Karyotype, Sex Determination, and Meiotic Chromosome Behavior in Two Pholcid (Araneomorphae, Pholcidae) Spiders: Implications for Karyotype Evolution

Adriana E. Golding, Leocadia V. Paliulis

Biology Department, Bucknell University, Lewisburg, Pennsylvania, United States of America

Abstract

There are 1,111 species of pholcid spiders, of which less than 2% have published karyotypes. Our aim in this study was to determine the karyotypes and sex determination mechanisms of two species of pholcids: Physocyclus mexicanus (Banks, 1898) and Holocnemus pluchei (Scopoli, 1763), and to observe sex chromosome behavior during meiosis. We constructed karyotypes for P. mexicanus and H. pluchei using information from both living and fixed cells. We found that P. mexicanus has a chromosome number of 2n = 15 in males and 2n = 16 in females with X0-XX sex determination, like other members of the genus Physocyclus. H. pluchei has a chromosome number of 2n = 28 in males and 2n = 28 in females with XY-XX sex determination which is substantially different from its closest relatives. These data contribute to our knowledge of the evolution of this large and geographically ubiquitous family, and are the first evidence of XY-XX sex determination in pholcids.

Introduction

Spiders display a wide range of chromosome numbers and sex determining systems, and very commonly have multiple X chromosomes. Of the spiders studied, the most common sex determining system is X1X20 (male)/X1X1X2X2 (female) [1]. Some systems have three or more X chromosomes and/or a Y chromosome (for examples, see [1,2]).

The spider family Pholcidae currently consists of 84 genera and 1,111 species [3]. Of these species, fewer than 2% have published karyotype data [4–16]. The previously studied pholcid species have diploid chromosome numbers between 2n = 15 and 2n = 32, with metacentric or submetacentric chromosomes [4–16]. While the majority of species have X1X20 (male)/X1X1X2X2 (female) sex determination, most studied pholcid species have X0 (male)/XX (female) sex determination, which is substantially different from its closest relatives. These data contribute to our knowledge of the evolution of this large and geographically ubiquitous family, and are the first evidence of XY-XX sex determination in pholcids.

Results

Physocyclus mexicanus and Holocnemus pluchei spermatocytes were observed in metaphase and anaphase of meiosis I and meiosis II to determine chromosome number and sex determination mechanism. Using observations of living and fixed cells, we were able to obtain a karyotype for each species (Figure 1). Karyotypes were constructed using images of Giemsa-stained fixed preparations of cells in anaphase I and metaphase II (chromosomes for karyotypes were obtained from images shown in Figure S1). Preparations from 30 individuals of each species were used to determine karyotypes. In both species, all chromosomes are either metacentric or submetacentric. P. mexicanus has a chromosome number of 2n = 15 in males and 2n = 16 in females with X0 (male)/XX (female) sex determination, though X1X20 (male)/X1X1X2X2 (female) and X1X2Y (male)/X1X1X2X2 (female) sex determination systems have also been observed [4–16].

Karyotype data (with information on sex determination) can be helpful in establishing evolutionary relationships between species and for differentiating species that otherwise look similar [2,17]. In this study we have determined the karyotypes and sex determining systems of two pholcids, Physocyclus mexicanus and Holocnemus pluchei using observations of living cells and stained fixed cells. We verified our observations of the sex determination mechanism using micromanipulation. We have compared chromosome number and sex determination mechanism with closely related species, and have found that P. mexicanus has the same chromosome number and sex determining system as other species of Physocyclus, while H. pluchei is the first observed example of a pholcid with XY (male)/XX (female) sex determination and has a different karyotype than other closely related species.
that remained near the center of the spindle (Figure 2B, arrows). Because our previous studies (Figure 2A) show that univalent sex chromosomes remain near one spindle pole through metaphase I and anaphase I in spiders, we suspected that the X chromosome was associated with a small Y chromosome, which was apparent in some images (Figure 2B, arrowheads). In addition, because we often find that it can be difficult to count the number of X chromosomes present in spermatocytes or to clearly see small Y chromosomes, we also used a small micromanipulation needle to move the sex chromosomes in meiosis I spermatocytes in both species. In organisms with multiple X chromosomes (beyond a single X chromosome, e.g. X;X;X;0 (male)/X;X;X;X;X (female)—Doan, Andreychik and Paliulis in preparation) it is possible to separate and count the number of sex chromosomes by this technique. Micromanipulation of the sex chromosome in male metaphase I in Physocyclus mexicanus revealed there was a single X chromosome, showing that males are X0 (Figure 3A, arrows) and confirming X0 (male)/XX (female) sex determination. Micromanipulation experiments were repeated five times, always showing that there are two spindle attachment sites on the sex chromosomes, and confirming XY (male)/XX (female) sex determination. Our data show that P. mexicanus has chromosome number of 2n = 15 in males and 2n = 16 in females, and that H. pluchei has a chromosome number of 2n = 28 in males and 2n = 28 in females (Figure 3).

Discussion

Our data show that Physocyclus mexicanus has a karyotype that is very similar to that of other species of Physocyclus. Previously published studies reveal that Physocyclus globosus [5], Physocyclus californicus, and Physocyclus enaulus [9] have 2n = 15 = 14+X in males and 2n = 16 = 14+XX in females, like we saw with P. mexicanus. All P. mexicanus, chromosomes appear to be metacentric or submeta-
araneomorph spiders (e.g. pholcids), in which one of the intermediates is an XY (male)/XX (female). In this proposed mechanism, the ancestral form is an X1X2Y (male)/X1X1X2X2 (female) system, which is observed in the pholcid Spermophora senoculata. According to the phylogeny of pholcids constructed by Bruvo-Madarić et al. [19], Spermophora senoculata is basal to Holocnemus pluchei. In S. senoculata, both X1 and X2 are metacentric and there is a very small metacentric Y chromosome [6]. Král et al. proposed that both X1 and X2 are converted from metacentric chromosomes to acrocentric chromosomes by pericentric inversions [6]. Then, a Robertsonian translocation between X1 and X2 forms a single metacentric X chromosome [6]. Král mentions an XY (male)/XX (female) sex determining system in the pholcid Smeringopus pallidus as unpublished data [6]. Smeringopus pallidus is basal to Holocnemus pluchei on the phylogenetic tree constructed by Bruvo-Madarić et al. [19], potentially explaining the presence of an XY (male)/XX (female) sex determining system in H. pluchei. Král et al. proposed that the small Y chromosome is lost in some lineages, leading to an X0 (male)/XX (female) sex determining system in Holocnemus caudatus.

Based on morphological characters, the genera Holocnemus and Physocyclus were placed in the subfamily Holocneminae [19,20]. However, recent molecular phylogenetic data show that they are far more distantly related than initially thought [19,20], which is supported by the significant differences in the karyotypes of Holocnemus and Physocyclus (i.e. large differences in chromosome number and morphology). Our current results in comparison with the previously obtained results of closely related species show that closely related species have similar chromosome number and structure (e.g., the different species of Physocyclus), but that key changes can happen concomitantly with or following speciation, as we have deduced by comparing chromosome number and sex chromosome behavior in Holocnemus pluchei with the previously obtained karyotypes of Holocnemus caudatus and Crossopriza lyoni [5,6]. In addition, in H. pluchei we have found the first evidence of an XY-XX sex determination system in pholcids. Further analysis will be required to determine whether the hypothesis of Král et al. explains the evolution of the sex determining system of Holocnemus pluchei. In addition, further study will be necessary to explain why H. pluchei has more autosomes than H. caudatus and C. lyoni, its two closest relatives.

These results add to the known karyotype information for the family Pholcidae, allowing further understanding of karyotype evolution in this family. When chromosome data for other pholcids are obtained, these results have the potential to elucidate the phylogeny for this family.

Materials and Methods

Living Physocyclus mexicanus and Holocnemus pluchei males and females were obtained from Spider Pharm Inc. (Yarnell, AZ). Spiders were collected in Yarnell, AZ, USA and identified by C. Kristensen. The authors verified the identification. Specimens are deposited in the National Museum of Natural History, Smithsonian Institution.

Giems staining of chromosomes

Adult Physocyclus mexicanus and Holocnemus pluchei testes were fixed in 6:3:1 ethanol:chloroform:mecetac acid for 10 minutes, testes were macerated in 45% acetic acid and pipetted using a Pasteur pipette to produce a cell suspension. The cell suspension was spread on a microscope slide and placed at 60°C until the cell suspension had dried. Chromosomes were stained with 5% Giemsa for 5 minutes, mounted, and observed using a Zeiss inverted microscope.
Living cell preparations

Living cell preparations of adult male testes were prepared at room temperature according to the method of Doan and Palulis [16]. Primary and secondary spermatocytes undergoing meiosis were filmed across multiple focal planes. To verify sex determination method, micromanipulation was used to position sex chromosomes in meiosis I spermatocytes so the number of pairs of sister chromatids could be determined. Tension was applied to determine whether meiosis I sex chromosomes were univalent or bivalent [18].

Supporting Information

Figure S1 Chromosome spreads used to derive karyotypes in Figure 1. A. Giemsa-stained spread of Physocyclus mexicanus metaphase II spermatocyte used to derive karyotype in Figure 1A, with eight chromosomes. Arrow points to X chromosome. Bar = 10 μm. B. Giemsa-stained spread of Holocnemus pluchei metaphase II spermatocyte used to derive all chromosomes but X chromosome in karyotype in Figure 1B, with 14 chromosomes. Arrowhead points to Y chromosome. Bar = 10 μm. C. Giemsa-stained spread of Holocnemus pluchei anaphase I spermatocyte used to derive X chromosome in karyotype in Figure 1B, with 28 chromosomes. Arrow points to X chromosome. Arrowhead points to Y chromosome. Bar = 10 μm.

(TIF)

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Author Contributions

Conceived and designed the experiments: AEG LVP. Performed the experiments: AEG LVP. Analyzed the data: AEG LVP. Contributed reagents/materials/analysis tools: AEG LVP. Wrote the paper: AEG LVP.

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