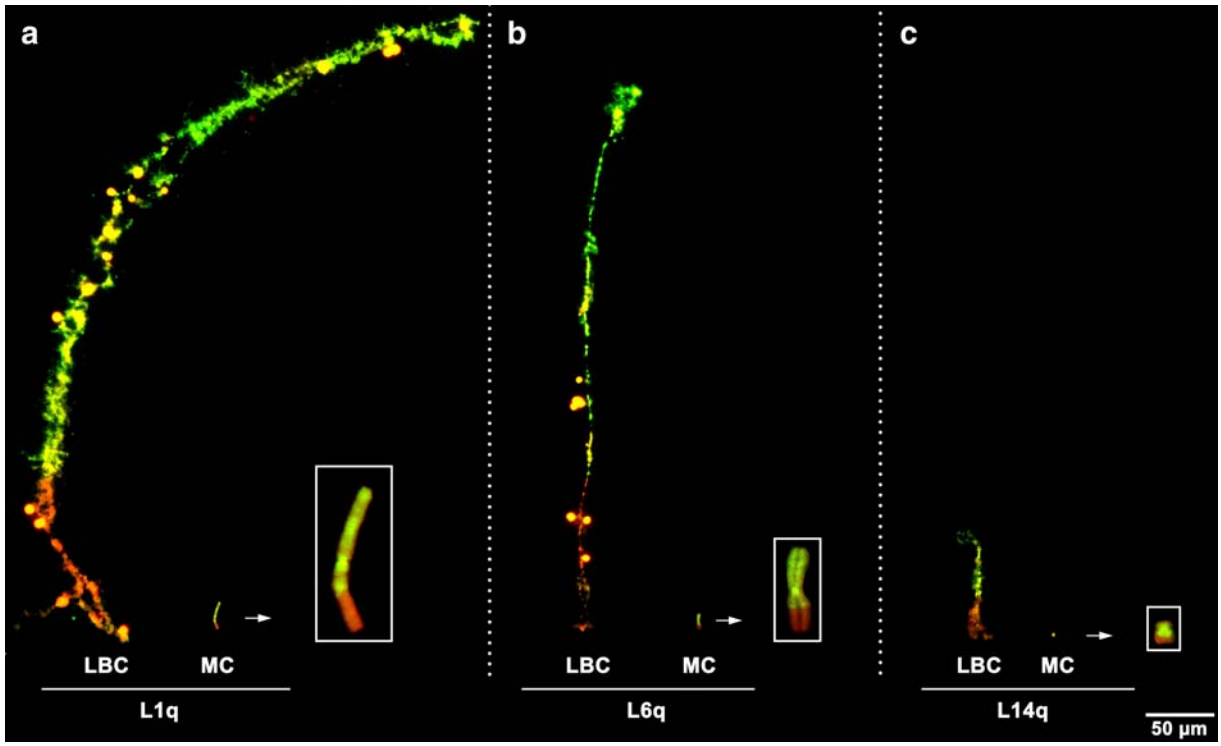

Fig. 4 Size comparison between exchanged LBCs and their corresponding mitotic metaphase chromosomes. GISH clearly distinguishes the *A. laterale* LBCs/segments (*painted green*) from the *A. jeffersonianum* LBCs/segments (*counterstained red*). In this figure, "MC" represents a mitotic metaphase chromosome. The photos of LBCs are homologous bivalents that contain exchanged segments and the corresponding mitotic metaphase exchanged chromosomes. Both were taken at the same magnification (×1,000). **a** L1q is one of the most widespread exchanged chromosomes found in many unisexual populations in southern Ontario. **b, c** L6q and L14q are unique exchanged chromosomes discovered in the unisexual population of Waterdown in southern Ontario. The *boxed* chromosome in each picture represents an enlarged mitotic metaphase chromosome (see Bi et al. 2008b) providing a clearer view of the exchanged segment. The *white scale bar* represents 50 μm

Unisexual female JPB40194 (LJJ) was collected and processed in fall 2009, so its oocytes should have only contained extended diplotenic LBCs. Interestingly, we found that the ovaries from this individual had oocytes that contained LBCs at distinctly different stages of contraction. Eight LBC preparations examined had extended "fall"-like LBCs, while six preparations all had condensed LBCs and resembled those in "spring" preparations. Through his examination of LBCs from 41 female *Ambystoma*, Bogart (2003) found that oocytes from the same female were always at the same developmental stage. The mechanism responsible for non-synchronic oogenesis is not known for *Ambystoma*, but other amphibians often have oocyte nuclei at different stages of meiotic condensation within the same ovary (Callan 1986). Using intergenomic exchanges as markers, we compare LBCs that were observed from oocytes at different meiotic stages in Fig. 5.

Lampbrush quadrivalents are initiated by homologous synapses

LBC quadrivalents have previously been observed in unisexual *Ambystoma* (Macgregor and Uzzell 1964; Bogart 2003). Macgregor and Uzzell (1964) hypothesized that LBC quadrivalents are generated through homologous synapses which allow gene exchanges between homologs. Quadrivalents have regular and predictable configurations (i.e., four arms; Fig. 6). They can easily be distinguished from overlapped bivalents even in poorly dispersed preparations. Bogart (2003) observed more LBC quadrivalents as well as some possible non-sister chromosome associations from sampled unisexual females. Bi et al. (2007a) proposed that LBC quadrivalents might be facilitated by intergenomic interactions. With the application of GISH to differentiate the genomes in unisexuals (Bi and Bogart 2006), we re-examined

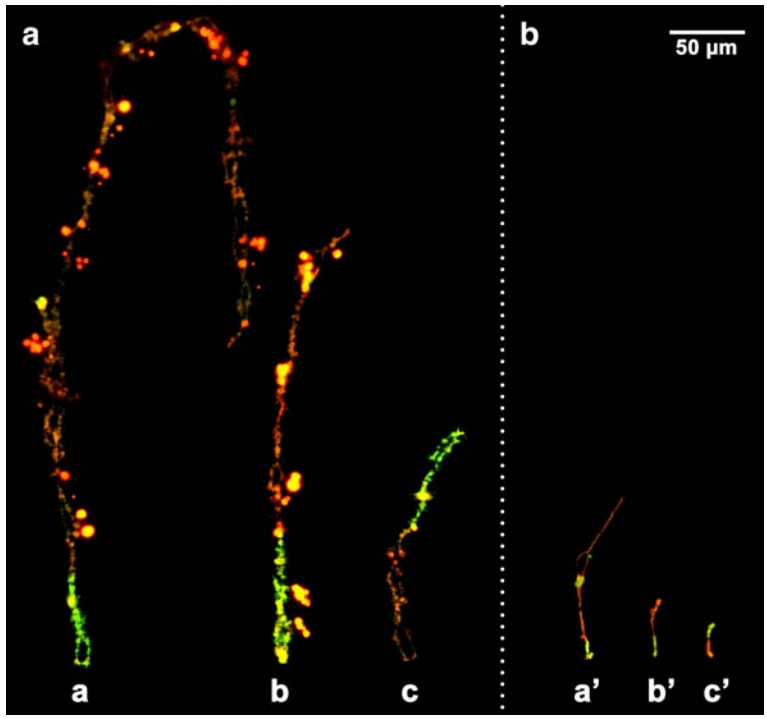

Fig. 5 Exchanged LBCs in two different oocytes from the same unisexual female JPB40194 (LJJ). *Ambystoma laterale* LBCs/segments were *painted green* and *A. jeffersonianum* LBCs/segments were *counterstained red*. The exchanged LBCs *a*, *b*, and *c* were found in the same oocyte (**a**) while the same exchanged chromosomes *a′*, *b′*, and *c′* (**b**) were observed in a different oocyte from the same specimen. These exchanged chromosomes were newly discovered by the present study. The *white scale bar* represents 50 μm

quadrivalent associations in order to determine if quadrivalents involve homologous or homeologous chromosomes and if any of the LBCs in a quadrivalent contained intergenomic exchanges.

We observed 13 LBC quadrivalents. Twelve were found in "fall" preparations and one from a "spring" preparation. Neither univalents nor trivalents were observed. LBC quadrivalents were observed in both *A. jeffersonianum* and *A. laterale* sperm-dependent unisexuals, but their distribution was not uniform by oocytes/females. For example, unisexual females JPB38873 (LLJ) and JPB40194 (LJJ) had the most (five) quadrivalents among all the salamanders that we examined. In *A. jeffersonianum* sperm-dependent specimens, four "fall" LBC quadrivalents were discovered from a single oocyte from unisexual female JPB40194 (LJJ). We could not confirm that some LBCs are more frequently involved in quadrivalent configurations than others because the size of the LBCs varied in different preparations (Fig. 6).

Each of the 13 quadrivalents had the same fluorescence by GISH, proving that they were actually homologous (L–L or J–J) rather than homeologous (L–J) associations (Fig. 6). Exchanged LBCs were present in JPB38873 (LLJ) and JPB40194 (LJJ), but they were not included in any quadrivalent. Our data clearly show that intergenomic interactions are not necessarily linked to quadrivalents, which supports the hypothesis of Macgregor and Uzzell (1964) that LBC quadrivalents in unisexuals are homologous synapses (Fig. 1a). These random homologous synapses demonstrate that crossovers between homologous chromosomes are possible, which may provide genetic recombination and subsequent variation among the offspring of unisexual females (Macgregor and Uzzell 1964; Bogart 2003).

Conclusions

GISH successfully distinguishes LBC homeologs from homologs in the unisexuals' oocytes and provides a powerful tool to examine the meiotic mechanism of intergenomic interaction. Meiotic homeologous synapses should be indispensable for intergenomic exchanges to occur, but our data show that the mechanics of intergenomic exchanges may not be as straightforward as we expected. The rarity of ongoing homeologous interactions, as demonstrated by GISH-LBC, contradicts observations of commonality

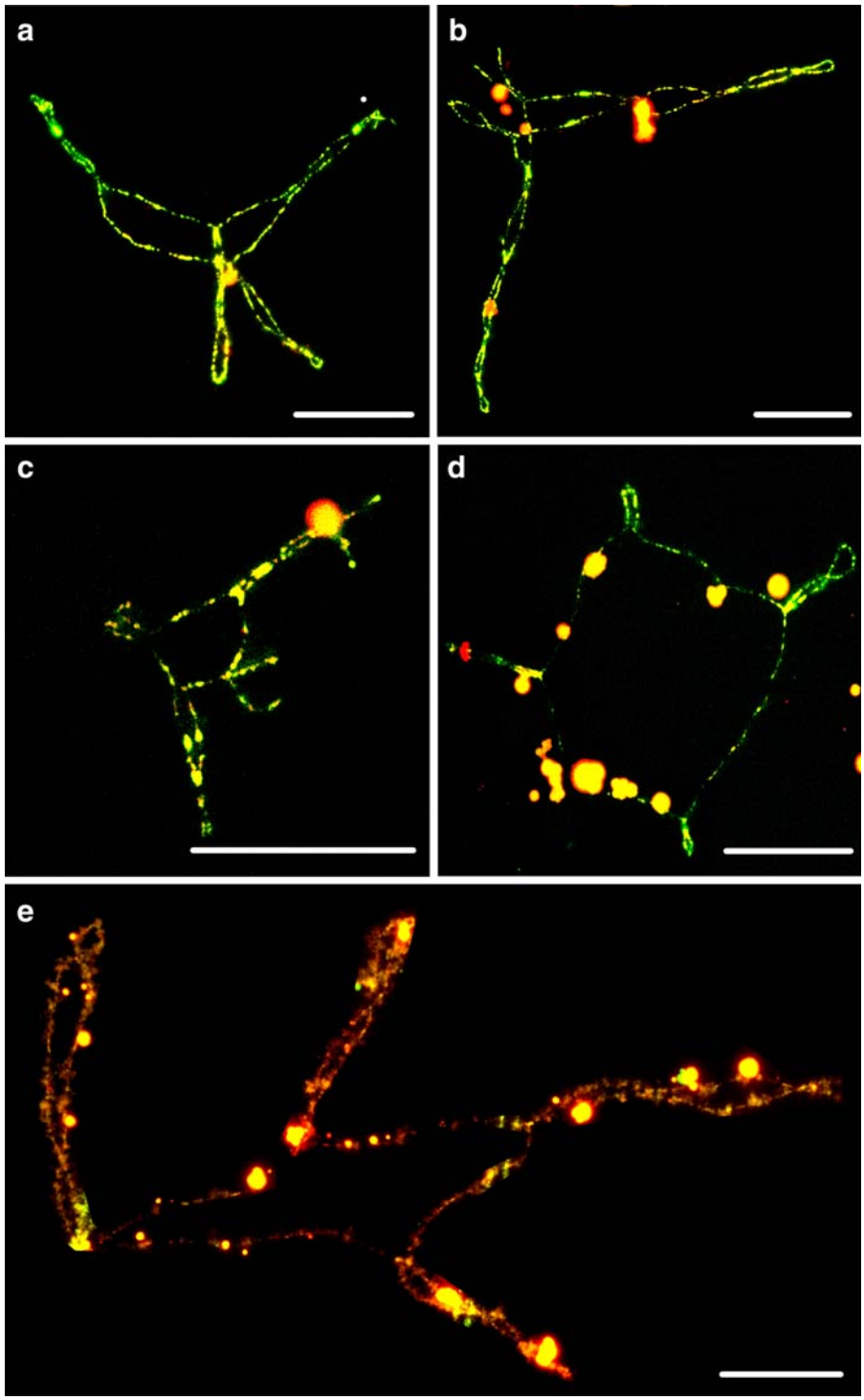

Fig. 6 The composition of LBC quadrivalents revealed by GISH. GISH demonstrates that the LBC quadrivalents observed by the present study were synapses between homologs rather than homeologs. Using GISH, quadrivalents had the same fluorescence (*painted green* or *counter-stained red*), indicating that they were either synapsed via L–L (**a**–**d**) or via J–J (**e**). No exchanged LBC was observed in any quadrivalents. The *white scale bar* in each picture represents 50 μm

of intergenomic exchanges throughout a wide range of unisexual populations (Bi et al. 2008b; Bi et al. 2009). The sporadic occurrence of contemporary intergenomic exchanges is less likely to be a major contributor to the extremely high embryonic mortality in unisexual populations. Our experiments also showed that exchanged LBCs were not involved in any quadrivalents via partial homologous synapses. The

LBC quadrivalents appear to be occasional associations between homologs, which allow a low level of gene exchanges between homologs and would only generate limited genetic variation among offspring.

To obtain a better understanding of intergenomic interactions, it would be important to know the exact timing of pre-meiotic endomitosis and whether homeologous synapses could take place prior to or after self-chromosome duplication. Although we were able to observe lampbrush chromosomes from triploid and tetraploid unisexuals from distant localities that involved four biotypes (Table 1), all of these females possessed only *A. laterale* and *A. jeffersonianum* genomes. It will be of interest to use GISH to examine meiotic chromosomes in diploid unisexuals and in biotypes that include genomic combinations of *A. texanum*, *A. tigrinum*, or *A. barbouri*.

The evolutionary implications of observed intergenomic exchanges in mitotic chromosomes (Bi et al. 2008b) are difficult to evaluate. The lingering questions pertaining to such issues include: (1) possible genome dosage compensation mechanisms in various unisexual biotypes; (2) the importance of an *A. laterale* (L) genome in every unisexual individual; (3) the consequences of having L genomes possessing intergenomic exchanges in vast non-*A. laterale*-dependent unisexual populations, where the L genome is irreplaceable (Bi et al. 2008a); and (4) why are some exchanged chromosomes much more commonly distributed than others. Application of GISH-type molecular cytogenetics in conjunction with other molecular techniques should help to quantify the proportion and types of exchanged segments that are involved in promoting or compromising the sustainability of unisexuals. Finding answers to these questions will not only improve our knowledge of reproductive and evolutionary aspects of unisexual *Ambystoma* but advances our fundamental understanding about the significance of genomic interactions and the regulation of genomic compatibility.

Acknowledgements The authors thank Dr. Brian Husband for providing assistance with the fluorescence microscope. Collection of specimens was assisted by J-F. Désrouches, J. Ball, and J. Pisapio. We thank the Ontario Ministry of Natural Resources (OMNR) and the Animal Care Committee of the University of Guelph for issuing permits for laboratory and field components of this study. A very special thanks to the late J. Kezer for mentoring JPB on lampbrush chromosome preparation. This research is supported by an NSERC discovery grant to JPB.

References

Austin C, Novikova N, Guacci V, Bellini M (2009) Lampbrush chromosomes enable study of cohesin dynamics. Chromosome Res 17:165–184

Avise JC (2008) Clonality: the genetics, ecology, and evolution of sexual abstinence in vertebrate animals. Oxford University Press, Oxford

Bi K, Bogart JP (2006) Identification of intergenomic recombinations in unisexual salamanders of the genus *Ambystoma* by genomic in situ hybridization. Cytogenet Genome Res 112:307–312

Bi K, Bogart JP, Fu J (2007a) Intergenomic translocations in unisexual salamanders of the genus *Ambystoma* (Amphibia, Caudata). Cytogenet Genome Res 116:289–297

Bi K, Bogart JP, Fu J (2007b) Two rare aneutriploids in the unisexual *Ambystoma* (Amphibia, Caudata) identified by GISH indicating two different types of meiotic errors. Cytogenet Genome Res 119:127–130

Bi K, Bogart JP, Fu J (2008a) The prevalence of genome replacement in unisexual salamanders of the genus *Ambystoma* (Amphibia, Caudata) revealed by nuclear gene genealogy. BMC Evol Biol 8:e158

Bi K, Bogart JP, Fu J (2008b) Genealogical relationships of southern Ontario polyploid unisexual salamanders (genus *Ambystoma*) inferred from intergenomic exchanges and major rDNA cytotypes. Chromosome Res 16:275–289

Bi K, Bogart JP, Fu J (2009) An examination of intergenomic exchanges in *A. laterale*-dependent unisexual salamanders in the genus *Ambystoma*. Cytogenet Genome Res 124:44–50

Bogart JP (2003) Genetics and systematics of hybrid species. In: Sever DM (ed) Reproductive biology and phylogeny of Urodela. Science, Enfield, pp 109–134

Bogart JP, Klemens MW (2008) Additional distributional records of *Ambystoma laterale*, *A. jeffersonianum* (Amphibia: Caudata) and their unisexual kleptogens in eastern North America. Am Mus Novit 3627:1–58

Bogart JP, Licht LE (1986) Reproduction and the origin of polyploids in hybrid salamanders of the genus *Ambystoma*. Can J Genet Cytol 28:605–627

Bogart JP, Lowcock LA, Zeyl CW, Mable BK (1987) Genome constitution and reproductive biology of hybrid salamanders, genus *Ambystoma*, on Kelleys Island in Lake Erie. Can J Zool 65:2188–2201

Bogart JP, Bi K, Fu J, Noble D, Niedzwiecki J (2007) Unisexual salamanders (genus *Ambystoma*) present a new reproductive mode for eukaryotes. Genome 50:119–136

Bogart JP, Bartoszek J, Noble DWA, Bi K (2009) Sex in unisexual salamanders: discovery of a new sperm donor with ancient affinities. Heredity 103:483–493

Callan HG (1986) Lampbrush chromosomes. Springer-Verlag, Berlin

Clanton W (1934) An unusual situation in the salamander *Ambystonia jeffersonianum* (Green). Occ Pap Mus Zool Univ Mich 290:1–14

Cuellar O (1971) Reproduction and the mechanism of meiotic restitution in the parthenogenetic lizard *Cnemidophorus uniparens*. J Morphol 133:139–166

Dawley RM (1989) An introduction to unisexual vertebrates. In: Dawley RM, Bogart JP (eds) Evolution and ecology of unisexual vertebrates. Albany: NY State Mus. Bull. 466, pp 1–18

Downs FL (1978) Unisexual *Ambystoma* from the Bass Islands of Lake Erie. Occ Pap Mus Zool Univ Mich 685:1–36

Galkina S, Deryusheva S, Fillon V, Vignal A, Crooijmans R, Groenen M, Rodionov A, Gaginskaya E (2006) FISH on avian lampbrush chromosomes produces higher resolution gene mapping. Genetica 128:241–251

Hedges SB, Bogart JP, Maxson LR (1992) Ancestry of unisexual salamanders. Nature 356:708–710

Itono M, Morishima K, Fujimoto T, Bando E, Yamaha E, Arai K (2006) Premeiotic endomitosis produces diploid eggs in the natural clone loach, *Misgurnus anguillicaudatus* (Teleostei: Cobitidae). J Exp Zool 305A:513–523

Kezer J, Leon P, Sessions SK (1980) Structural differentiation of the meiotic and mitotic chromosomes of the salamander, *Ambystoma macrodactylum*. Chromosoma 81:177–197

King M (1990) Amphibia, vol. 4, Chordata 2. In: John B (ed) Animal cytogenetics. Gebrüder Borntraeger, Berlin Stuttgart, pp 1–241

León P, Kezer J (1990) Loop size in newt lampbrush chromosomes. Chromosoma 99:83–86

Lowcock LA, Licht LE, Bogart JP (1987) Nomenclature in hybrid complexes of *Ambystoma* (Urodela: Ambystomatidae): no case for the erection of hybrid 'species'. Syst Zool 36:328–336

Mable BK (2007) Sex in the postgenomic era. Trends Ecol Evol 22:559–561

Macgregor HC (1993) An introduction to animal cytogenetics. Chapman and Hall, London

Macgregor HC, Uzzell TM (1964) Gynogenesis in salamanders related to *Ambystoma jeffersonianum*. Science 143:1043–1045

Morris MA (1985) A hybrid *Ambystoma platineum*×*A. tigrinum* from Indiana. Herpetologica 41:267–271

Petranka JW (1998) Salamanders of the United States and Canada. Smithsonian Institution, Washington DC

Piersol WA (1910) Spawn and larvae of *Ambystoma jeffersonianum*. Am Natur 44:732–738

Raina SN, Rani V (2001) GISH technology in plant genome research. Methods Cell Sci 23:83–104

Sambrook J, Russell DW (2001) Molecular cloning: a laboratory manual, 3rd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor

Schwarzacher T, Leitch AR, Bennet MD, Heslop-Herrison JS (1989) In situ hybridization of parental genomes in a wide hybrid. Ann Bot 64:315–324

Sessions SK (1996) Chromosomes: molecular cytogenetics. Chapter 5. In: Hillis DM, Moritz C, Mable BK (eds) Molecular systematics. Sinauer, Massachusetts, pp 121–168

Sessions SK (2008) Evolutionary cytogenetics in salamanders. Chromosome Res 16:183–201

Spolsky CM, Phillips CA, Uzzell T (1992) Antiquity of clonal salamander lineages revealed by mitochondrial DNA. Nature 356:706–708

Uzzell TM (1963) Natural triploidy in salamanders related to *Ambystoma jeffersonianum*. Science 139:113–115

Uzzell TM (1964) Relations of the diploid and triploid salamanders of the *Ambystoma jeffersonianum* complex (Amphibia, Caudata). Copeia 1964:257–300

Uzzell TM (1970) Meiotic mechanisms of naturally occurring unisexual vertebrates. Am Nat 104:433–445