

Variation in the Shape of Genital Appendages Along a Transect Through Sympatric and Allopatric Areas of Two Brachypterous Grasshoppers, *Parapodisma setouchiensis* and *Parapodisma subaistris* (Orthoptera: Podisminae)

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ABSTRACT Divergence in genital traits between closely related species is occasionally greater in sympatry than in allopatry, possibly because directional selection facilitates differentiation so as to prevent unfit hybridization. Here, we report for the first time that the shape of a functional genital structure, cercus, differs markedly between sympatrically and allopatrically occurring individuals in two brachypterous grasshopper species, *Parapodisma setouchiensis* Inoue 1979 and *Parapodisma subaistris* Huang 1983 (Orthoptera: Podisminae). Although in areas of allopatry the cerci were straight in both species, in areas of sympatry the cerci of *P. setouchiensis* had an almost orthogonal bend. Furthermore, the angles of curvature of the cerci of *P. setouchiensis* varied continuously along a transect through sympatric and allopatric areas. We also observed copulation behavior in pairs and found that the apical part of the cerci was inserted into the space between the posterior edges of the seventh abdominal sternite and the seventh abdominal tergite of the female. We suggest that the orthogonally bent cerci allows the male to obtain a firm grip on the female's abdomen and propose that the conspicuous variation in the cerci of *P. setouchiensis* contributes to the strength of reinforcement of a premating isolation system.

KEY WORDS *Parapodisma*, geographic variation, cercus, character displacement

When an incipient allopatric species expands its range before the establishment of prezygotic isolation mechanisms, selection acting against maladaptive hybrids or against costly interspecific matings may facilitate indirect selection pressures that favor trait divergence or assortative mating (Lukhtanov et al. 2005). In particular, phenotypic divergence tends to be greater for traits that are useful for species recognition or reproduction, such as the features of the genitalia. For instance, in closely related beetle species, the range of overall body size showed considerable overlap in the sympatric area, whereas there was no overlap of genital size in any sympatric location (Kawano 2002, 2003). This phenomenon may be a consequence of reproductive character displacement resulting from directional selection so as to prevent unnecessary cost before reproduction.

The genus *Parapodisma* (Orthoptera: Acrididae: Podisminae) is endemic to Japan and East Asia and consists of 13 species. Among all the species of *Parapodisma*, *Parapodisma setouchiensis* Inoue 1979 is the most widely distributed (from Aomori Prefecture in northernmost Honshu to Yakushima Island in south-

ern Japan; Fig. 1). Two species of the genus, *P. setouchiensis* and *Parapodisma subaistris* Huang 1983, are distributed sympatrically and allopatrically in the Kinki district in western Japan (Fig. 1). These species differ little in external morphology and ecological traits, such as habitat, bionomics, and feeding habits, but they have distinctively different internal genitalia (Kanô 1983, 1985; Kawakami 2002). So far, there have been no reports of interspecific hybridization between these two species in the areas of sympatric occurrence (Kanô 1985, Tominaga et al. 1996, Kawakami 1999), suggesting the presence of reproductive isolation mechanisms in these species.

P. setouchiensis is polytypic with respect to male characters and is classified into four geographic forms based on the shapes of genital parts, namely, Basic, Yamato, Tanba, and Mt. Hyonosen forms. This leads to some controversy with respect to the taxonomic status of these forms (Kawakami 1999, Kawakami and Hoshikawa 2006, Tominaga and Ito 2006). One of the diagnostic characters that is used to distinguish the four forms is the shape of the cerci in males. The cercus bends orthogonally in the Tanba form, whereas it is nearly straight in other forms as well as in females (Kawakami 1999). The bent cercus is presumed to be an apomorphic trait (Kawakami 1999) and intergrades between the Tanba and Mt. Hyonosen forms found at the eastern boundary of the latter's distribution range.

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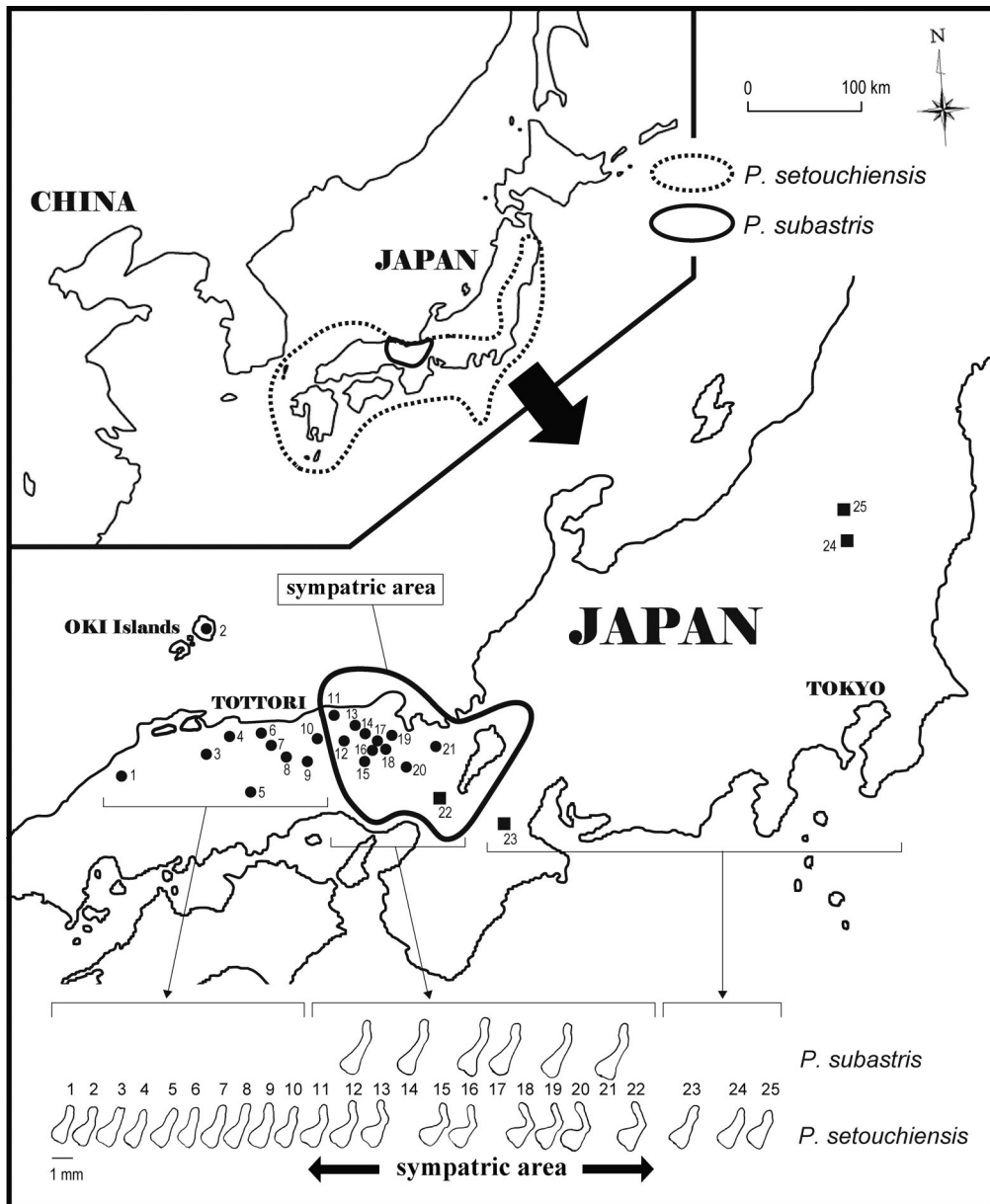


Fig. 1. Map showing the distributions of *P. setouchiensis* and *P. subaistris* in Japan (top left) and the locations of our sampling sites. Representative shapes of left cerci of males (lateral view) in each population of the two species are also shown. The region indicated by the solid line is the range where *P. setouchiensis* and *P. subaistris* occur sympatrically. Angles of the bent cerci of individuals collected from sites 22–25 (solid squares) were measured on the basis of the figures reported by Kanô (1983, 1986) and Tominaga and Kanô (1989).

In contrast to *P. setouchiensis*, *P. subaistris* males do not show such a polymorphism, and the cerci are almost straight (Kanô 1985; Tatsuta et al. 2001).

To explore the taxonomic status of each form in *P. setouchiensis*, we investigated the pattern of variation in the shape of the male cerci of *P. setouchiensis* and *P. subaistris* collected from transect areas of sympatric and allopatric occurrence. We also observed features of genital coupling in a pair of *P. setouchiensis* and considered the possible function of the male cerci.

Materials and Methods

Sampling Sites. During 1995–2002, 114 males in total, of which 91 belonged to *P. setouchiensis* and 23 to *P. subaistris*, were collected from 21 sites in western Honshu; these sites included areas of both allopatric and sympatric occurrence (Fig. 1). The samples were pooled because annual fluctuation does not significantly affect the shape of male cerci (Kawakami 1999). Most of the samples were preserved in 80% ethanol,

Table 1. Sampling locations and arithmetic means and CVs of the angles of curvature of the cerci of *P. subastris* and *P. setouchiensis*

No.	Locality	Altitude (m)	Date collected	Allopatric or sympatric	<i>P. setouchiensis</i> males			<i>P. subastris</i> males		
					N	Mean	CV	N	Mean	CV
1	Mt. Sanbe, Shimane	325	13-VIII-1996	Allopatric	1	137.0				
2	Oki Islands, Shimane		9-VIII-2000	Allopatric	2	139.0				
3	Saihaku-cho, Tottori	320	14-VIII-1995	Allopatric	5	144.2	3.2			
4	Tohaku-cho, Tottori	490	29-VIII-1995	Allopatric	7	139.4	4.0			
5	Kamogawa-cho, Okayama	240	9-VIII-1995	Allopatric	2	143.0				
6	Misasa-cho, Tottori	180	24-VII-1995	Allopatric	6	138.8	4.1			
7	Ningyō Pass, Okayama	600	10-VIII-1995	Allopatric	5	135.2	5.2			
8	Mt. Nagi, Okayama	500	17-VIII-1995	Allopatric	7	131.4	5.5			
9	Shibiki Pass, Hyogo	470	23-VIII-1995	Allopatric	5	129.0	5.5			
10	Mt. Hyonosen, Tottori	750	14-IX-1996	Allopatric	5	121.2	4.0			
11	Kan-nabe Valley, Hyogo	480	25-VIII-1995	Sympatric	4	106.0	7.7			
12	Yabu-cho, Hyogo	90	15-VII-2001	Sympatric	6	104.7	6.4	1	136.0	
13	Izushi-cho, Hyogo	140	7-VIII-1995	Sympatric	5	94.2	5.2			
14	Tantō-cho, Hyogo	230	16-VII-2001	Sympatric				4	140.3	3.8
15	Aogaki-cho, Hyogo	130	19-VII-1995	Sympatric	8	94.3	6.5			
16	Daimon, Fukuchiyama-shi, Kyoto	80	15-VIII-2001	Sympatric	4	81.3	7.8	6	133.7	4.0
17	Nagao, Fukuchiyama-shi, Kyoto	90	13-VIII-2002	Sympatric				4	134.0	2.7
18	Ōe-cho, Kyoto	50	31-VII-1995	Sympatric	7	80.3	5.3			
19	Futasegawa Stream, Kyoto	170	13-VIII-2002	Sympatric	7	90.6	8.4	6	134.3	4.0
20	Miwa-cho, Kyoto	160	20-VII-1995	Sympatric	5	79.0	3.6			
21	Miyama-cho, Kyoto	160	16-VII-2001	Sympatric				2	135.0	
22	Mt. Ponpon, Osaka		4-VIII-1977	Sympatric	1	78.0				
23	Aoyama-cho, Mie		23-VII-1981	Allopatric	1	138.0				
24	Kuriyama-cho, Tochigi			Allopatric	1	147.0				
25	Hieki-son, Fukushima		14-VIII-1980	Allopatric	1	140.0				

N, number of individuals. CV, coefficient of variation in populations where more than three males were collected. Data for populations 22, 23, 24, and 25 were obtained from figures reported by Tominaga and Kanō (1989) (p. 7, fig. 18), Kanō (1983) (p. 40, fig. 3), Kanō (1986) (p. 19, fig. 25), and Kanō (1983) (p. 40, fig. 26), respectively.

and some were preserved as dried and pinned specimens. Details of sample collection are summarized in Table 1.

Measurements of Genital Appendages. The specimens were dissected, and the left cercus of each male was drawn in lateral view using a camera lucida and a

binocular microscope; the angle between the distal protuberance and the basal part was measured using a protractor (see Fig. 2 for details). Additional data were obtained from the figures provided by Kanō (1983, 1986) and Tominaga and Kanō (1989). The angles of curvature in the cerci of sympatrically oc-

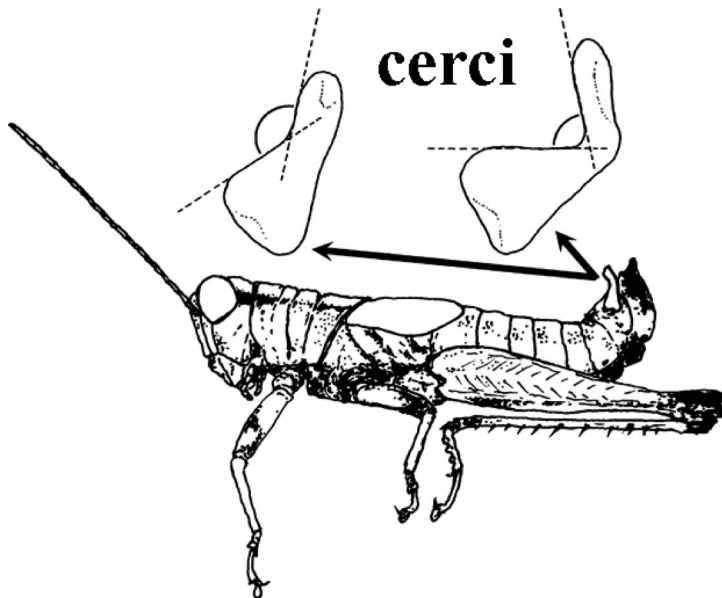


Fig. 2. Measurement of the angle of the bent cerci of males of to the two *Parapodisma* species. The degree of curvature of the cerci was represented by the angle between the inner margin of the protuberance and that of the basal part.

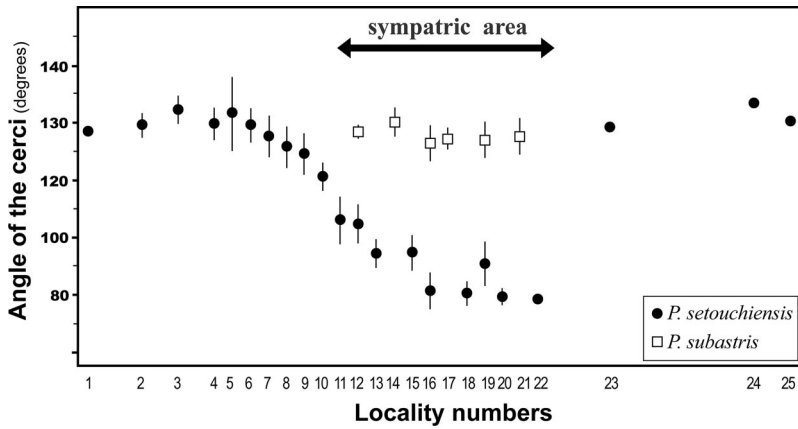


Fig. 3. The angles of bent cerci of *P. setouchiensis* (closed circles) and *P. subaestris* (open squares) males in areas where they occur allopatrically and sympatrically. The locality numbers, corresponding to those shown in Table 1 and Fig. 1, are approximately aligned from west (left) to east (right). Each population is represented by the mean (circle and square for *P. setouchiensis* and *P. subaestris*, respectively) ± 1 SD (bar).

curing individuals were compared with those of allopatrically occurring individuals (Fig. 1) by using the Watson-Williams procedure, which tests whether the mean angles of cerci are equal for each of the two species (Zar 1999).

Observation of Genital Coupling. We observed the copulation behavior of *P. setouchiensis* in the laboratory. Nymphs were collected from the following areas: Daimon, Fukuchiyama-shi, Kyoto prefecture, 80-m altitude, on 29 June 1997 and 6 and 18 July 1997 (site 16 in Table 1 and Fig. 1) and Ohmi, Miwa-cho, Kyoto prefecture, 160-m altitude, 29 June 1997 (site 20). Nymphs were reared separately in plastic cages (100

by 150 by 150 mm) to prevent mating. Individuals were provided daily with leaves of herbaceous and shrubby plants such as *Petasites japonicus* (Siebold & Zucc.) Maxim., *Hydrangea macrophylla* L., and *Artemisia princeps* Pampan. We used 5- to 7-d-old adult grasshoppers for observations of mating.

Results and Discussion

The angles of curvature of the cerci of *P. setouchiensis* and *P. subaestris* males are listed in Table 1 and Fig. 3, and representative shapes of the cerci of the two species are illustrated in Fig. 1. The angles of the bent



Fig. 4. Features of genital coupling in *P. setouchiensis* (view from the right side). Male cerci holding the edge of a female's seventh abdominal sternite. The apical part of the cercus cannot be seen because it was inserted into the space inside the seventh abdominal sternite. Refer to the text for further clarification. (Online figure in color.)

cerci ranged between 134.0 and 140.3° in *P. subastrictis* males, whereas those of in *P. setouchiensis* males showed greater variation (78.0–147.0°). The range of coefficients of variation of the angles was wider in *P. setouchiensis* (range, 3.2–8.4%) than in *P. subastrictis* (range, 2.7–4.0%; Table 1). A conspicuous and highly significant difference was observed in the angles of curvature of the cerci between *P. setouchiensis* males obtained from areas of sympatric occurrence and those obtained from areas of allopatric occurrence ($F_{1,93} = 2398.97$; $P < 0.0001$). The angles of curvature in the cerci of *P. setouchiensis* varied continuously rather than discretely among site 1–22, suggesting that there is no reason to partition individuals in corresponding areas into different species.

Figure 4 shows an illustration of genital coupling. The male cerci were observed to be fixed and immobilized at the female's seventh abdominal sternite, holding the edge of the female's abdomen from both the sides during copulation. The apical part of each cercus was inserted into the space between the posterior edges of the seventh abdominal sternite and the seventh abdominal tergite of the female. In *P. setouchiensis*, the orthogonally bent cerci had a firm grip on the female's abdomen and the bent tip could be inserted inside the seventh abdominal sternite as a hook (Fig. 4), suggesting that the highly bent form of the cerci helps in strengthening the attachment and providing a firm grip. In a related group of species in North America, the genus *Melanoplus*, male cerci may help in obtaining access to the genital chambers of females by pressing against a flap of the ventral surface of the female and pinching a side of the oviduct to expose the genital chamber (Sirof 2003). This physical amalgamation for better coupling of genital chambers might be the primary function of male cerci.

To understand how variations in the shape of the cerci in *P. setouchiensis* have evolved, it should first be demonstrated whether cerci work against unfit hybrids, because they are known to do for the copulatory piece of carabid beetles (Sota and Kubota 1998). Provided the cerci function as a recognition system for partners, cercus variation in *P. setouchiensis* is probably due to the reinforcement of a premating isolation system as a result of directional selection against maladaptive hybridization during expansion of the species. If this were the case, it would be expected that individuals of *P. setouchiensis* from a sympatric area would recognize *P. subastrictis*, thus allowing hybridization to be prevented, whereas those from an allopatric area would not discriminate between species. This hypothesis could be tested by pairing individuals from sympatric and allopatric areas.

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References Cited

- Kanô, Y. 1983. Genus *Parapodisma* around Nahari City (2). *Hirakura* 27: 37–48.
- Kanô, Y. 1985. Genus *Parapodisma* around Nahari City (3). *Hirakura* 29: 51–64.
- Kanô, Y. 1986. Podismini grasshopper in the Tohoku region and its neighborhood (2). *Tôhoku-no-Shizen* 18: 18–23.
- Kawakami, Y. 1999. Geographic variation of the brachypterous grasshopper *Parapodisma setouchiensis* group in western Honshu, with its taxonomic revision. *Spec. Divers.* 4: 43–61.
- Kawakami, Y. 2002. A possible reproductive interference and male size difference between two species of brachypterous grasshoppers, *Parapodisma setouchiensis* and *P. subastrictis*. *Tettigonia* 4: 1–6.
- Kawakami, Y., and K. Hoshikawa. 2006. Possible evidences of secondary contact between parapatric forms of *Parapodisma setouchiensis* (Orthoptera: Acrididae). *New Entomol.* 55: 9–13.
- Kawano, K. 2002. Character displacement in giant rhinoceros beetles. *Am. Nat.* 159: 255–271.
- Kawano, K. 2003. Character displacement in stag beetles (Coleoptera, Lucanidae). *Ann. Entomol. Soc. Am.* 96: 503–511.
- Lukhtanov, V. A., N. P. Kandul, J. B. Plotkin, A. V. Dantchenko, D. Haig, and N. E. Pierce. 2005. Reinforcement of pre-zygotic isolation and karyotype evolution in *Agrodiactes* butterflies. *Nature* 436: 385–389.
- Sirof, L. K. 2003. The evolution of insect mating structures through sexual selection. *Fla. Entomol.* 86: 124–133.
- Sota, T., and K. Kubota. 1998. Genital lock-and key as a selective agent against hybridization. *Evolution* 52: 1507–1513.
- Tatsuta, H., K. Miura, and S. Akimoto. 2001. Morphological differentiation among neighboring populations in the brachypterous grasshopper *Parapodisma subastrictis* (Orthoptera: Acrididae). *Entomol. Sci.* 4: 315–320.
- Tominaga, O., and Y. Kanô. 1989. A new *Parapodisma* species (Orthoptera: Catantopidae) from Japan. *Akitsu New Ser.* 102: 1–10.
- Tominaga, O., S. Y. Storozhenko, and Y. Kanô. 1996. Two new species and a subspecies of the genus *Parapodisma* (Orthoptera, Acrididae) from Japan. *Tettigonia*. 1: 1–23.
- Tominaga, O., and G. Ito. 2006. Subfamily Melanoplinae (=Podisminae), pp. 514–527. *In* The Orthopterological Society of Japan [eds.], *Orthoptera of the Japanese Archipelago in Color*. Hokkaido University Press, Sapporo, Japan.
- Zar, J. H. 1999. *Biostatistical analysis*, 4th ed. Prentice-Hall, NJ.

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