Evolution of karyotype, sex chromosomes, and meiosis in mygalomorph spiders (Araneae: Mygalomorphae)

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Spider diversity is partitioned into three primary clades, namely Mesothelae, Mygalomorphae, and Araneomorphae. Mygalomorph cytogenetics is largely unknown. Our study revealed a remarkable karyotype diversity of mygalomorphs. Unlike araneomorphs, they show no general trend towards a decrease of 2n, as the chromosome number was reduced in some lineages and increased in others. A biarmed karyotype is a synapomorphy of mygalomorphs and araneomorphs. Male meiosis of some mygalomorphs is achiasmatic, or includes the diffuse stage. The sex chromosome system X1X20, which is supposedly ancestral in spiders, is uncommon in mygalomorphs. Many mygalomorphs exhibit more than two (and up to 13) X chromosomes in males. The evolution of X chromosomes proceeded via the duplication of chromosomes, fissions, X–X, and X-autosome fusions. Spiders also exhibit a homomorphic sex chromosome pair. In the germinal of mygalomorph males these chromosomes are often deactivated; their deactivation and pairing is initiated already at spermatogonia. Remarkably, pairing of sex chromosomes in mygalomorph females is also initiated at gonial cells. Some mygalomorphs have two sex chromosome pairs. The second pair presumably arose in early-diverging mygalomorphs, probably via genome duplication. The unique behaviour of spider sex chromosomes in the germline may promote meiotic pairing of homologous sex chromosomes and structural differentiation of their duplicates, as well as the establishment of polyploid genomes. © 2013 The Linnean Society of London, Biological Journal of the Linnean Society, 2013, 109, 377–408.


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INTRODUCTION

Spiders are one of the most diverse animal orders. They are divided into three primary lineages, including the suborder Mesothelae and two infraorders, Mygalomorphae and Araneomorphae, which together form the suborder Opistothelae. Mesothelae and mygalomorphs show more plesiomorphic characters than derived araneomorphs, which are much more diverse in species number and morphology.

Karyotypes of nearly 700 spiders belonging to 64 families have been reported so far (Kořínková & Král, 2013). In spite of this progress, almost all chromosome data refer to araneomorphs only. Spiders show considerable karyotype diversity, reflecting their high species diversity and deep evolutionary history. Male diploid numbers range from seven (haploxyne araneomorph Ariadna lateralis Karsch, 1881, Segestriidae; Suzuki, 1954) to 110 (mygalomorph Pocilotheria formosa Pocock, 1899, Theraphosidae; Král et al., 2011). Most entelegyne araneomorphs and the only representative of Mesothelae studied so far exhibit acrocentric karyotypes. In contrast, most haploxyne araneomorphs (Král et al., 2006) possess biarmed (metacentric/submetacentric) chromosomes. Finally, the haploxyne superfamily Dysderoidea displays holocentric chromosomes (Král et al., 2006).

An unusual feature of spider karyotypes is the predominance of multiple sex chromosome systems. The ancestral system is assumed to be $X_1Y$, $X_2Y$, $X_1X_2Y$, where 0 indicates the absence of a Y chromosome. The $X_1X_2Y$ system is common in entelegynes and mygalomorphs, showing more plesiomorphic characters than derived araneomorphs, which are much more diverse in species number and morphology.

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An unusual feature of spider karyotypes is the predominance of multiple sex chromosome systems. The ancestral system is assumed to be $X_1X_2/\varnothing X_1X_2X_0$, where 0 indicates the absence of a Y chromosome. The $X_1X_2Y$ system is common in entelegynes (Král et al., 2006) and has been found in other major clades of spiders (Suzuki, 1954). At male prophase I, X chromosomes show heterochromatinization; consequently, they stain more intensively than autosomes (so-called positive heteropycnosis; Král, 2007). The origin of this system is unresolved. Both X chromosomes are supposedly non-homologous, based on their acentric pairing during male meiosis. One group of hypotheses suggests the origin of the $X_1X_2Y$ system by fission of a single X chromosome (Bole-Gowda, 1950; Suzuki, 1954; White, 1973). An alternative explanation suggests non-disjunction of an X chromosome and differentiation of the newly formed sex chromosome (Postiglioni & Brum-Zorrilla, 1981). The same scenario is hypothesized for the derived systems with three or four X chromosomes found in some entelegynes and mygalomorphs (Kořínková & Král, 2013). The hypothesis of multiple X chromosomes originating from duplications has recently been supported by an unique behaviour of these chromosomes in female meiosis, which includes the deactivation and association of sex chromosome pairs during prophase I (Král, 2007; Král et al., 2011). Other derived sex chromosome systems include the X0 system, probably resulting from a fusion of the X chromosomes (Bole-Gowda, 1950), and systems originated by X-autosome fusions (Maddison, 1982; Rowell, 1985; Král et al., 2006; Řezáč et al., 2006). X-autosome fusions may possibly also have produced the $X_1X_2Y$ system found in the early-diverging araneomorph family Hypochilidae, as well as in the haploxyne families Drymusidae, Filistatidae, Pholcidae, and Sicariidae. Specific features of the $X_1X_2Y$ system suggest that these families form a monophyletic group (Král et al., 2006).

Recent studies of male meiosis in spiders have revealed a considerable complexity in their sex chromosome systems. Besides multiple X chromosomes, or their derivatives, spider karyotypes also include a sex chromosome pair (SCP) in which chromosomes lack morphological differentiation. In entelegynes, the SCP has been found in families Agelenidae and Lycosidae, being only detectable by transmission electron microscopy (TEM) (Král, 2007). Ultrastructural data on male meiosis in other spider lineages are lacking; however, the SCP has been detected in two haploxyne and two mygalomorph families by light microscopy, based on specific behaviour in the male germ line (Král et al., 2006, 2011).

In this article we investigate karyotypes, sex chromosomes, and meiosis of a representative sample of mygalomorph spiders. This infraorder includes more than 2700 species, classified into 16 families (Bond et al., 2012; Platnick, 2013). In spite of this diversity, karyotypes of only six species from three families, namely Atypidae (Suzuki, 1954; Řezáč et al., 2006), Dipluridae, and Theraphosidae (Král et al., 2011), have been comprehensively described. Fragmentary data on 13 other species have been reported (see Řezáč et al., 2006 for a review). Our study includes 31 species belonging to 13 families, nine of which are studied for the first time (Table S1). Our data suggest non-disjunctions of mygalomorph sex chromosomes. Furthermore, they indicate an ancient duplication of the mygalomorph genome, including the sex chromosomes. These events probably contributed to the considerable karyotype diversity of mygalomorphs demonstrated in this study.

MATERIAL AND METHODS

MATERIAL

Collection data are presented in Table S1. Some taxa were only determined to genus level because the available specimens were juveniles, females (reported features are sometimes not sufficient to distinguish congeneric females), or undescribed species. Specimens are preserved in 96% ethanol. All cytogenetic
data are reported for the first time, except for 2n of Brachypelma albopilosum Valerio, 1980 (Vitková et al., 2005).

The testes of subadult males were found to be optimal for study. Testes contained dividing cells such as spermatogonial mitoses, as well as meiotic cells. Juveniles were thus reared until preferably the subadult stage, and then dissected; however, subadult males were not easily distinguishable from the preceding instars. Therefore, adult males were also often used, although their testes contained relatively fewer dividing cells. Male meiosis of some mygalomorphs was almost (Cyphonidia; Macrothele inumai Shimojana & Haupt, 1998) or fully completed (Ancylostria pf. fosor Simon, 1889; Antrodiaetus; Iberesia) just after the final molt. The pair of long testes usually showed a convoluted or tortuous morphology; deferent ducts were reduced or absent. A different morphology is likely to be ancestral in spiders. Female chromosomes were obtained from the ovaries or intestines of juveniles or adults. Concerning female meiosis, only prophase I or complete sequences of the first meiotic division were found.

**Chromosome Preparations and Evaluation of Karyotype**

Chromosome preparation was based on Dolejš et al. (2011), with two modifications. First, as mygalomorph cells proved to be relatively resistant to hypotonization, they were accordingly hypotonized for 25 min. In some cases, hypotonization was reduced to 10 min to preserve sex chromosome association. Second, the voluminous tissues of large species were fixed in three changes (6, 10, and 30 min) of fixative. C-banding was conducted following the method described by Král et al. (2008). The detection of nucleolar organizer regions (NORs) by silver followed Dolejš et al. (2011), except for the prolonged time of staining (7 min). Silver detects the NORs transcribed in the previous cell cycle only (Miller et al., 1976). This technique often visualized the kinetochores and meiotic sex chromosomes as well. Slides were stained with 5% Giemsa solution in Sörensen buffer (pH 6.8) for 25 min (for standard preparations), 75 min (for C-banding), or were left unstained (for the detection of NORs).

Slides were inspected under a Zeiss Jenaval microscope. Images were photographed on Kodak Technical Pan black and white film and digitized using a Super Coolscan 5000 ED scanner (Nikon) and Nikon Scan 4 software. Alternatively, preparations were observed under an Olympus BX 50 microscope and black and white images were recorded using an Olympus DP 71 CCD camera. In both cases an oil immersion lens was used (100×). For most species, chromosomes of five mitotic metaphases or metaphases II were measured to construct the karyotype (Table S1). In the latter case, the plates containing both sister metaphases were used except for Paratropis species. Relative chromosome lengths were calculated as a percentage of the total chromosome length of the diploid set, including the sex chromosomes (%TCL). Measurements were carried out using IMAGE TOOL 3.0 (University of Texas, San Antonio, TX, USA). Karyotypes were constructed using Corel PHOTO-PAINT X3.

**Detection of NORs by Fluorescent in Situ Hybridization (FISH)**

Genomic DNA (gDNA) of Brachypelma was extracted using the DNeasy Blood & Tissue kit (Qiagen GmbH, Hilden, Germany), or phenol-chloroform DNA extraction, for 18S rDNA probe amplification. The gDNA was used as a template for PCR; conditions and probe biotin labelling were performed according to the method described by Novotná et al. (2011). FISH was performed following the method described by Sahara, Marec & Traut (1999), with some modifications (Novotná et al., 2011). Slides were observed under a Zeiss Axioplan 2 microscope using a 100× oil immersion lens. Black and white images were captured with an F-View CCD camera and AnalySIS 3.2 (Soft Imaging System GmbH, Münster, Germany). Images were obtained separately for each fluorochrome, pseudocoloured, and superimposed with Adobe PHOTOSHOP 5.0.

**Placement of Data into a Phylogenetic Context**

Our proposed scheme of mygalomorph karyotype evolution is based mostly on the phylogenetic hypothesis of Bond et al. (2012), who recognized two primary mygalomorph lineages: the superfamilies Atypoidea and Avicularioidea. Most mygalomorph species diversity is contained within the latter group, which consists of several early-diverging groups and two diverse sister clades: Crassitarsae and Domiothelina.

**Results**

**Atypoidea**

Antrodiaetidae

In Aliatypus californicus (Banks, 1896) (2n♂ = 27, X0), most chromosomes were metacentric except for
two submetacentric pairs (nos 2 and 5). The first autosome pair was much longer than the second pair (17.6 versus 11.2% of TCL), and the size of the remaining pairs decreased gradually. The X chromosome was medium sized (Fig. 1A), and its reliable determination was possible only during meiosis (Fig. 2D). Two small pairs (nos 8 and 11) included a terminal NOR, and one chromosome contained two terminal NORs (Fig. 2A). Autosomes of *Antrodiaetus riversi* (O.P.-Cambridge, 1883) (2n♂ = 47, X0) were mostly acrocentric; only three pairs (nos 3, 17, and 22) were probably biarmed (Fig. 1B). The metacentric sex chromosome was nearly twice as long as the chromosomes of the first pair (5.5 versus 3.2% of TCL). The longest and one medium-sized pair contained a terminal NOR (Fig. 2C). The NOR of the longest pair corresponded to a conspicuous secondary constriction (Figs 1B and 2B), and was remarkable for its large size (Fig. 2C). The antrodiaetid gonosomes showed common features in the germ, namely the positive heteropycnosis and parallel attachment of X-chromosome arms in early prophase I (i.e. leptotene

![Figure 1. Male karyotypes of antrodiaetids (A, B) and mecicobothriids (C) (based on spermatogonial metaphases). Open arrowheads indicate secondary constrictions. A, Aliatypus californicus. B, Antrodiaetus riversi. C, Megahexura fulva. Scale bars: 10 μm (A, C); 5 μm (B).](image-url)

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to pachytene) in males (Fig. 2D), and the parallel arrangement of chromosomes of the X pair at oogonal mitosis in females (Fig. 2B, C). The following stage of prophase I was only observed in male Antrodiaetus riversi, where autosomes were partially despiralized, and the X chromosome retained its heteropycnosis (the so-called diffuse stage; Fig. 2E).

Mecicobothriidae

In Megahexura fulva (Chamberlin, 1919) (2n♂ = 43, X0) all autosomes were biarmed (Fig. 1C). The metacentric X chromosome (4.5% of TCL) was somewhat longer than the autosomes of the first pair (3.7% of TCL). Two pairs (nos 2 and 4) contained terminal achromatic segments (Fig. 1C), which corresponded to a large NOR in the case of one pair (Fig. 2F).

AVICULARIOIDEA

Early-diverging Avicularioidea

Dipluridae

The karyotype of Euagrus lynceus Brignoli, 1974 (2n♂ = 59, X1X2X3X4X0) was biarmed (Fig. 3C). Male meiosis was modified as a result of the absence of chiasmata. One or two bivalents contained a heteropycnotic NOR knob (Fig. 3A, B). These knobs were also observed during the second meiotic division (Fig. 3C). At the beginning of meiosis, the X chromosomes formed a peripheral sex chromosome body (SCB; Fig. 3A), which exhibited a curious migration into the middle of the plate at prometaphase I. A similar migration of X chromosomes was also observed in other representatives of the superfamily Avicularioidea for which male meiosis was available.

Figure 2. Atypoidea, nucleolar organizer regions (NORs) and chromosome behaviour in the germline. Unless otherwise indicated, based on male plates; arrow, X chromosome; open arrowhead, secondary constriction/NOR. A, Aliatypus californicus, mitotic metaphase, silver staining (*chromosome with two terminal NORs; 1, chromosomes of the largest pair). B, C, Antrodiaetus riversi, female, oogonal metaphase containing the association of chromosomes of the X pair, Giemsa (B), and silver staining (C). B, one pair includes a large secondary constriction at one end. C, two pairs (1, 2) bear terminal NORs. D, Aliatypus californicus, pachytene: the arms of the metacentric X chromosome are positively heteropycnotic and arranged in parallel. E, Antrodiaetus riversi, diffuse stage, the X chromosome is positively heteropycnotic. F, Megahexura fulva, mitotic metaphase, silver staining. Note the ample terminal NOR of one pair. Scale bars: 10 μm.
This event was followed by the disintegration of the SCB (not shown). At metaphase I, loop gonosomes composed a cluster. Ends of gonosomes were directed towards the centre of the cluster (Fig. 3B).

The karyotype of Ischnothele caudata Ausserer, 1875 (2nC=14, XY) was metacentric, except for the two submetacentric pairs (nos 3 and 4). The gonosomes were medium sized and exhibited a similar size (Fig. 4A). Three metacentric pairs (nos 1, 2, and XY) contained a subterminal NOR (Fig. S1A). The autosome and gonosome NORs differed by their pattern of activity. Silver staining revealed that the autosome NORs were active mostly at spermatogonia. Despite the heterochromatinization of gonosomes at premeiotic interphase and prophase I, their NORs were also active during this period (Fig. 3F). Gonosomes paired only at one end at pachytene (Fig. 3D). After pachytene, nuclei entered the diffuse stage: autosomes despiralized considerably and gonosomes formed a heteropycnotic body in the middle of the plate (Fig. 3E). Following autosome recondensation at the onset of diakinesis, all bivalents were chiasmatic. At diakinesis the heteropycnotic mass of the X chromosome reappeared for the part forming the chiasma (Fig. 3G). The heteropycnotic segment of the X chromosome included an interstitial NOR at prophase II: this region was formed by the major part of the long arm (Fig. 3H).

The autosomes of Linothele megatheloides Paz & Raven, 1990 (2nC=86, X,X,X,X,X,X,X,X,X,X,X,X,X,X) bore a terminal NOR (Fig. 3I). Moreover, one small X chromosome included an interstitial NOR (Fig. 3J). The SCP showed a precocious chromatid separation at the spermatogonial metaphase (Fig. 3I). Early meiotic cells contained two SCBs (Fig. S2A). At early diplotene, one body was transformed into a cluster of X univalents. In the course of diplotene, these elements condensed considerably, adopting a rod-like morphology (Figs 3J and S2B). The second body evolved into the SCP (Fig. S2B). At metaphase II, the gonosomes could be identified by their delayed chromatid separation (X univalents) or slight underspiralization (SCP), respectively (Fig. 4B).

Hexathelidae

The chromosomes of Macrothele gigas Shimojana & Haupt, 1998 (2nC=85, X,X,X,X,X,X,X,X,X,X,0) were biarmed, except for five short pairs (nos 28 and 36–39). Conversely, among X chromosomes only the two longest were biarmed. The X chromosomes were among the smallest elements at metaphase II (1.2–0.5% of TCL). In contrast to other diplurids, an SCP was detected in the male germline of Linothele. These metacentric chromosomes were the largest elements of the set (Fig. 4B). One metacentric (no. 29) and one acrocentric (no. 35) autosome pair bore a terminal NOR (Fig. 3I). Moreover, one small X chromosome included an interstitial NOR (Fig. 3J). The SCP showed a precocious chromatid separation at the spermatogonial metaphase (Fig. 3I). Early meiotic cells contained two SCBs (Fig. S2A). At early diplotene, one body was transformed into a cluster of X univalents. In the course of diplotene, these elements condensed considerably, adopting a rod-like morphology (Figs 3J and S2B). The second body evolved into the SCP (Fig. S2B). At metaphase II, the gonosomes could be identified by their delayed chromatid separation (X univalents) or slight underspiralization (SCP), respectively (Fig. 4B).

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which exhibited biarmed morphology (Fig. 5E, inset). The chain was composed of two chromosomes, X and Y, and female (118) diploid number, as well as segregation at male metaphase II (Fig. S1B), revealed that the X chromosomes show a delayed chromatid separation. The X univalents adopted a rod-like morphology at late prophase I (i.e. diplotene). The comparison of male and female (118) diploid number, as well as segregation at male metaphase II (Fig. S1B), revealed that the chain was composed of two chromosomes, X and Y, which exhibited biarmed morphology (Fig. 5E, inset).

The SCP showed the same meiotic behaviour as in the previous paratropidid species, except for the absence of heteropycnosis during late prophase I and the second meiotic division.

Crassitarsae

Theraphosidae

Karyotypes: Members of five theraphosid subfamilies were studied. The chromosome set of *Psalmopoeus cambridgei* Pocock, 1895 (2\(n\)\(\sigma\) = 84, X, X0) (Psalomopoidea) contained biarmed pairs, except nine medium-sized and small acrocentric pairs. The metacentric sex chromosomes differed considerably in size (X1 2.3% and X2 1.6% of TCL; Fig. 6A); each of them contained a centromeric block of CH. Moreover, one arm of the X1 chromosome included a block of intercalar CH (Fig. 8A). The set of *Pelinobius muticus* Karsch, 1885 (2\(n\)\(\sigma\) = 67, X, X0) (Eumenophorinae) was acrocentric, except for two long X chromosomes and nine long biarmed pairs. The gonomosomes showed metacentric (X1, 2.2% of TCL), subtelocentric (X2, 1.7% of TCL), and acrocentric (X3, 1.2% of TCL) morphologies (Fig. 6B).

One ischnocoline was investigated, *Ischnocolus jickelli* L. Koch, 1875 (2\(n\)\(\sigma\) = 85, X, X0), as well as three theraphosines, *Brachypelma albopilosum* (2\(n\)\(\sigma\) = 74, X, X0), *Holothoele cf. longipes* (L. Koch, 1875) (2\(n\)\(\sigma\) = 73, X, X0), and *Grammostola rosea* (Walckenaer, 1837) (2\(n\)\(\sigma\) = 72, X, X0) (Figs 7A, S3, and S4). The karyotype of *Ischnocolus* consisted only of biarmed chromosomes (Fig. S3); the remaining species exhibited between one and three monoarmed chromosome pairs (Figs 7A and S4). X chromosomes were biarmed, large or medium-sized chromosomes (Figs 7A, S3, and S4). The FISH technique revealed one metacentric pair with a large terminal NOR in *Brachypelma* (Fig. 8K).

Our study included two harpactirines that showed a reduced number of chromosomes, particularly gonomosomes, compared with the other subfamilies studied. Both species display an X0 system; the metacentric X was the largest element of the set (Fig. 7B, C). Chromosomes of *Pterinochilus murinus* Pocock, 1897 (2\(n\)\(\sigma\) = 43, X0) were metacentric, except for three large submetacentric pairs (nos 8, 9, and 10). The chromosome pairs can be divided into three size groups: ten large, four medium-sized, and seven small.

Paratropididae

Karyotypes of the two *Paratropis* species studied contained approximately the same portion of biarmed and monoarmed pairs. Almost all small pairs were monoarmed (subtelocentric/acrocentric) (Figs 5A and S1B). At the beginning of meiosis, the male nuclei of *Paratropis* sp. from Colombia (2\(n\)\(\sigma\) = 115, X, X0) contained two positively heteropycnotic SCBs. At transition to diplotene, one body was transformed into a cluster of loop X univalents at late diplotene; their ends were directed to the centre of the cluster (Fig. 3M). In contrast to other Avicularioidea, including *M. yaginumai* (Fig. 3K), the X univalents of *M. gigas* returned to the periphery of the nucleus at diplotene (Fig. 3L–N).

The constitutive heterochromatin (CH) was mostly condensed than the other pairs. The X chromosomes show a delayed chromatid separation. Both groups of gonosomes displayed a loose association (Fig. 5B). The X univalents adopted a rod-like morphology at late prophase I (i.e. diplotene and diakinesis; Fig. 5C). The metacentric SCP belonged to the largest pairs, being also heteropycnotic at prophase and metaphase II (Fig. 5A). Male diplotene of *Paratropis* sp. from Mexico (2\(n\)\(\sigma\) = 112, X, X0) contained a cluster of six loop X univalents and a chain associated at one end with the univalents (Fig. 5D, E). The comparison of male and female (118) diploid number, as well as segregation at male metaphase II (Fig. S1B), revealed that the chain was composed of two chromosomes, X and Y, which exhibited biarmed morphology (Fig. 5E, inset).

(2\(n\)\(\sigma\) = 77, X, X0) was biarmed (not shown). Besides being mostly biarmed, the *Macrothele* species also exhibited other common features. The chromosome pairs, as well as the multiple X chromosomes, decreased gradually in size. One pair bore an ample terminal secondary constriction (Fig. 4C). The constitutive heterochromatin (CH) was mostly restricted to the centromeric regions (Fig. 3N). Male meiosis included a short diffuse stage. During this period, SCBs migrated from the periphery to the centre of the nucleus. The diffuse stage contained an enormous SCB associated with two lesser positively heteropycnotic SCBs (Fig. 3L, inset) that were transformed into two SCPs of similar size at early diplotene (Fig. 3L). The enormous body disintegrated into a group of loop X univalents at late diplotene; their ends were directed to the centre of the cluster (Fig. 3M). In contrast to the other Avicularioidea, including *M. yaginumai* (Fig. 3K), the X univalents of *M. gigas* returned to the periphery of the nucleus at diplotene (Fig. 3L–N).

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Figure 5. Paratropidid karyotype and meiosis, male; arrows, X chromosome(s); open arrow, positively heteropycnotic sex chromosome pair (SCP); arrowheads, centromeric knobs. A–C, Paratropis sp. (Colombia). A, haploid karyotype without sex chromosomes (metaphase II). Note the monoarmed chromosomes (nos 16, 17, 20, 22, 23, 26–29, 33, 35, 37–39, and 41–54). B, transition to diplotene, the loose association of the SCP and the cluster of loop X chromosomes. C, diakinesis composed of 54 autosome bivalents, the SCP, and the cluster of seven X univalents placed in the middle of the plate. D, E Paratropis sp. (Mexico), diplotene containing X chromosome cluster in the middle of the plate. Note the association of the cluster with a chain (c). D, early diplotene. E, late diplotene (52 bivalents, cluster of the six X univalents, and a chain). Inset: diakinesis, the chain consisting of two biarmed chromosomes joined by terminal chiasma (ch). Scale bars: 10 μm, except the inset of E (5 μm).
Figure 6. Male karyotypes of psalmopoeine (A) and eumenophorine (B) theraphosids (based on metaphases II). A, *Psalmopoeus cambridgei*. B, *Pelinobius muticus*. Scale bars: 5 μm.
Figure 7. Male karyotypes of theraphosine (A) and harpactirine (B, C) theraphosids (based on metaphases II). A, *Brachypelma albopilosum*. The pairs 28, 33, and 35 are subtelocentric. Three X chromosomes (X₁, X₃, and X₄) show a slightly delayed chromatid separation. B, *Pterinochilus murinus*, the X chromosome is positively heteropycnotic. C, *Idiothele mira*, the X chromosome exhibits negative heteropycnosis. Scale bars: 10 µm.
pairs (Fig. 7B). The second pair bore a terminal NOR (Fig. 8B). *Idiothele mira* Gallon, 2010 (*2n♂ = 25, X0*) also exhibited a metacentric set, except for one subtelocentric (no. 5), one submetacentric (no. 8), and one acrocentric (no. 9) pair. The chromosome pairs decreased gradually in size, except for the last pair (Fig. 7C). The seventh pair contained a pericentric NOR (Fig. 8C).

**Sex chromosomes in the male germline: X chromosomes:** The X chromosomes of theraphosids formed a positively heteropycnotic SCB at premeiotic interphase and prophase I (Fig. 8D). Silver staining revealed a loop morphology of the X chromosomes, which were associated with each other by their ends on the nuclear periphery (Fig. 8E). The end-to-end pairing of X chromosomes was mostly retained after SCB disintegration (Fig. 8F, I). The same behaviour was found in theraphosids with a single X chromosome, the arms of which aligned with each other (Fig. 8G, J). In some theraphosids (*Brachypelma*, *Ischnocolus*, and *Psalmopoeus*), the end-to-end association of multiple X chromosomes persisted, often up to metaphase I (Fig. 8K). In contrast, the loop X chromosomes of *Pelinobius* (*X*,*X*,*X*,0) completely paired at the transition to diplotene (Fig. 8H), and adopted a rod-like morphology during diakinesis (Fig. 8H, inset). The X chromosomes of theraphosids migrated into the middle of the nucleus at the transition to diplotene (Fig. 8G, H). The X chromosomes of some species also exhibited specific behaviour at prophase and metaphase II, namely delayed condensation (Fig. S3) and chromatid separation (Figs 7A and S3), and positive (Fig. 7B) or negative (Fig. 7C) heteropycnosis. Apart from the germinal cells, the X chromosomes were also heteropycnotic and were associated with each other in the endopolyplid cells of the testes, namely in the genera *Grammostola*, *Holothele*, and *Psalmopoeus*. The homologous chromosomes of *Psalmopoeus* were usually arranged in parallel (Fig. 8L).

SCPs and their association with X chromosomes: Analysis of the germ revealed SCPs in all theraphosids, except *Brachypelma*. Old World species exhibited one SCP (Figs 8G, H, J, 9F, G, and 14), whereas New World species possessed two SCPs (Figs 8F, 9B–E, H, I, and 14). These chromosomes were mostly metacentric and belonged to the largest chromosomes of the complement. This pattern was found in all theraphosids with one SCP (Fig. 9F, G) and one species with two SCPs (*Holothele cf. longipes*). Both *Holothele* pairs possessed a similar morphology and size (Fig. 9E). Two other species with two SCPs, *Grammostola rosea* and *Psalmopoeus cambridgei*, displayed a more complicated pattern: one pair was metacentric and belonged to the largest chromosomes, whereas the second pair was formed by middle-sized submetacentrics (Fig. 9D, I). The SCPs did not differ from the other chromosomes in the distribution and low level of CH (Fig. 9I).

The SCPs displayed a specific behaviour in the germline. These chromosomes were associated with each other at spermatogonial prophase, forming a positively heteropycnotic body in the middle of the nucleus (Fig. 9A). During the disintegration of the body at late prophase and prometaphase, the SCP chromosomes retained heteropycnosis, also showing a delayed condensation (Fig. 9B, C). Their attachment was restricted to one end (Fig. 9C), which was sometimes also observable at metaphase (Fig. 9G). In species with two SCPs, the prometaphase attachment included all of the chromosomes of these pairs (Fig. 9C). In contrast, only the connection or association of the homologous chromosomes was observed at metaphase in these species, whereas these chromosomes were often also aligned side by side by homologous regions (Fig. 9E, I). However, a loose association of all SCP chromosomes at metaphase was observed more frequently (Fig. 9D), but in this case the association of the homologues remained more stable. The SCP chromosomes exhibited a precocious chromatid separation at metaphase (Fig. 9D, F) (in some species from prometaphase). The chromatid separation of all chromosomes of the karyotype was synchronized at the late metaphase (not shown). SCP chromosomes continued clustering at the spermatogonial anaphase (Fig. 9H). From the premeiotic interphase they formed a single heteropycnotic SCB on the nuclear periphery (Fig. 8D). At the transition to diplotene, this SCB was transformed into chiasmatic bivalent(s) (Fig. 8H, J). In some species SCP(s) retained positive heteropycnosis up to diplote, or even diakinesis or metaphase I (Fig. 8H). The SCPs of harpactirines exhibited a complicated pattern of meiotic heteropycnosis. In *Pterinochilus*, only the centromere region and proximal parts of the arms were heteropycnotic (Fig. 8J). The SCP of *Idiothele* was completely heteropycnotic at early prophase I (Fig. 8G). At the transition to the diplote stage, heteropycnosis was restricted to one arm and the proximal part of the other arm (Fig. 8G, inset). Chiasmata always occurred outside the heteropycnotic region (Fig. 8G, inset and J).

During the premeiotic interphase and early prophase I, nuclei often contained an association of two SCBs, one formed by SCP(s) and another by X chromosomes (Fig. 8D). Details of the association were observable in the following genera only, namely during transformation of one body into SCP bivalent(s). During this period, the SCPs of *Idiothele*, *Ischnocolus* (one SCP), *Grammostola*, and *Holothele* (two SCPs) exhibited loop morphology. Their ends
Figure 8. Theraphosids, detection of nucleolar organizer regions (NORs), constitutive heterochromatin, and gonosomes in the male germline; arrows, X chromosomes; open arrows, the sex chromosome pairs (SCPs); arrowheads, primary constrictions; open arrowheads, NORs. A, Psalmopoeus cambridgei, C-banded diakinesis, association of X univalents. Note the centromeric (c) and intercalar (i) heterochromatin. B, Pterinochilus murinus, mitotic metaphase, silver staining. One large arm bears a terminal NOR. C, Idiothele mira, mitotic metaphase, silver staining. One pair includes a pericentric NOR. D, Holothoele cf. longipes, premeiotic interphase. The X chromosome cluster on the nuclear periphery is associated with a positively heteropycnotic body formed by the two SCPs. E, Psalmopoeus cambridgei, premeiotic interphase, silver staining. Two loop X chromosomes are associated by their ends on the nuclear periphery (*nucleolus). F, Grammostola rosea, transition to diplotene. The two X univalents and two SCPs show a loop morphology and positive heteropycnosis, forming a single cluster. Inset: scheme of the cluster (black – autosome bivalents). G, Idiothele mira, transition to diplotene. The X chromosome and the heteropycnotic SCP are associated by their ends in the middle of the plate. Inset: diakinesis, SCP. Heteropycnosis is restricted to one arm (1) and the proximal part of the other arm (2) (c, centromeric knob; ch, chiasma). H, Pelinobius maticus, transition to diplotene. The cluster in the middle of the plate is formed by positively heteropycnotic SCP and three loop X univalents. Inset: diakinesis, the cluster formed by the SCP and the rod-like X univalents arranged in parallel. I, Ischnocolus jickelii, diplotene, end-to-end pairing of loop X univalents. J, Pterinochilus murinus, transition to diplotene. Note the association of the X univalent and the SCP (*). Middle part of the SCP including centromeric knobs is positively heteropycnotic. Note the chiasmata outside of the deactivated region. Inset: scheme of the X univalent (brown) and the SCP (blue, regions without deactivation; red, deactivated segment; ch, chiasma); c, centromeric knob. K, Brachypelma alboipolosum, metaphase I, fluorescent in situ hybridization (FISH) with rDNA probe. Note the NOR bivalent and four loop X univalents (three form a cluster and one is separated) in the middle of the plate. Inset: metaphase II, metacentric chromosome bearing terminal NOR. L, Psalmopoeus cambridgei (X,X,0), octoploid endomitotic nucleus containing a cluster of four X,X sets. Homologous X chromosomes are associated in parallel. Scale bars: 10 μm, except for G (20 μm), J (5 μm), and the inset of K (2 μm).

were associated with the ends of the loop X univalents (Fig. 8F, G). The SCPs migrated along with the X univalents into the middle of the plate at the transition to diplotene (Fig. 8G, H). The SCPs and X univalents showed only loose or no association at late prophase I (Fig. 8H).

Barychelidae

The karyotype of Cyphonisia sp. (2n♂ = 40) was acrocentric, except for four subtelocentric (nos 1, 4, 6, and 7) and two submetacentric/subtelocentric pairs (nos 2 and 3; Fig. 10A). Male meiotic segregation (showing the same chromosome number in sister metaphases II) as well as a comparison of the sexes (the same 2n in males and females) suggests the XY system. However, the XY pair did not differ from the autosomes in morphology (all pairs were composed by two chromosomes with the same morphology), pairing (all bivalents were chiasmatic), and pycnosis (all chromosomes were isopycnotic) at available meiotic stages (diplotene–metaphase II; Fig. 11A).

Nemesiidae

Acanthogonatus pissii (Simon, 1889) (2n♂ = 61, X,X,X,0) exhibited only metacentric pairs, except for two submetacentric (nos 17 and 24) and six acrocentric (nos 23 and 25–29) pairs. The X chromosomes displayed a metacentric (X1 and X2) or subtelocentric (X3) morphology. Chromosomes X1 and X2 were much longer (2.6 and 2.3% of TCL, respectively) than the X3 chromosome (1.3% of TCL). The silver staining of metaphase II cells revealed extensive terminal NOR at metacentric pairs 1 and 14 (Fig. 10B). Pachytene nuclei contained three positively heteropycnotic bodies that were often associated (Fig. 11B). At transition to diplotene, two were converted into large metacentric SCPs (Fig. 11C). The third body only disintegrated at diakinesis, namely into the X univalents that remained paired at one end until metaphase I (Fig. 11D). They remained less spiralized than the other chromosomes throughout meiosis (Fig. 10B). In Iberesia machadoi Decae & Cardoso, 2006 (2n♂ = 76), only mitotic cells were available. A comparison of male and female plates suggests an X,X,0 system. The autosomes were mainly subtelocentric, except for five acrocentric (nos 1, 3, 30, 33, and 34), three submetacentric (nos 9, 27, and 31), and three metacentric (nos 21, 24, and 35) pairs. The presumed chromosomes X were monoarmed, and differed considerably in size. The karyotype included two SCPs. They were the longest elements of the set, whereas the longer metacentric pair was more than twice the size of the submetacentric pair (Fig. 5). One chromosome of the metacentric SCP contained terminal NOR at both ends, one of which was remarkable for its large size (Fig. 11E). Chromosomes of both SCPs were often associated separately at the metaphase (Fig. 11F), showing precocious chromatid separation (Fig. 11E).
Figure 9. Behaviour of theraphosid sex chromosome pairs (SCPs) at spermatogonial mitosis. The open arrow indicates chromosomes of the SCP (1, long SCP; 2, short SCP). A–D, Grammostola rosea. A, B, prophase, chromosomes of the two SCPs are positively heteropycnotic, being located in the middle of the plate. At the beginning of division, they form a body (A) that is disintegrated into a cluster of chromosomes at the late prophase (B). C, prometaphase, SCP chromosomes persist in the middle of the plate showing association at one end (*) and delayed condensation. Insets: magnified association of the SCP chromosomes (left) and scheme of this association (right) (black, chromosomes of long SCP; grey, chromosomes of short SCP). D, metaphase containing a cluster of SCP chromosomes. They show a precocious chromatid separation. Chromosomes of the two SCPs (1, 2) differ considerably in their size (arrows – primary constrictions). E, Holothele cf. longipes, transition to anaphase. The two metacentric SCPs have a similar size; chromosomes of each pair are associated separately. Note the homologous attachment of chromosomes of the bottom pair. F, G, Idiothele mira, late metaphase. Metacentric SCP chromosomes exhibit precocious chromatid separation. They are often associated by homologous regions (G). H, Grammostola rosea, anaphase. Chromosomes of the SCP form a cluster in the middle of the plate and exhibit slightly different condensation. I, Psalmopoeus cambridgei, incomplete spermatogonial metaphase containing long SCP, C-banding (c, centromere; arrowhead, heterochromatin block). The metacentric SCP chromosomes are associated by homologous regions in the middle of the plate. Inset: C-banded SCPs, compiled from two metaphases. Note the considerable size difference of both pairs. They show a low content of heterochromatin. Scale bars: 10 μm, except D (5 μm).
Figure 10. Male karyotypes of barychelids (A), nemesiids (B), and microstigmatids (C). A, Cyphonisia sp. (metaphase II), the XY pair is unidentifiable. B, Acanthogonatus pissii (metaphase II, silver staining). Kinetochores, nucleolar organizer regions (NORs, open arrowheads), and X chromosomes are argentophilic. The X chromosomes exhibit a delayed condensation and separation of chromatids (arrowhead – kinetochore). C, Microstigmata zuluensis (mitotic metaphase), note monoarmed pairs (nos 23, 25, 27–31, and 35). Scale bars: 10 μm.
The chromosomes of the species under study are described. The karyotype of the genus *Microstigmata* is discussed, with a focus on the sex chromosome behaviour. The sex chromosome system is suggested to be X0, with the putative X being submetacentric. The chromosomes of the sex chromosome pairs (SCPs) are analyzed, with a particular focus on the SCP chromosomes. These chromosomes show a delayed condensation during metaphase, and their segregation is delayed at anaphase. The SCP chromosome retained both heteropycnosis and delayed segregation at the anaphase.

**Figure 11.** Karyotype analysis and sex chromosome behaviour in germlines of barychelids (A), nemesiids (B–F), and microstigmatids (G–I). Unless otherwise indicated, based on male plates; arrows, X chromosomes; open arrows, chromosomes of the sex chromosome pairs (SCPs); arrowheads, primary constrictions (centromeric knobs); open arrowheads, nucleolar organizer regions (NORs); 1, metacentric SCP; 2, submetacentric SCP. A, Cyphopsis sp., diplotene composed of 20 chiasma bivalents. All bivalents are without heteropycnosis, the XY bivalent is unidentifiable. B–D, Acanthogonatus pissii. E, spermatogonial metaphase. Chromosomes of the SCP are grouped at the periphery of the plate. Inset: SCP chromosomes, magnified. Note the slightly precocious separation of chromatids. F, early metaphase. Chromosomes of particular SCPs (1, 2) are associated separately. G, Microstigmata amatola, spermatogonial metaphase. Chromosomes of the SCP are grouped at the periphery of the plate. Inset: SCP chromosomes, magnified. Note the slightly precocious separation of chromatids. H, Microstigmata zuluensis, part of telophase I. Note the group of five acrocentric X chromosomes showing positive heteropycnosis. I, Microstigmata amatola, female, oogonial metaphase. Scale bars: 10 μm, except H (5 μm).

**Microstigmatidae**

The two studied *Microstigmata* species had very different karyotypes. The set of *Microstigmata amatola* (Griswold, 1985; 2n♂ = 110) was monoarmed except for eight biarmed pairs (Fig. 11I), whereas the set of *M. zuluensis* (Lawrence, 1938; 2n♂ = 75, X,X,X,X,X,X,0) contained both biarmed and monoarmed chromosomes, with the latter comprising approximately three-quarters of the karyotype. The SCP chromosomes were middle-sized acrocentrics of similar size (Figs 10C and 11H). The karyotypes of both species included a metacentric SCP. These chromosomes were the largest pair of the set, exhibiting precocious chromatid separation at the spermatogonial metaphase (Fig. 11G). The pachytene nuclei contained two SCBs. The body formed by X chromosomes disintegrated only at diakinesis, namely into rod-like chromosomes that segregated to the same pole (Fig. 11H).

**Cyrtacteniidae**

The chromosomes of *Ancylotrypa cf. fossor* (2n♂ = 42) were mostly acrocentric, with two subtelocentric pairs (nos 2 and 13) and submetacentric SCP. Chromosomes of the SCP were the largest elements of the set (5.3 and 4% of TCL; Fig. 12A). Chromosomes of the remaining pairs decreased gradually in size from 3.1 to 1.8% of TCL. At the beginning of spermatogonial mitosis, the SCP formed a positively heteropycnotic body at the periphery of the plate (Fig. 13A). During the disintegration of the body at the prometaphase, the SCP chromosomes showed a delayed condensation, being associated with each other at one end (Fig. 13B). At metaphase, they continued this association or exhibited a parallel arrangement, whereas their chromatids separated precociously (Fig. 12A). At the transition to anaphase, the SCP chromosomes were positively heteropycnotic, and segregation of their chromatids was delayed (Fig. 13C). One SCP chromosome retained both heteropycnosis and delayed segregation at the anaphase (Fig. 13D). The karyotype of *Ancylotrypa sp.* (2n♂ = 86) was also acrocentric, except for the three longest pairs, which exhibited a metacentric morphology (Fig. 13E).

**Domiiothelina**

**Ctenizidae**

The karyotypes of *Cyrtocarenum cuniculairum* (Olivier, 1811; 2n♂ = 74, X,X,X,0) and *Ummidia* sp. (2n♂ = 53, X0) were mostly biarmed (Figs 12B and S6A). The largest *Cyrtocarenum* pair was the metacentric SCP (7.7% of TCL); the remaining pairs decreased gradually in size from 3.9 to 1.6% of TCL. A comparison of male and female diploid number suggested the X,X,0 system; the putative X chromosomes showed metacentric (X, 2.4% of TCL) and submetacentric (X0, 1.2% of TCL) morphologies (Fig. 12B). The SCP chromosomes showed precocious chromatid separation at the spermatogonial metaphase (Fig. 13G). Moreover, they were often associated at gonial mitoses of both sexes (Figs 13H and S7). The comparison of *Ummidia* sexes suggested the X,X,0 system, with the putative X being submetacentric (Fig. S6A). A remarkably high chromosome number was found in *Cyclocosmia siamensis* Schwendinger, 2005; 2n♂ = 128). The five longest chromosomes displayed metacentric morphology, whereas three other long chromosomes were subtelocentric. The karyotype included both biarmed and monoarmed pairs (Fig. 13F), with the former being more numerous than the latter (~2:1).

**Idiopidae**

The karyotype of *Idiops syriacus* O.P.-Cambridge, 1870; 2n♂ = 61) was mostly biarmed (Fig. S6B). The data obtained are not sufficient to determine the sex chromosome system, but the odd, largest chromosome
Figure 12. Male sets of cyrtaucheniiids (A), ctenizids (B), and migids (C) (based on spermatogonial metaphases). Chromosomes of the first two species are arranged into pairs tentatively because their sex chromosome system is unknown. A, Ancylotrypa cf. fossor. The original relative position of chromosomes of the sex chromosome pair (SCP) is preserved (dashed line – primary constriction). B, Cyrtocarenum cunicularium (subtelocentric pairs: nos 5, 8, and 15). C, Poecilomigas abrahami. Scale bars: 10 μm.
Figure 13. Gonial mitoses of cyrtaucheniids (A–E), ctenizids (F–H), and migids (I). Unless otherwise indicated, based on male plates; arrows, X chromosomes; open arrows, chromosomes of the sex chromosome pair (SCP); arrowheads, primary constrictions. A–D, Ancylotrypa cf. fossor, behaviour of SCP at spermatogonial mitosis. A, B, prometaphase. The body on the periphery of the plate (A) disintegrates into SCP chromosomes (B). They are associated at one end (*). Inset: scheme of association of SCP chromosomes. C, transition to anaphase. The SCP chromosomes show a delayed chromatid segregation and positive heteropycnosis. D, anaphase. One SCP chromosome continues heteropycnosis and delayed segregation. E, Ancylotrypa sp., female, oogonial metaphase. The three largest pairs are metacentric. F, Cyclocosmia siamensis, spermatogonial metaphase. Note the five largest chromosomes showing metacentric morphology (arrowheads) and three large subtelocentrics (st). G, H Cyrtocarennum cunicularium. G, spermatogonial metaphase. SCP chromosomes display slightly precocious chromatid separation. H, female, oogonial metaphase. SCP chromosomes are associated with each other (arrows 1 point to the ends of one chromosome and arrows 2 to the ends of the second chromosome). I, Moggridgea peringueyi, female, oogonial metaphase. Metacentric X chromosomes are arranged in parallel. Arrowheads indicate primary constrictions of another biarmed pair. Scale bars: 10 µm.
(7.6% of TCL) showing a metacentric morphology could be an X chromosome. Two metacentric pairs included large secondary constrictions (Fig. S6B).

Migidae
A common feature of the analysed migids was a relatively low 2n and a predominance of acrocentric chromosomes. Poecilomigas abrahami (O.P.-Cambridge, 1889) (2n♂ = 33, X0) exhibited only two biarmed autosome pairs (nos 2 and 6). The metacentric X chromosome was the longest element of the karyotype (7.1% of TCL), being 1.8 times longer than the chromosomes of the first pair (Fig. 12C). The female set of Moggridgea peringueyi Simon, 1903 (2n♀ = 36) contained only two biarmed pairs. The first pair exhibited a metacentric morphology and considerable length (12.8% of TCL, i.e. 1.5 times longer than the second pair). These patterns suggest that it is formed by X chromosomes. Chromosomes of the X pair were often arranged in parallel at the oogonial metaphase (Fig. 13I).

DISCUSSION
DIPLOID NUMBERS AND AUTOSOME MORPHOLOGY
Although mygalomorphs exhibit less morphological and species diversity than entelegyne araneomorphs, their karyotypes are much more differentiated. Considerable karyotype variability has also been found in haplogyne araneomorphs (Král et al., 2006). Increased rates of chromosome evolution in haplogyne araneomorphs and mygalomorphs could be related to their less efficient dispersal capabilities. Specifically, the ability to disperse by air, a specific spider strategy employed to spread over long distances, is reduced or absent in haplogyne (Beatty, 1970) and mygalomorphs (Coyle et al., 1985). Consequently, isolated populations of these spiders might accumulate chromosome rearrangements.

Mygalomorphs show a wide range of male diploid numbers, ranging from 14 (Atypus affinis Eichwald, 1830, Atypidae; Rézák et al., 2006; and Ischnothele caudata, Dipluridae, this study) to 128 (Cyclocosmia siamensis, Ctenizidae, this study). Our data show that the diploid numbers in mygalomorphs are generally much higher than in araneomorphs. The diploid number of Cyclocosmia is the highest found in spiders so far. Considerable variability in diploid numbers has also been found in many families and even in some genera of mygalomorphs. Extreme ranges of 2n were found in the families Ctenizidae (2n♂ = 53–128), Dipluridae (2n♂ = 14–90), and Theraphosidae (2n♂ = 25–110) (Král et al., 2011; this study). The considerable variability of 2n in the diplurids may reflect their paraphyly, which has been previously indicated through morphological (Goloboff, 1993) and molecular phylogenetic analyses (Hedin & Bond, 2006; Bond et al., 2012). In contrast, theraphosids as well as the ctenizid genera studied appear to be monophyletic (Fig. 14, Bond et al., 2012). In the case of congeneric mygalomorph species, their karyotypes always differ in one or more basic characters, namely diploid number, chromosome morphology, and/or sex chromosome system (based on Rézák et al., 2006; Král et al., 2011; this study). Remarkably, we have not found intraspecific variability of these characters, suggesting that karyotype data may be an effective method to distinguish closely related species of these morphologically conservative spiders.

Most mygalomorphs studied so far exhibit mostly biarmed chromosomes (Rézák et al., 2006; Král et al., 2011; this study), which also prevail in phylogenetically early-diverging clades of the infradorder Araneomorphae (Král et al., 2006). In contrast, mesotheles exhibit a predominance of acrocentrics (Král et al., 2010). This pattern indicates that the prevalence of biarmed elements may be ancestral for the suborder Opisthothelae. Interestingly, a substantial portion of acrocentrics was found in all mygalomorphs with male diploid numbers higher than 100, i.e. Cyclocosmia siamensis (Ctenizidae), Microstigmata amatolata (Microstigmatae), Paratropis species (Paratropidiae; this study), and Poecilotheria formosa (Theraphosidae; Král et al., 2011). According to our hypothesis, these karyotypes have evolved independently from ancestral biarmed complements by fissions. This view is supported by a comparison of the two Microstigmata species (this study). The karyotype of M. amatolata (2n♀ = 110) contains a much greater proportion of monoarmed chromosomes than the set of M. zuluensis (2n♂ = 75), which is dominated by biarmed chromosomes. The small size of the monoarmed chromosomes in M. zuluensis indicates that they have also been generated by fission. Some other mygalomorphs with a prevalence of biarmed chromosomes are also noted for the small size of their monoarmed chromosomes, namely Acanthogonatus (Nemesiidae), Linothele (Dipluridae), and Psalmopoeus (Theraphosidae) (this study). Their monoarmed chromosomes could also have originated by fission. We suggest that mygalomorph sets showing low diploid numbers arose by an opposite process, namely by fusions. In this context, it is remarkable that some karyotypes showing a low 2n contain pair(s) that are much smaller than the others, namely in the atypid Atypus affinis (Rézák et al., 2006) and harpactirine theraphosids (this study). These chromosomes may represent unfused relics of the original set. The reduction of 2n is a basic trend of araneomorph karyotype evolution (Král et al., 2006).
Some mygalomorph families also include species with a predominance of monoarmed chromosomes, namely Antrodiaetidae (*Antrodiaetus riversi*) (this study), Atypidae (*Atypus karschi* Dönitz, 1887) (Suzuki, 1954), Cyrtarachnidae (*Ancylotrypa* spp.) (this study), Dipluridae (*Diplura* cf. petrunkevitchi) (Caporaciaco, 1955) (Král et al., 2011), Nemesiidae (*Iberesia machadoi*), and Theraphosidae (*Pelinobius muticus*) (this study). Moreover, all of the barychelids (one species) and migids (two species) studied possess a predominance of monoarmed chromosomes (this study). Monoarmed karyotypes could arise from the hypothesized original biarmed set by pericentric inversions. This hypothesis conforms to the karyotype pattern found in the two clades for which data from multiple species are available: Atypoidea and Theraphosidae. Representatives of Atypoidea and theraphosids showing a predominance of acrocentric chromosomes possess similar 2n values as most members with biarmed karyotype belonging to these clades (Rézác et al., 2006; this study). The evolution of some monoarmed sets probably includes a substantial reduction of diploid numbers, as suggested by two *Ancylotrypa* species (Cyrtarachnidae) that differ considerably in 2n and exhibit a prevalence of monoarmed chromosomes (this study).

**NUCLEOLAR ORGANIZER REGIONS**

Nucleolar organizer regions (NORs) have only been detected in several araneomorph families so far (Dolejš et al., 2011). To study the evolution of mygalomorph NORs, their position has been determined in representatives of the families Antrodiaetidae, Dipluridae, Meicobothriidae, Nemesiidae, and Theraphosidae. The mygalomorphs studied display one or two pairs of NORs, usually placed at the ends of the autosomal arms. Notably, most araneomorphs exhibit the same range as well as predominant position of NORs, suggesting that this pattern is a symplesiomorphy of araneomorphs and mygalomorphs. Interestingly, enormous secondary constrictions/NORs on one or even two autosomal pairs have been found in many mygalomorphs, belonging to disparate families (Rézác et al., 2006; Král et al., 2011; this study). Less pronounced expansion of NORs has also been revealed in some entelegynes (Dolejš et al., 2011). The ample mygalomorph NORs are stained completely by silver. As silver staining is confined to the active rRNA genes (Miller et al., 1976), these results indicate transcriptional activity of the whole NOR. We hypothesize that in mygalomorphs the NORs have expanded independently on multiple occasions to compensate for their low number.

In general, meiotic activity of NORs is restricted to the prophase and metaphase I, respectively (Sumner, 2003). Male NORs of *Acanthogonatus* (*Nemesiidae*) are also visualized by silver during the second meiotic division, which indicates transcription of rRNA genes at the preceding interphase and prophase nucleus, i.e. during interkinesis and prophase II. With respect to other animals, such patterns of NOR activity have only been reported in other arthropod groups (Král et al., 2008).

Interestingly, NORs have also been detected on spider sex chromosomes. Location of NORs on multiple sex chromosomes and their derivatives is frequent in haplogynes (Král et al., 2006), being a possible synapomorphy for certain clades (Král et al., 2006; Oliveira et al., 2007). In contrast, it appears to be uncommon in entelegynes (Král et al., 2011). In mygalomorphs, this NOR position has been demonstrated only in the diplurids *Ischnothele* (Ischnothelinidae) (chromosomes X and Y) and *Linothele* (Diplurinae) (one X chromosome; this study). Despite the heterochromatinization of the gonosomes during male prophase I, their NORs were active during this period. As morphological analysis does not support the placement of subfamilies Ischnothelinidae and Diplurinae into a monophyletic family (Goloboff, 1993; Fig. 14), sex chromosome-linked NORs of these diplurids probably arose independently. The NORs could also be present on the X chromosome of *Aliatypus* (X0), as indicated by the presence of an odd NOR-chromosome in this species (this study). Finally, we revealed SCP-linked NORs in the nemesiid *Iberesia*, where one SCP chromosome of the males contains NOR at both ends, whereas its homologue bears NO NOR. One SCP-linked NOR exhibits large size. This pattern could arise by the loss of NORs from one SCP chromosome, promoting an expansion of one remaining SCP-linked NOR. Besides the sex chromosome-linked NORs, the karyotype of *Iberesia* contains only one pair of autosomal NORs (J. Král, unpubl. data).

**SEX CHROMOSOME EVOLUTION**

*Multiple sex chromosomes and their derivatives*

The multiple X chromosomes of most mygalomorphs are biarmed. Except for *Linothele* (this study), X chromosomes showing predominantly monoarmed morphology have only been reported in species in which autosomal complements show a considerable portion, or even dominance, of monoarmed chromosomes (Suzuki, 1954; Král et al., 2011; this study). This correlation suggests the operation of the same rearrangements within autosomal and X chromosome complement of these species.

All representatives of Atypoidea are reported to exhibit the X0 system (Rézác et al., 2006; this study), except for *Atypus karschi* (X;X0 system; Suzuki,
The phylogeny used to map cytogenetic characters represents a compilation of hypotheses based on different character data, namely molecular and morphological (Bond et al., 2012; black branches), morphological (diplurids, Goloboff, 1993; theraphosids, Raven, 1985; Smith, 1990; Samm & Schmidt, 2010; Cyrtocarenium, Raven, 1985; black dashed branches), morphological and cytogenetic (Atypus, Suzuki, 1954; Řezáč et al., 2006; grey branches), and cytogenetic (theraphosids, Král et al., 2011; this study; grey dashed branches). Theraphosid taxonomy follows Raven (1985), except for the restriction of Ischnocolinae to Old World taxa (Smith, 1990) and the removal of Psalmopoeus from the subfamily Selenocosmiinae (Samm & Schmidt, 2010). This taxonomic arrangement is in the best accordance with cytogenetic data. Theraphosid subfamilies included: Eumenophorinae (E), Harpactirinae (H), Ischnocolinae (I), Psalmopoeinae (P), Selenocosmiinae (S), and Theraphosinae (T).

Character sets

C, chromosome number and morphology

Ancestral male karyotypes (prevalence of biarmed chromosomes)

- C1, 2nC0 = 40–50;
- C2, 2nC0 = 47;
- C3, 2nC0 = 43;
- C4, 2nC0 = 41;
- C5, 2nC0 = 70–90 (origin by duplication of genome?);
- C6, 2nC0 = 70.

Evolution of chromosome morphology

- C7, considerable reduction of 2n [chromosome morphology of resulting karyotype: A, prevalence of biarmed chromosomes retained; B, prevalence of monoaarmed chromosomes retained (operation of tandem fusions or cycles of centric fusions and subsequent pericentric inversions); C, prevalence of monoaarmed chromosomes, chromosome morphology of ancestral set unclear];
- C8, chromosome fissions [A, limited operation of fissions → most chromosomes remain biarmed (most monoaarmed chromosomes exhibit small size)];
- C9, frequent operation of pericentric inversions → prevalence of monoaarmed chromosomes.

M, male meiosis

- M1, suppression of chiasmata (A, no suppression, chiasmatic meiosis, ancestral; B, expression of chiasmata delayed, cryptochiasmatic meiosis; C, suppression of chiasmata, achiasmate meiosis);
- M2, diffuse stage (A, no, ancestral; B, yes).

N, nucleolar organizer region (NOR)

- N1, low number of NORs;
- N2, secondary constrictions/NORs located at distal/subdistal parts of arms of chromosome pairs;
- N3, pericentric NOR;
- N4, sex chromosome-linked NOR (location: A, X chromosome; B, neo-XY chromosomes; C, SCP);
- N5, expansion of secondary constrictions/NORs of one or two chromosome pairs;
- N6, meiotic activity of NORs (A, restricted to prophase and metaphase I, ancestral; B, also during interkinesis and onset of the second meiotic division).

P, sex chromosome pair (SCP)

- P1, supposed ancestral homomorphic SCP without specific behaviour in male germline;
- P2, supposed two homomorphic SCPs without specific behaviour in male germline (metacentric morphology and similar size of both pairs), second SCP arose by duplication of the ancestral SCP?;
- P3, evolution of two SCPs [A, male germline contains one SCP exhibiting specific behaviour and morphological changes, respectively (1, partial meiotic heterochromatinization; 2, complete meiotic heterochromatinization; 3, submetacentric SCP)];
- P4, male germline contains two SCPs exhibiting specific behaviour and morphological changes, respectively (1, one SCP is reduced and submetacentric); C, male germline contains one enormous SCP exhibiting specific behaviour → origin by fusion of two SCPs?; D, initial morphological differentiation of SCP chromosomes.

S, multiple X chromosomes and their derivatives

- S1, ancestral X,X10 system;
- S2, fusion of X1 and X2 chromosomes → X0 system;
- S3, X-autosome(s) fusion → XY system containing neo-Y chromosome;
- S4, X,X,X,X10 system (origin by duplication of X,X,X0 system?); and its evolution [1, ancestral sex chromosome system unclear; IX, ancestral multiple X chromosome system unclear; A, increase of X chromosome number (supposed origin of X chromosomes: 1, non-disjunctions of X chromosomes; 2, non-disjunctions and fissions of X chromosomes; 3, fissions of X chromosomes); B, systems originated by X chromosome fusions (1, X,X,X10; 2, X,X0; 3, X0); C, X-autosome rearrangements without production of sex chromosome; D, XY system containing neo-Y (evolution of system included X-autosome fusion); E, multiple sex chromosome system containing neo-Y (origin by X-autosome fusion)].

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origin of the X0 system by fusion is also supported by the large size of X chromosome in most representatives of Atypoidea. The XY system of *A. affinis* presumably originated from the X0 system by X-autosome fusion(s) (Rezáč et al., 2006).

Members of the superfamily Avicularioidea display a considerable diversity of sex chromosomes. Data on Domiothelina are scarce: they suggest only the presence of X0 and X2X0 systems. Most members of early-diverging Avicularioidea and Crassitarsae possess multiple X chromosomes consisting of more than two elements (Král et al., 2011; this study). Although the X,X,X0 and X,X,X,X0 systems have also been found in some entelegynes (Kořínková & Král, 2013), those composed of higher numbers of X chromosomes have only been discovered in some early-diverging Avicularioidea (this study). The largest numbers of X chromosomes were found in the genera *Macrothele* and *Paratropis*. *Macrothele gigas* exhibits 13 X chromosomes, representing a multiple X system with the largest number of chromosomes known so far. The peculiar behaviour of the X,X,X,X,X0 system during the female meiosis of Avicularioidea suggests that this system originated by duplication of the X,X0 system: four sex chromosomes pairs form two associations, each consisting of two pairs with a similar structure (Král et al., 2011). This pattern has been found in two distantly related families, diplurids and theraphosids, suggesting that the X,X,X,X,0 system is ancestral in the superfamily Avicularioidea. Systems with lower numbers of X chromosomes have probably originated by X–X fusions. This hypothesis is supported by two observations. First, the X0 system occurs only in karyotypes with a low 2n that have been, with all probability, derived by fusions. Second, intercalar CH found in one X chromosome of *Psalmopoeus* (X,X0) could evolve from the centromeric CH of a chromosome involved in fusion. Intercalar CH is otherwise rare in mygalomorphs (J. Král, unpubl. data). Our results suggest frequent fusions of X chromosomes during the evolution of Avicularioidea, especially in the family Theraphosidae. In the subfamily Theraphosinae, the number of male X chromosomes decreased from a hypothesized ancestral four (*Brachypelma*) to two elements (*Grammostola*). In harpactirines, X chromosomes were reduced even to a single element (Fig. 14). X chromosome fusions have also been frequent during araneomorph evolution (Kořínková & Král, 2013).

Systems with more than four X chromosomes could evolve by fissions or non-disjunctions of X chromosomes, or a combination of these modes. Fissions are supposed to take part in the formation of complements comprising a high number of mostly (*Linothele*) or exclusively (*Microstigmata*) acrocentric gonosomes. This hypothesis is supported by the small size of sex chromosomes in these genera. The two largest X chromosomes of *Linothele* are biarmed, and may represent the original biarmed chromosomes unaffected by fission. In contrast to *Linothele* and *Microstigmata*, sex chromosomes of the two *Macrothele* species studied are biarmed, as in most other species of Avicularioidea. This pattern does not correspond to the operation of fissions. Another possibility to explain the increase in sex chromosome numbers are X-autosome rearrangements. However, this hypothesis is not consistent with: (1) the absence of Y chromosome(s); (2) a similar number of autosomes arms in *Macrothele yaginumai* (nine X chromosomes, 136 arms) and *M. gigas* (13 X chromosomes, 138 arms); and (3) end-to-end pairing of meiotic sex chromosomes. Terminal sex chromosome segments formed by autosome material, as well as sex chromosomes of autosomal origin, do not exhibit end-to-end pairing in mygalomorphs (see below). Finally, sex chromosomes of *Macrothele* could arise by non-disjunctions, as suggested for multiple X chromosomes of entelegyne spiders composed of two, three, or four elements (Kořínková & Král, 2013). In this context, it is interesting that the increase in the number of sex chromosomes is not accompanied by an expansion of their CH. The reduction of some sex chromosomes may reflect the exclusion of redundant DNA.

The Y chromosome has been found in several members of Avicularioidea, namely *Cyphonisia* sp. (XY) (Barychelidae), *Ischnothele caudata* (XY) (Dipluridae), and *Paratropis* sp. (X,X,X,X,X,X,X,Y) (Paratropidae) (this study). Chiasmatic pairing between X and Y chromosomes suggests that the origin of these systems is X-autosome rearrangement(s). In *Ischnothele* males, the X is partially heterochromatic during some meiotic stages. The heterochromatic region probably corresponds to the original X chromosome material, which retains its pycnotic behaviour. Finally, the system of *Paratropis* sp. (Mexico) arose presumably from the X,X,X,X,X,X,0 system found in *Paratropis* sp. (Colombia), specifically by an X-autosome fusion. The part of the neo-X chromosome corresponding to the original sex chromosome retains its meiotic association with the other X chromosomes. Despite the overall similarity in behaviour of X chromosomes in the male germline of all opisthotele spiders, there are two traits that are specific for mygalomorphs (this study). First, in contrast to araneomorphs, the X chromosomes of mygalomorphs are never heteropycnotic at spermatogonia, and only rarely exhibit any differential behaviour at this stage. Second, the X chromosomes of early-diverging Avicularioidea and Crassitarsae migrate from the periphery to the middle of the nucleus at the transition to
diplotene, and persist in this area until metaphase I. This migration could be common to all mygalomorphs or at least Avicularioidea; however, data from the other mygalomorphs (Atypoidea, Domiothelina) are missing. In some taxa, X chromosome migration is delayed (Euagrus) or completed earlier (Macrothele gigas). The cause of this peculiar movement is unknown. In contrast, the X chromosomes of araneomorphs are reported to be placed on the plate periphery until metaphase I.

During male prophase I of most Avicularioidea, each X chromosome forms a loop, with the ends of the sex chromosomes forming an achiasmatic cluster (Král et al., 2011; this study). Interestingly, the metacentric univalent of the X0 system also demonstrates a loop morphology and terminal association of the chromosome arms. Achiasmatic pairing of sex chromosomes can be accomplished in various ways, including association by NORs or nucleolus (see Wolf, 1994 for a review). Data from FISH suggest that the end-to-end pairing of X univalents in mygalomorphs is not ensured by NORs or nucleolar material (this study; J. Král, M. Forman, and I. M. Ávila Herrera, unpubl. data). This end-to-end pairing, also discovered in some haplogynes and early-diverging entelegynes, is probably ancestral in opisthtothele spiders (so-called X1X2Y-like pairing; Král et al., 2011). Acrocentric X chromosomes of most entelegynes are attached in parallel and associated by their centromeric regions with a centrosome, as revealed by TEM (Král et al., 2011). The same association could also be maintained by the cluster of sex chromosome ends in spiders with X1X2Y-like pairing.

Meiotic pairing of X chromosomes is modified in some species of Avicularioidea. Pairing of the predominantly monoarmed X chromosomes (Linothele, Microstigmata, and Pelinobius) is already completed during the disintegration of the SCB. The mode of their pairing within SCB is unknown. In Acanthognatus, following SCB disintegration, the pairing of two metacentric and one subtelocentric X chromosomes is restricted to one end. A similar pairing pattern was detected in the haplogyne spider Pholcus (X1X2Y) (Král et al., 2006). The submetacentric X2 of Pholcus also pairs by the end of one arm only, but unlike Acanthognatus, both ends of metacentric chromosomes X1 and Y are involved in the pairing. Another cause of early completion of X chromosome pairing could be an addition of autosome material to one end of the X chromosomes (Král et al., 2011). In this context, it should be noted that Avicularioidea sex chromosomes derived from autosomes do not take part in end-to-end pairing (this study). These observations indicate that the X chromosome ends of Avicularioidea contain specific information ensuring their meiotic association.

Sex chromosome pairs

Besides X chromosomes, an SCP has previously been detected in some opisthotele spiders (Král et al., 2006; 2011; Král, 2007). The origin of the SCP is unresolved. Its presence in two primary clades of spiders (araneomorphs and mygalomorphs), as well as low differentiation, suggest that it could represent the original spider gonomes. This pair could produce multiple X chromosomes by non-disjunctions (Král, 2007).

The SCP of some haplogynes and mygalomorphs shows specific behaviour in the male germ, including deactivation by heterochromatinization. Remarkably, this behaviour is somewhat different in both groups (Král et al., 2006; 2011; this study). Furthermore, in mygalomorphs it is detectable already at the spermatogonial prophase (Král et al., 2011; this study), whereas in haplogynes it only appears during the premeiotic interphase (Král et al., 2006). Heterochromatinization of the SCP is much more frequent in mygalomorphs than in haplogynes. It has been found in more than half of the mygalomorphs for which the male germ line has been investigated to date, namely in eight families of Avicularioidea. Some families include both species with or without SCP (Fig. 14). We suggest that the apparent absence of SCP in some mygalomorphs results from its undifferentiated state and the absence of specific behaviour in the male germ. Consequently, it is not detectable by light microscopy, much as in entelegyne spiders. This hypothesis is supported by similar morphology of the SCP in all mygalomorphs. This pair is always formed by large biarmed chromosomes. In particular families the heterochromatinization of SCP is often restricted to some species only. Therefore, one can assume that it has evolved independently on multiple occasions during mygalomorph evolution. Notably, the heterochromatinic SCP of haplogynes shows the same pattern of distribution and is also formed by large biarmed chromosomes (Král et al., 2006). The meiotic silencing of the SCP presumably prevents the differentiated chromosomes of this pair from recombining (Král et al., 2011). This hypothesis is corroborated by the morphological differentiation of the SCP chromosomes in the mygalomorphs Ancylotrypa and Iberesia. Besides this, SCP chromosomes of Ancylotrypa differ from each other by the pattern of heterochromatinization at spermatogonial mitosis, which presumably also reflects their structural differentiation (this study).

Unexpectedly, some members of Avicularioidea exhibit two SCPs, namely most New World theraphosids and all of the hexathelids and nemesiids analysed (Fig. 14). Both SCPs exhibit standard segregation of chromosomes to the opposite poles during anaphase I, suggesting that each pair is formed by an
X and Y chromosome. The second pair could arise from fission of a single biarmed SCP; however, this would contradict our findings. First, both pairs are always biarmed. Second, their size is not substantially reduced in comparison with the original single SCP; one or both pairs are large chromosomes. A more plausible explanation of this pattern is that the second pair arose by duplication. This idea is supported by the often similar size and morphology of both pairs, as well as by a peculiar pairing behaviour of the SCP chromosomes during spermatogonial mitosis (see below). Remarkably, the families exhibiting two SCPs are not closely related. Provided that Avicularioida with none, one, or two deactivated SCPs represent various stages of the gradual differentiation of two SCPs, the original SCP was most probably duplicated in the common ancestor of this superfamily (Fig. 14). This can be traced back more than 200 Myr, as the first known avicularioid, a representative of ancient hexathelids, was found in Middle Triassic formations (Penney & Selden, 2011). Remarkably, our data suggest that the XXY0 system has also undergone a duplication in ancient Avicularioida (Král et al., 2011); therefore, the most probable scenario of the sex chromosome evolution in Avicularioida is a simultaneous duplication of multiple X chromosomes and SCP via polyploidization of the genome.

In general, sex chromosomes form a considerable barrier to polyploidization. Their duplication can lead to a disruption of sex-determining mechanisms (Mable, 2004); however, the data available indicate frequent duplications of sex chromosomes during spider evolution (Kofínková & Král, 2013; this study). Tolerance of spider genomes to sex chromosome duplications could facilitate the establishment of polyploidy in mygalomorphs. We hypothesize that chromosomes of the original mygalomorph SCP were largely undifferentiated, as found in entelegynes, and contained few genes involved in sex determination. These features could facilitate the integration of the duplicated pair into the genome. This process could be accelerated by structural differentiation of the newly formed SCP, including the loss of dispensable sequences, which could explain the reduction of one SCP in two genera of South American theraphosids and nemesiid Iberesia. It is remarkable that the karyotypes of these spiders contain fewer X chromosomes, which is also supposed to be a derived character of sex chromosome evolution in Avicularioida (Fig. 14). In spite of the reduction, the SCP does not show an increased level of CH and retains its biarmed morphology. Interestingly, biarmed morphology of the SCP is also retained in predominantly monoarmed sets, and thus is probably essential for the function or stability of these chromosomes in mygalomorphs.

Regardless of the process generating the copy of the SCP, duplicated chromosomes could affect meiosis by pairing with chromosomes of the original pair, forming a multivalent. To reduce the negative consequences of chromosome duplications, polyploid plants have evolved genetic systems promoting the meiotic pairing of true homologues. They include the formation of clusters comprised of homologous and homeologous chromosomes, which evolve into homologous associations (Martinez-Perez et al., 2003). A similar structure is the unique end-to-end association of deactivated X chromosome bivalents in spider females during the premeiotic interphase and prophase I, which presumably ensures the pairing of homologous X chromosomes (Král et al., 2011). Our data suggest that the attachment of homologous X chromosomes as well as homologous SCP chromosomes is already initiated at oogonial mitosis of spider females. A similar end-to-end mechanism probably discriminates between homologous and homeologous SCP chromosomes in spermatogonia of mygalomorph males. The association of the deactivated SCP chromosomes found at the onset of spermatogonial mitosis is resolved after the prometaphase, namely into pairs formed by homologous chromosomes (this study). The association of SCP chromosomes appears very early in spermatogonia of mygalomorphs. It has already been observed in the first nymphal instars of Crassitarsae and Domiothelina (J. Král, unpubl. data). It is possible that spider systems ensuring meiotic pairing of homologous sex chromosomes can also promote pairing of homologous autosomes. If so, they can take part in the maintenance of allopolyploid genomes, namely in ensuring the formation of autosome bivalents composed of homologous chromosomes.

The mygalomorph SCPs show similar behaviour to X chromosomes during male meiosis. They exhibit a species-specific pycnotic cycle and migrate into the nuclear centre, along with X chromosomes. Furthermore, they are associated with X univalents during the premeiotic interphase and prophase I. However, this association was not observed in all plates, which suggests its fragility. Similar to X univalents, the association of SCPs with other elements is ensured by their ends. This supports the hypothesis that X chromosomes have originated by non-disjunctions of SCP chromosomes (Král, 2007). Interestingly, the SCP of harpactirine theraphosids is only partially heterochromatic during male meiosis: the chiasmata are localized outside the deactivated region. We propose two hypotheses to explain this pattern. First, it may represent an early stage of differentiation of SCP chromosomes. The heterochromatic segment could undergo extensive structural differentiation that leads to its meiotic deactivation, including the inhibition of recombination; however,
this hypothesis is in conflict with the fact that SCPs of other mygalomorphs are completely heterochromatic but undergo recombination. Alternatively, harpactirine SCP could arise by fusion of the original SCP, which retains deactivation, with autosomal material showing standard meiotic behaviour. This explanation seems to be more probable: the frequent fusion processes during the evolution of harpactirines are indicated by low number of autosomes and X chromosomes in these spiders in comparison with other theraphosids. Rearrangements between the SCP and autosomes have already been demonstrated in the entelegyne genus Malthonica (Král, 2007). A peculiar meiotic behaviour of the SCP was also found in Diplura, the biarmed SCP of which is remarkable for its enormous size. In contrast to other spiders, the arms of the SCP are associated with each other at the beginning of male meiosis (Král et al., 2011). According to our hypothesis, this pair arose by centric fusion of two acrocentric SCPs. This idea is supported by the large size of the SCP, as well as the acrocentric morphology of most of the other elements of the set. The association of the arms may reflect the homeology of the original SCPs.

MODIFICATIONS OF MEIOTIC DIVISION

Males of some mygalomorphs exhibit modifications of the first meiotic division. Suppression of chiasma has been observed in two diplurid genera. Whereas in Diplura the chiasma only exhibit delayed expression (cryptochiasmate meiosis) (Král et al., 2011), they are completely absent in Euagrus (achiasmate meiosis; this study). Cryptochiasmate meiosis is probably an evolutionary intermediate between chiasmate and achiasmate meiosis (White, 1973). The other diplurids studied exhibit standard meiosis; however, the suppression of chiasma could be frequent in diplurids because the genera in which it has been observed belong to different subfamilies. Suppression of chiasma has so far only been reported in two haplogyne families (Benavente & Wettstein, 1980), but these instances were only illusive. Chiasma were present but not visible until diakinesis because of the considerable despiralization of the bivalents after the pachytene (the so-called diffuse stage; Král et al., 2006). The diffuse stage, until now observed in various organisms, is characterized by enhanced transcription (Klášterská, 1977). Because of the need for enhanced synthesis in oocytes, it is more common in females, but has occasionally been found in males as well (Benavente & Wettstein, 1980). In spiders, the male diffuse stage has been reported in some entelegynes (Mittal, 1966), all studied haplogynes (Král et al., 2006), and three mygalomorph taxa, namely the genera Antrodiaetus (Antrodiaetidae) and Macarothele (Hexathelidae), as well as diplurid subfamily Ischnothelineae (this study; J. Král, unpubl. data). The X chromosomes and SCP(s) stay silenced at the diffuse stage, which suggests the suppression of their transcription.

KARYOTYPE EVOLUTION OF MYGALOMORPHS

Hypotheses on mygalomorph karyotype evolution are summarized in Figure 14. The remarkable karyotype diversity discovered in this study supports the recognition of two primary mygalomorph lineages: the superfamilies Atypoidea and Avicularioidea. Atypoidea is characterized by relatively low diploid numbers (2n ≤ 47); all species probably possess the X0 system, or its derivatives. The ancestral male karyotype of this clade probably consisted of 40–50 predominantly biarmed chromosomes, including a single X chromosome (Fig. 4). This condition is still common in Atypoidea (Rezáč et al., 2006; this study). Diploid numbers were reduced considerably in some species of the Antrodiaetidae (this study) and Atypidae (Rezáč et al., 2006). Most representatives of Avicularioida are distinguished by high diploid numbers (2n > 70) and multiple X chromosomes containing more than two elements.

Furthermore, our data allow us to determine some basic features of karyotype evolution in spiders and mygalomorphs. Mesotheles show a high number (2n ≤ 96) of predominantly acrocentric chromosomes (Suzuki, 1954; Král et al., 2010). In contrast, the original complement of opisthotheles presumably comprised approximately 40–50 chromosomes and showed a prevalence of biarmed chromosomes. This karyotype is suggested to be ancestral, both in mygalomorphs (this study) and araneomorphs (Král et al., 2006). It probably arose by saturation of the mostly monoarmed complement found in mesotheles by centric fusions. We hypothesize that the ancestral opisthothele set was duplicated in ancestral Avicularioida. This hypothesis is in accordance with high diploid numbers and the structure of sex chromosome systems in this superfamily. Major trends in the subsequent evolution of avicularioi karyotypes were decreases and increases of numbers of autosomes and sex chromosomes, as well as conversions of biarmed chromosomes to monoarmed chromosomes by pericentric inversions. Furthermore, cytogenetic data indicate a deep split within theraphosids, namely into New World and Old World clades (Fig. 14).

Despite the determination of fundamental trends of karyotype evolution in mygalomorphs, a cladistic analysis of these spiders using cytogenetic characters is impossible at this time. First, mygalomorphs possess many unique cytogenetic features. Our
hypotheses explaining these traits should be considered a base for future research, which would also make use of cytogenetic data for cladistic analysis. Second, data about some families or large mygalomorph clades (e.g. Domiothelina) remain limited. Because of the considerable species and karyotype diversity of mygalomorphs, a cytogenetic-based cladistic analysis would only be effective after the inclusion of more species of each family. Third, data on some characters are also fragmentary in the other spider clades. As a consequence, the possibility to compare mygalomorphs and outgroup taxa is limited.

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**REFERENCES**


SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

Figure S1. Male sets of diplurids (A) and paratropidids (B). A, Ischnothele caudata, karyotype, silver staining (mitotic metaphase). Three pairs (nos 1, 2, and XY) contain a subterminal NOR. B, Paratropis sp. (Mexico), haploid set without X chromosomes (metaphase II). Note monoarmed chromosomes (nos 17, 19, 24–30, 32, 33, 35, 37–40, 42–46, and 48–53). Two chromosomes are submetacentric/subtelocentric (nos 41 and 47). Scale bars: 10 μm.

Figure S2. Linothele megatheloides (Dipluridae): prophase I of male; arrow, X chromosome(s); open arrow, SCP. A, transition to diploptene. Note a SCB composed by X chromosomes on the nuclear periphery and a positively heteropycnotic SCB formed by SCP; B, diakinesis. The central part of the plate contains X chromosomes and SCP, which is the largest bivalent of the set. Scale bars: 10 μm.

Figure S3. Male set of Ischnocolus jickelii (Theraphosidae, Ischnocolinae): metaphase II. Karyotype consists of metacentrics, except for six submetacentric pairs (nos 11, 20, 24, 32, 33, and 38). The sex chromosomes show a slightly delayed condensation and chromatid separation. Scale bar: 10 μm.

Figure S4. Male karyotypes of the theraphosid subfamily Theraphosinae, based on metaphases II. A, Holothele cf. longipes, chromosomes are biarmed, except for two monoarmed pairs (nos 2 and 35). B, Grammostola rosea, chromosomes are metacentric, except for two submetacentric (nos 12 and 19) and one subtelocentric (no. 32) pairs as well as submetacentric X chromosome. Scale bars: 10 μm.
Figure S5. Male karyotype of *Iberesia machadoi* (Nemesiidae): mitotic metaphase. Scale bar: 10 μm.

Figure S6. Male sets of ctenizids (A) and idiopids (B), based on mitotic metaphases. A, *Ummidia* sp. (monoarmed pairs: nos 16 and 19). B, *Idiops syriacus* (submetacentric/subtelocentric pairs: nos 23–25). Chromosomes are arranged into pairs tentatively because the sex chromosome system is unknown. The odd element is the putative X chromosome. Open arrowheads – large terminal secondary constrictions. Scale bars: 10 μm.

Figure S7. Male of *Cyrtocarenum cunicularium*: late spermatogonial metaphase. Note association of SCP chromosomes (open arrows). Scale bar: 10 μm.

Table S1. Basic data concerning collection (localities, sex, and instar of specimens used) and evaluation (*families studied for the first time, 1relative chromosome lengths were not determined, 2relative lengths of X chromosomes were not determined*). CA, California; Co., county; MX, Mexico; NR, nature reserve; RSA, Republic of South Africa. Unless otherwise indicated, deposited in collection of J. Král (BD, B. Drolshagen; NMP, National Museum, Prague; RJR, R.J. Raven). f, female; fn, female nymph; m, male; mn, male nymph; sf, subadult female; sm, subadult male.