Geographic Variation of Chromosomes in the Japanese Harvestman, Gagrellopsis nodulifera, with Special Reference to a Hybrid Zone in Western Honshu

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ABSTRACT - Chromosomes of Gagrellopsis nodulifera Sato and Suzuki (Arachnida, Opiliones, Phalangiidae) were surveyed for 28 populations in Honshu, Japan. Chromosome numbers were found to vary among and sometimes within populations with a fairly wide range from 2n=14 to 22. This variation in chromosome number can be mainly attributed to centric fusion or fission. A hybrid zone of about 10-15 km width was found in the eastern part of Tottori Prefecture, western Honshu, where two forms with different chromosome numbers, 2n=16 and 2n=22, meet. Presumable formation process of the hybrid zone is presented on the supposition that origin of the zone dates back to about 8,000-10,000 years B.P. during the postglacial period.

INTRODUCTION

Gagrellopsis nodulifera Sato and Suzuki (Phalangiidae, Gagrellinae) is one of the common harvestmen of moderate size in Japan and mainly inhabits mountainous areas in the temperate deciduous forest zone. Unlike most phalangiid harvestmen, this species overwinters in the juvenile stage and matures in early May, with a duration of about two months as adults.

Chromosomes of this species were first studied by Tomohiro [1] based on the material collected from Mt. Gokurakuji, near Hiroshima city, western Honshu. In this pioneer work on cytological studies of Japanese harvestmen, he reported the chromosome number as 2n=16. On the other hand, one of the authors (N. T.) found a population having 2n=18 chromosomes in 1982 at a site on the Shiojiri Pass, Nagano Prefecture, central Honshu. Occurrence of geographic variation in chromosome number of this species was, thus, suggested. To elucidate the case in the area intervening between the two distant populations, we performed a chromosomal survey in Tottori Prefecture and its adjacent areas in western Honshu during June 1989. The results revealed that the chromosomes vary in number with an unexpectedly wide range from 2n=14 to 2n=22. In addition, a hybrid zone was found in the eastern part of Tottori Prefecture where two forms with 2n=16 and 2n=22, respectively, meet. In this paper, we will describe the overall pattern of the chromosomal variation with special reference to the nature of the hybrid zone. The proposed cause of the zone and its formation process will also be briefly discussed.

MATERIALS AND METHODS

The chromosomal data were obtained from air-dry preparations of testes of adult males. For the details of the method, see [2, 3]. Chromosomal spreads (spermatogonial metaphase) obtained were generally few since the survey was mainly conducted during the period past the peak of their reproductive activities. Diploid chromosome number was, therefore, inferred also from the first and second meiotic metaphase plates. As a result, chromosome numbers of 97 specimens from 28 populations were determined (Table 1, Figs. 1-3). Data on the specimens examined are listed in the appendix, since the detailed distribution of this
species in Japan has never been published.

RESULTS

Karyotypes

Chromosome number of this species was found to vary enormously among and sometimes within populations (Table 1, Figs. 1–3). The lowest number was 2n=14, obtained for the Itoi Valley population (Loc. No. 26 in Fig. 2; Hyogo Pref.); while the highest was 2n=22 for several populations in mountainous area surrounding Mt. Hyōnoson (1,510 m height) which is located on the boundary between Tottori and Hyogo prefectures (Fig. 2). The chromosome numbers of other populations were between the two extremes (Table 1). Among these, 2n=16 appeared to be prevalent because this number was shared by many populations of Chūgoku district, ranging from the western part of Hiroshima Pref. to the eastern part of Tottori Pref., and by a population of Lake Misuzu (Loc. No. 28 in Fig. 1), Nagano Pref., Chūbu district (Figs. 1, 2).

Some representative karyotypes are shown in Figures 4 and 5. It appears to be futile to describe each karyotype in detail, since only a few spreads sufficient for karyotype analysis were obtained. Figures 4 and 5 show three karyotypes with 2n=16, 22, and 24 chromosomes, although no such chromosomes could be detected in the others (Figs. 4B, 5D). Differences in size between the two chromosomes of a heteromorphic pair is eminent in some cases. For example, in the heteromorphic pair of karyotypes of the Amedaki Fall population (Loc. No. 23 in Fig. 2), one chromosome was consistently the largest meta- or submetacentric chromosome among the component chromosomes and about three times larger than the other of the heteromorphic pair (Fig. 4D). A large chromosome which has fewer acrocentrics than those with higher numbers. For example, no acrocentric chromosome is observed in the karyotype with 2n=16 of the Daisen Fall population (Loc. No. 1 in Fig. 2), comprising mainly metacentrics (Fig. 4B). On the other hand, the one with 2n=22 of the Forest Park of Hattō Town (Loc. No. 22 in Fig. 2) contains four pairs of acrocentrics and one acrocentric chromosome that was tentatively regarded as a component of sex chromosomes (Fig. 4C). However, this correlation is not so clear, since component chromosomes vary to some extent even among populations with the same number of chromosomes (e.g., compare among Figs. 4A, B, 5A, and B and between Fig. 4C and D).

2) Some karyotypes (Figs. 4A, 4C, 5A) contained a pair of heteromorphic chromosomes which probably represent X and Y sex chromosomes, although no such chromosomes could be detected in the others (Figs. 4B, 5D). Differences in size between the two chromosomes of a heteromorphic pair is eminent in some cases. For example, in the heteromorphic pair of karyotypes of the Amedaki Fall population (Loc. No. 23 in Fig. 2), one chromosome was consistently the largest meta- or submetacentric chromosome among the component chromosomes and about three times larger than the other of the heteromorphic pair (Fig. 4D). A large chromosome which

Table 1. Chromosome numbers of males in 28 populations of Gagrellopsis nodulisera

<table>
<thead>
<tr>
<th>Location13)</th>
<th>Date</th>
<th>No. indiv. obs.</th>
<th>2n chrom. number</th>
<th>No. modal cells2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Daisen Fall (T)</td>
<td>4-VI-1989</td>
<td>5</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>2) Fukumoto-Sekigane (T)</td>
<td>11-VI-1989</td>
<td>3</td>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td>3) Bessho, Chiku (O)</td>
<td>11-VI-1989</td>
<td>3</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>4) Tawara, Misasa (T)</td>
<td>11-VI-1989</td>
<td>3</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>5) Mt. Jūbō, Shikano (T)</td>
<td>3-VI-1989</td>
<td>5</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>6) Hata, Chizu (T)</td>
<td>8-VI-1989</td>
<td>5</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td>7) Monomi Pass, Chizu (T)</td>
<td>8-VI-1989</td>
<td>5</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>8) Miyamoto, Chizu (T)</td>
<td>22-VI-1989</td>
<td>2</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>9) Kōzūhara, Chizu (T)</td>
<td>22-VI-1989</td>
<td>3</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td>10) Mt. Nagi, Chizu (T)</td>
<td>22-VI-1989</td>
<td>1/4</td>
<td>15/16</td>
<td>1/1</td>
</tr>
<tr>
<td>11) Higashi-UNaka, Chizu (T)</td>
<td>22-VI-1989</td>
<td>1/4</td>
<td>18/189</td>
<td>1/2</td>
</tr>
<tr>
<td>12) Kuroko Pass, Nagi (O)</td>
<td>8-VI-1989</td>
<td>3</td>
<td>20</td>
<td>9</td>
</tr>
<tr>
<td>13) Ute Pass, Chizu (T)</td>
<td>8-VI-1989</td>
<td>3/1/1</td>
<td>18/19/20</td>
<td>11/4/-</td>
</tr>
<tr>
<td>14) Komagarii, Chizu (T)</td>
<td>22-VI-1989</td>
<td>1</td>
<td>22</td>
<td>2</td>
</tr>
<tr>
<td>15) Yakōdani, Chizu (T)</td>
<td>29-VI-1989</td>
<td>1/1</td>
<td>16/17</td>
<td>1/-</td>
</tr>
<tr>
<td>16) Ashizu, Chizu (T)</td>
<td>8-VI-1989</td>
<td>2/3</td>
<td>16/17</td>
<td>5/3</td>
</tr>
<tr>
<td>17) Mitaka Dam, Chizu (T)</td>
<td>29-VI-1989</td>
<td>2</td>
<td>22</td>
<td>16</td>
</tr>
<tr>
<td>18) Tōsen Lodge, Chizu (T)</td>
<td>29-VI-1989</td>
<td>1/2</td>
<td>21/22</td>
<td>2/-</td>
</tr>
<tr>
<td>19) Ōdōri Pass, Wakasa (T)</td>
<td>6-VI-1989</td>
<td>3</td>
<td>22</td>
<td>1/-</td>
</tr>
<tr>
<td>20) Ochitori, Wakasa (T)</td>
<td>6-VI-1989</td>
<td>2</td>
<td>22</td>
<td>1/-</td>
</tr>
<tr>
<td>21) Mt. Hyōnoson (T)</td>
<td>6-VI-1989</td>
<td>4</td>
<td>22</td>
<td>6</td>
</tr>
<tr>
<td>22) Forest Park, Hanō (T)</td>
<td>6-VI-1989</td>
<td>5</td>
<td>22</td>
<td>25</td>
</tr>
<tr>
<td>23) Amedaki Fall (T)</td>
<td>1-VI-1989</td>
<td>8</td>
<td>22</td>
<td>18</td>
</tr>
<tr>
<td>24) Mt. Myōken (H)</td>
<td>20-VI-1989</td>
<td>3</td>
<td>22</td>
<td>1</td>
</tr>
<tr>
<td>25) Inanba, Hidaka (H)</td>
<td>20-VI-1989</td>
<td>1</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>26) Itoi Valley (H)</td>
<td>20-VI-1989</td>
<td>5</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>27) Shiojiri Pass (N)</td>
<td>1-VII-1982</td>
<td>1</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td>28) Lake Misuzu (N)</td>
<td>28-VI-1984</td>
<td>3</td>
<td>16</td>
<td>9</td>
</tr>
</tbody>
</table>

1) Location number corresponds to those on Figs. 1–3. Prefecture names abbreviated in parentheses: T=Tottori, O=Okayama, H=Hyogo, N=Nagano.
2) Number of modal cells in spermatogonial metaphase.
3) Individual considered to be of hybrid origin.

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Fig. 1. Distribution and chromosome numbers (gothic numerals) of Gagrellopsis nodulisera in Japan. Circled numbers denote locality codes and correspond to those in Table 1 and Appendix. 2n=16 at left shows Mt. Gokurakujį population, Hiroshima, reported by Tomohiro [1].

Fig. 2. Distribution of chromosome numbers (gothic numerals) of Gagrellopsis nodulisera in the area outlined by a rectangle in Fig. 1. The area stretches over Tottori, Okayama, and Hyogo prefectures. Open circles and solid circles denote populations with 2n=16 and 22, respectively. Populations with other numbers are depicted with double circles. Locality codes (circled numbers) correspond to those in Table 1 and Appendix.
Hybrid zone

A hybrid zone was detected in the mountainous areas surrounding Chizu Town, eastern Tottori Pref., where two populations with $2n=16$ and $2n=22$ chromosomes meet (Figs. 2, 3). Samples collected from five populations west of the Haji River, a branch of the Sendai River (Loc. Nos. 6-10 in Fig. 3), have $2n=16$ except for one specimen with $2n=15$ (Fig. 6) found in the northern foot of Mt. Nagi (Loc. No. 10). On the other hand, chromosome number increases toward the east and reaches the maximum with $2n=22$ in the vicinities of Mt. Tōsen and Mt. Okinosen. Six populations in this intergradation zone showed intrapopulation variation in chromosome number, being accompanied frequently by karyotypes with odd numbers. For instance, of five males studied for the Ute Pass population (loc. no. 13), three showed $2n=18$, and two showed 19 and 20, respectively.

Although meiosis in animals with odd chromosome number, such as $2n=17, 19, 21$, was carefully examined, no signs of meiotic disorder could be detected. In those cases, trivalents were usually observed in the first meiotic metaphases. As an example, meiotic divisions in a male with $2n=15$ from the population at the northern foot of Mt. Nagi are shown in Figure 6. Each first spermatocyte...
cytoplasmic variation of a Harvestman

271

ozygous karyotypes that are frequently found in the contact zone between 2n=16 and 2n=22 populations. These facts suggest that the diversity in chromosome number can be attributed to centric fusions/fissions, which only slightly reduce heterozygote fertility [4, 5]. Disparity among the number of chromosome arms (FN: found mental number) in various karyotypes may be caused partly from the addition of heterochromatin on the centromeric regions of some acrocentric chromosomes. Future study using C-banding analysis is needed to verify this possibility.

Spatial distribution of chromosome numbers in a nested pattern [cf. 6, 7] suggests that the widespread number is plesiomorphic and the central

**DISCUSSION**

Probable mode of rearrangements responsible for the change in chromosome number

Current study revealed remarkable geographic diversity of the chromosome number in *Gagrellopsis nodulifera*, ranging from 2n=14 to 22. Comparison among their karyotypes was not performed sufficiently due to the paucity of good spermatogonial metaphase plates obtained. However, limited data available suggested that there are concomitant increase and decrease in numbers, between metacentrics and acrocentrics. In addition, any meiotic disorder that renders a heterozygote sterility could not be detected in individuals with heterozygous karyotypes that are frequently found in the contact zone between 2n=16 and 2n=22 populations. These facts suggest that the diversity in chromosome number can be attributed to centric fusions/fissions, which only slightly reduce heterozygote fertility [4, 5]. Disparity among the number of chromosome arms (FN: found mental number) in various karyotypes may be caused partly from the addition of heterochromatin on the centromeric regions of some acrocentric chromosomes to form short arms of submetacentric chromosomes. Future study using C-banding analysis is needed to verify this possibility.

Spatial distribution of chromosome numbers in a nested pattern [cf. 6, 7] suggests that the wide ranging number is plesiomorphic and the central
number is the apomorphic state. In the present system, the wide ranging 2n=16 is considered the plesiomorphic state and the most derived state is 2n=22.

Properties and origin of the hybrid zone between populations with 2n=16 and 22

In this study, a narrow zone of intergradation in chromosome number was found in the eastern part of Tottori Pref., where populations with 2n=22 and those with 2n=16 abut. This zone is considered to be a hybrid zone derived from secondary contact of two populations that differentiated during a period of allopatry [8–10] for the following two reasons: First, several, rather than one, chromosomes participate concordantly in the difference between the two karyotypes. It is unlikely that such a case would arise through parapatric differentiation. Secondly, some other species of harvestmen show parallel patterns of intergradation of geographic variation in this area. They include *Gagrellula ferruginea* (Loman) (*Gagrellidae*) [11, Tsurusaki and Shimokawa, unpubl.], *Nelima nigricoxa* Sato and Suzuki (*Leiobuniniae*) (Tsurusaki et al., unpubl.), and *Leiobunum montanum* Suzuki (*Leiobuniniae*) (Tsurusaki and Murakami, unpubl.). Congruent geographic intergradations in the same area are also suggested in two species of damselflies, *Mnais nawai* Yamamoto and *M. pruinosus* Selys [12]. These facts strongly suggest the past occurrence of some extrinsic barrier.

Taking these points into consideration, the history of the formation of the hybrid zone in *Gagrellopsis nodulifera* may be envisaged as follows (Fig. 8):

**Stage 1.** Ancestral population of *G. nodulifera* had 2n=16 chromosomes throughout the range of the species. A new karyotype with 2n=22 occurred somewhere in the area surrounded by theSendai and Maruyama Rivers, and displaced a karyotype with 2n=16 during or before the Pleniglacial. This shift may have been proceeded by either the range expansion of individuals with the new 2n=22 karyotype to the vacant space left by the local disappearance of the population with 2n=16 or by gradual displacement of 2n=16 karyotype by 2n=22 karyotype forming a narrow hybrid zone on their front of contact. Either way, the 2n=16 and 2n=22 forms in the eastern part of Tottori Pref. may have been geographically separated by the Sendai River during the Pleniglacial (ca. 70,000–13,000 years B.P.). Climate of the area during the period is thought to have been about 5°C cooler than the present. Palynological and archaeological evidence reveals that the upper portion of the mountainous area that is now in the warm-temperate or cool-temperate zones and accompanied mainly by afforested Japanese Red Cedar (*Cryptomeria japonica*) forest and by natural beech (*Fagus crenata*) forest was covered with subarctic coniferous forest [13, 14]. Because *G. nodulifera* requires temperate zone habitats, the species distribution was shifted downward (i.e., to the north) and bilateral gene flow would be greatly hindered by the Sendai River.

**Stage 2.** As the climate became warmer after the termination of the glacial period (ca. 10,000 years B.P.), ranges of both forms gradually moved upward on the slopes of the mountains to meet each other at the upper reaches of the Sendai River, and thus hybridization occurred. A gradualcline in number from 2n=16 to 2n=22 would have been created through the succeeding backcrosses of the hybrids with each of their parental forms. If the chromosomes for which the races differ are neutral for selection, the number of generations (years in a univoltine animal like *G. nodulifera*) since contact (T) can be estimated by the following formula: \[ T = \frac{1}{2} \left( \frac{s^2}{\sigma^2} + 1 \right) \] where \( s \) is the width of the cline and \( \sigma \) is the standard deviation of individual dispersion [10, 15]. Since the width of the hybrid zone in *G. nodulifera* is rather wide as compared to other reports of hybrid zones [cf. 9, 16, 17] and no evidence of heterozygote inferiority has been observed, it is possible that this case approximates the situation explained by neutral diffusion [15]. Although data on the lifetime dispersal of this species are not available, \( \sigma \) is unlikely to be greater than 100 m. The \( s \) value is suggested by limited data on distance of individual movements in a limited time obtained for other opilionid species of comparable size [18, 19]. Therefore, if we assume that the cline in *G. nodulifera* is 15 km wide and individual dispersion (\( \sigma \)) is 100 m/generation, the number of years since contact (T) will be 7,875 years. This value seems to be fairly consistent with the biogeographic evidence based upon the climatic change during the Quaternary period discussed above. A field survey on the individual dispersal abilities, using mark-recapture method, is currently underway.

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APPENDIX

List of specimens of Gagrellidae:

- Nishio-Azuma, Shiraba Spa route, 600–1000 m, 2juv., 31-VIII-1980, NT.
- NAGANO PREF.: Kake, Ina-mura, Kogashi-mura, 1180 m Lk, 1juv., 5-VII-1988, NT.
- Gokasho, Ichikawa-mura, 1430 m Lk, 2juv., 8-VII-1989.


- Gokasho, Ichikawa-mura, 1430 m Lk, 2juv., 8-VII-1989.


- Gokasho, Ichikawa-mura, 1430 m Lk, 2juv., 8-VII-1989.