

Chromosomes of the Ragweed Beetle *Ophraella communa* (Coleoptera: Chrysomelidae) Invaded to Japan

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Abstract Chromosomes of the ragweed beetle *Ophraella communa* LE SAGE (Chrysomelidae: Galerucinae) were studied based on materials collected from four different sites in Tottori Prefecture, Honshu, Japan. Both males and females showed diploid chromosome number (2n) with 34, of which two were sex chromosomes X and Y in males and XX in females. The number is different from 2n=36 for the same species reported by PETITPIERRE *et al.* (1990) but conforms with 2n=34 by PETITPIERRE *et al.* (1993); the both based on the materials from New York State, the United States. The karyotype of the species is presented for the first time. Direction of the evolutionary change of the chromosome number in the genus *Ophraella* is briefly discussed.

Key words: chromosomes, karyotype, *Ophraella communa*, introduced species

Introduction

The ragweed beetle, *Ophraella communa* LE SAGE, 1986 (Chrysomelidae: Galerucinae: Galerucini) (Fig. 1B–D), is a species introduced to Japan in the late 1990's from North America (MORIYA & SHIYAKE, 2001; MORIYA *et al.*, 2002). The species mainly feeds on three introduced weeds of the family Asteraceae that are native to North America, *Ambrosia artemisiifolia* (Fig. 1A), *A. trifida*, and *Xanthium occidentale*, in Japan. The species was also found from Taiwan, Korea and China shortly after the first record of the species in Japan (SHIYAKE & MORIYA, 2005). Recently, the species has landed also on southern Europe and has been devastating common ragweed *Ambrosia artemisiifolia* which is alien also in Europe (BOSIO *et al.*, 2014, MÜLLER-SCHÄRER *et al.*, 2014).

Many studies have been made for *Ophraella communa* because the species provides a rare opportunity to study speciation processes with host plant associations (FUTUYAMA & MCCAFFERTY, 1990; FUNK *et al.*, 1995; KNOWLES *et al.*, 1999), ecological mechanisms of the invasion process that enable rapid expansion (TANAKA & YAMANAKA, 2009; ZHOU *et al.*, 2013), and rapid evolutionary response of the beetles to new environments (TANAKA, 2009, TANAKA, *et al.*, 2015), and evolutionary response to herbivore by the host plant *Ambrosia artemisiifolia* (FUKANO *et al.*, 2013).

We studied chromosomes of *Ophraella communa* since numerous flesh adult beetles just after eclosion were available during the survey of distribution in Tottori Prefecture, Honshu, Japan, at the initial stage of range expansion of the species in the area (see “Materials and Methods”). Chromosomes of the species have been studied for native North American populations and are reported to be 2n=36 (PETITPIERRE *et al.*, 1990) or 2n=34 (PETITPIERRE *et al.*, 1993). However, detailed karyotype of the species has remained to be unknown since those studies were made

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mainly based on meiotic metaphases obtained by squashing technique with aceto-orcein alone.

In this paper, we describe the karyotypes of both males and females of the species.

Materials and Methods

Ophraella communa was first found in four prefectures in the Kanto District (Chiba, Tokyo, Ibaraki, and Kanagawa) in 1996 in Japan and rapidly spread to various areas in the Kanto and Kinki Districts by the end of 1997 (MORIYA & SHIYAKE, 2001). In Tottori Prefecture of the San-in District, Honshu, the species was first found in 2000 at Shinosaka, Chizu-cho, southeastern part of the prefecture (MORIYA & SHIYAKE, 2001) and secondly found in 2001 from Yonago City, western part of the prefecture (KADOWAKI, 2001). We surveyed spread of *O. communa* mainly in eastern part of Tottori Prefecture just after the first discovery of the species at the entrance of Tottori Airport on 25 August 2002 and found that the species had already settled in various sites from Iwami-cho, the easternmost Town in the prefecture, to the Azo Forest Park in Tottori City (Fig. 2). No *Ambrosia artemisiifolia* infested by *O. communa* had been found during the surveys made in Iwami-cho in 2001 (KONDO, 2001) and in the Azo Forest Park by one of us (NT) in 2000. Thus, it is concluded that the species first arrived at these sites in 2001 or 2002.

Specimens (adults, pupae, and larvae) were collected from four different sites in Tottori Prefecture during the distributional survey in 2002 (Fig. 2 and Table 1). They were reared in the laboratory and adults newly hatched from pupae were used for chromosome preparation. Presence (male) or absence (female) of an apical notch on the last abdominal sternite (figs. 16 and 19 on page 11 in LE SAGE, 1986) was used for distinction of the sex in adults. Testes or ovaries dissected out from newly emerged adults were used as materials. Testis of the family Galerucinae is unique in forming a single mass of which right and left testes were fused together (SUZUKI, 1988). Testis of *O. communa* was no exception and it was an orange-colored bean-like organ firmly packed. Karyological data were obtained by air-drying method with dissociation process of cells using lactic acid (TSURUSAKI et al., 1993; TSURUSAKI, 2007). Chromosomes were observed using an Olympus BH-2 microscope and photographed with an Olympus PM-10ADS camera. Idiograms were made based on percentage ratios of each component chromosome to the total length of all the haploid autosomes and an X chromosome (total chromosome length, TCL). Nomenclature of chromosomes follows LEVAN *et al.* (1964).

Results and Discussion

Numerous well-spread spermatogonial metaphase plates and a few mitotic metaphase plates from ovaries obtained from the materials from four different sites invariably showed $2n=34$ (Fig. 3). All the autosomes were metacentric except for only two submetacentric pairs (large No. 1 which occupies nearly 11% of the total chromosome length and No. 11). The sex chromosome composition was XY (male)–XX (female), of which X is the largest (over 11% to the TCL) and Y is small (only 4% to the TCL) (Figs. 3–4). No significant difference was found among karyotypes obtained from specimens from four sites examined.

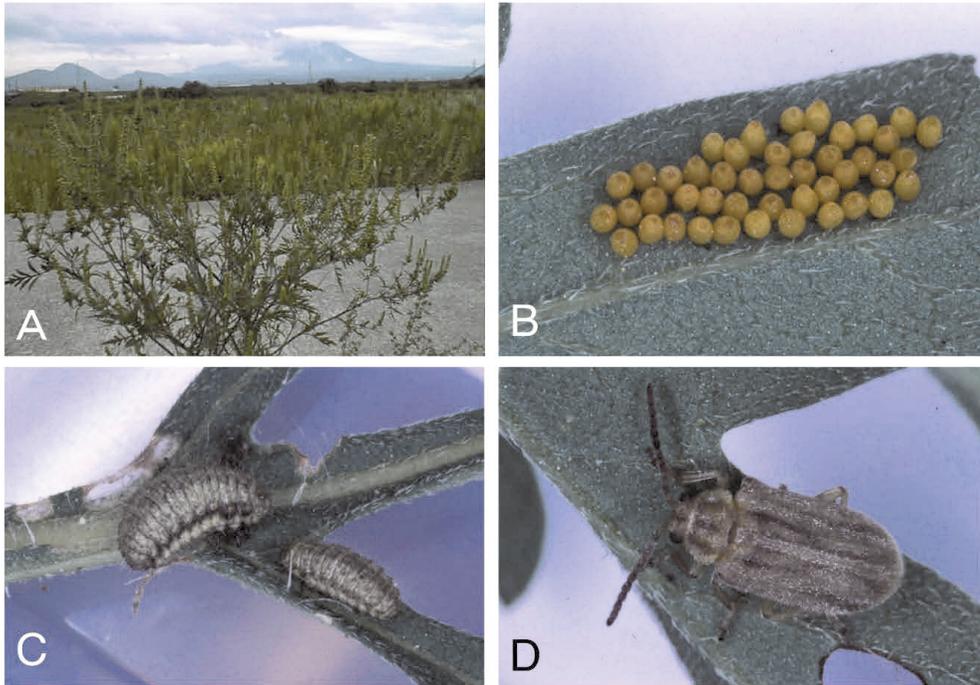


Fig. 1. *Ophraella communa* and its commonest host plant *Ambrosia artemisiifolia*. A: host plant, *Ambrosia artemisiifolia* (Kan-nonji, Yonago City, 27 September 2002). B-D: eggs (B), Second and third instar larvae (C) and an adult (D) of *Ophraella communa* (Entrance of Tottori Airport, Koyama-cho-kita, Tottori City, 25 August 2002).

Table 1. Materials of *Ophraella communa* from Tottori Prefecture in 2002 used for chromosome preparations. All the materials were collected on *Ambrosia artemisiifolia* (Asteraceae).

Locality	No. indiv. obs.	Date collected	Date fixed
Iwado, Tottori City	4♂8♀	23 Oct.	30 Oct. & 2 Nov.
Entrance of Tottori Airport, Koyama-cho Kita, Tottori City	7♂4♀	17 & 19 Sept.	20-29 Sept.
Fukurogawara, Tottori City	16♂6♀	25 Oct.	25 Oct. and 2 Nov.
Riverbed of Hino River, Kan-nonji, Yonago City	3♂4♀	27 Sept.	29 Sept.

The number of chromosomes of *O. communa* was first reported to be $2n=36$ and $n=18$ ($=17+XY$) by PETITPIERRE *et al.* (1990), though the diploid number was changed into 34 in PETITPIERRE *et al.*, (1993) without any explanation for the change. The present results showed the latter is the correct chromosome number for the species. It seems that we do not need to consider possibility of interpopulation polymorphism for the chromosome number since the first meiotic metaphase plate (MI) presented by PETITPIERRE *et al.* (1990, fig. 2 on page 691), which was alleged to show 18 similar bivalents, does not look enough to determine the chromosome number exactly. Furthermore, another photo that shows the second meiotic metaphase (fig. 12 on page 691) in the same paper can also be interpreted as $n=17$ instead of $n=18$.

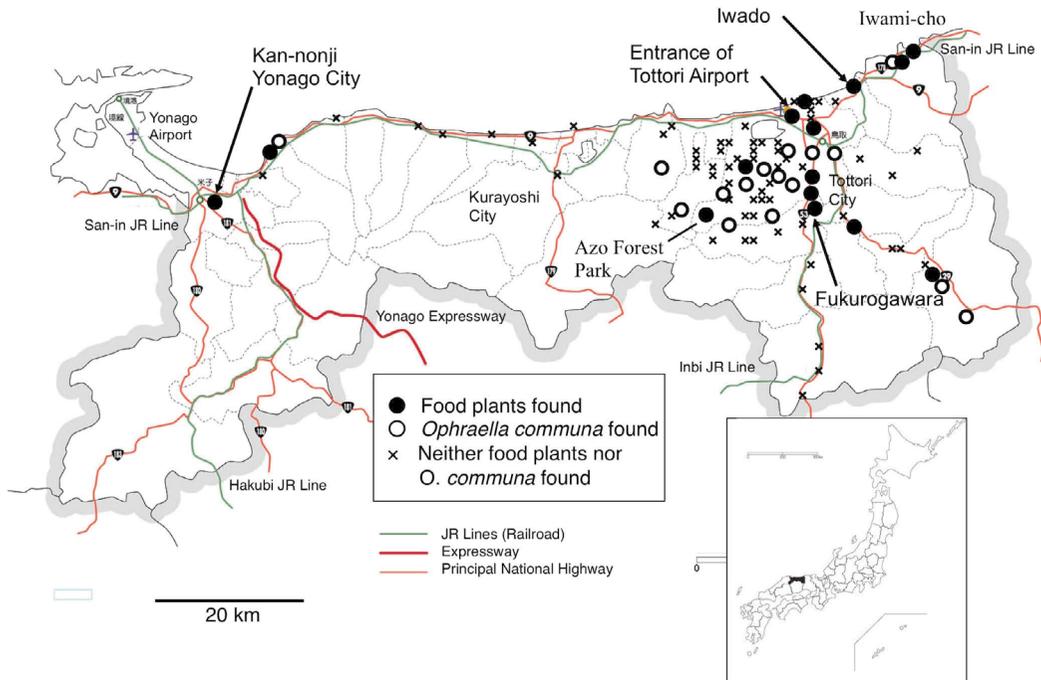


Fig. 2. Distribution of *Ophraella communa* in Tottori Prefecture in 2002 showing sources of materials used for chromosome observation (arrowed). The survey period was from 25 August to 31 October in 2002.

Of a total of 14 species described in the genus *Ophraella* (LESAGE, 1986; FUTUYMA, 1990, 1991), the chromosome number has been reported for 9 species (PETITPIERRE *et al.*, 1988, 1990, 1993, Table 2). The diploid number varies in the genus from 24 to 36, though the sex chromosome system is invariably XY-XX (male heterogametic). The number $2n=34$ shown by *O. communa* is shared by only a single species *O. pilosa* LESAGE, 1986 that occurs in Canada and adjacent northernmost areas in the United States and feeds on *Solidago* (LESAGE, 1986) in the genus. According to the phylogeny based on mitochondrial DNA of *Ophraella* (FUNK *et al.*, 1995), the closest sister species of *O. communa* is *O. bilineata* (KIRBY, 1837) that occurs in prairies of southern Canada and the northern United States and uses *Chrysopsis villosa* (Asteraceae) and *O. arctica* LESAGE, 1986 from *Solidago multiradiata scopulorum* in the arctic northwesternmost area in Canada (Fig. 5). The chromosome number of *O. bilineata* reported is $2n=36$ (PETITPIERRE *et al.*, 1990). However, it seems that re-examination of the chromosomes is necessary also for this species because the photo of the first meiotic metaphase (fig. 1 on page 691 in PETITPIERRE *et al.*, 1990) does not look good enough to get precise counts of chromosomes.

Figure 5 shows phylogenetic relationship of 12 species of *Ophraella* based on mtDNA depicted by FUNK *et al.* (1995) that incorporates known diploid chromosome numbers. From this figure, we can safely conclude that $2n=24$ shared by three species (*O. conferta*, *O. sexvittata*, and *O. cribrata*) forming a monophyletic lineage is apomorphic in the genus by outgroup comparison. Monophyly of the lineage is also supported by the phylogeny based on both morphological and

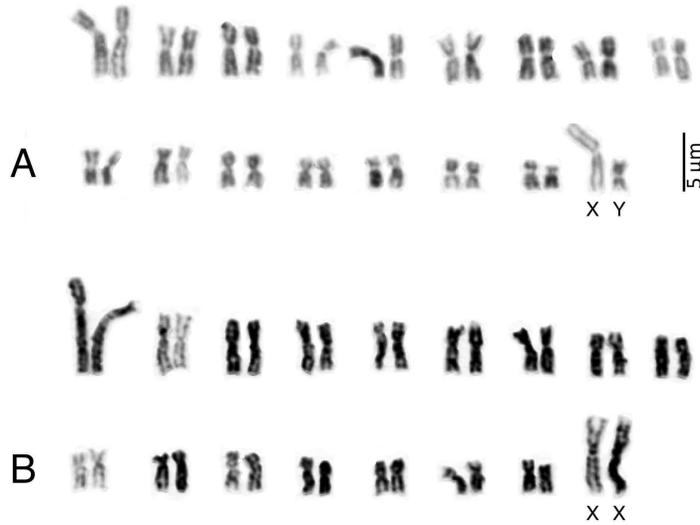


Fig. 3. Chromosomes of *Ophraella communa*. Karyotypes based on a male from Fukurogawara, Kawabara-cho, Tottori City (above) and a female from entrance of Tottori Airport, Koyama-cho Kita, Tottori City, both collected on *Ambrosia artemissifolia* (Asteraceae).

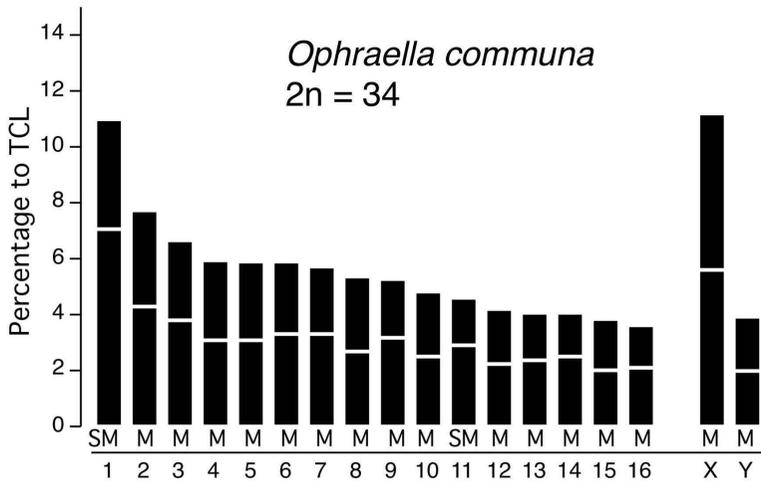


Fig. 4. Idiogram of the karyotype of *Ophraella communa* based on a male karyotype (Fig. 3).
 TCL=total chromosome length (=total length of haploid set of autosomes plus an X chromosome).

allozyme data (FUTUYMA & McCAFFERTY, 1990). On the contrary, the origin of $2n=34$ in *O. communa* is unclear. The fact that the number is shared by *O. pilosa* that is placed at the outermost position in the phylogeny favors the idea that $2n=34$ is plesiomorphic to $2n=36$. However, this scenario requires multiple evolution of $2n=36$ from $2n=34$ in the phylogeny. Unfortunately, reliability of chromosome counts in the former studies may not be so high as suggested in the case

Table 2. A list of species of the genus *Ophraella* and their numbers of chromosomes.

Species ¹	Locality	2n (♂/♀)	n (♂) ²	meiotic formula	References
<i>O. arctica</i>		-/-	-	-	Chromosomes not studied
<i>O. bilineata</i>	Saskatchewan, Canada		18	17+XY	PETITPIERRE <i>et al.</i> 1990
<i>O. communa</i>	New York State	36/-	18	17+XY	PETITPIERRE <i>et al.</i> 1990
	New York State	34/-	(17)	?	PETITPIERRE <i>et al.</i> 1993
	Tottori, Japan	34/34	17	16+XY	Present study
<i>O. californiana</i>		-/-	-	-	Chromosomes not studied
<i>O. artemisiae</i>		-/-	-	-	Chromosomes not studied
<i>O. nuda</i>	Alberta, Canada	-/-	18	17+XY	PETITPIERRE <i>et al.</i> 1990
<i>O. notulata</i>	?	-/-	18	17+XY	PETITPIERRE <i>et al.</i> 1988
	Los Angeles	36/-	18	17+XY	PETITPIERRE <i>et al.</i> 1990
	New York State	36/-	18	17+XY	PETITPIERRE <i>et al.</i> 1990
<i>O. slobodkini</i>		-/-	-	-	Chromosomes not studied
<i>O. conferta</i>	?	-/-	12	11+neoXY	PETITPIERRE <i>et al.</i> 1988
	New York State	-/-	12	11+neoXY	PETITPIERRE <i>et al.</i> 1990
<i>O. sexvittata</i>	?	-/-	12	11+neoXY	PETITPIERRE <i>et al.</i> 1988
	Florida	24/-	12	11+neoXY	PETITPIERRE <i>et al.</i> 1990
	Los Angeles	24/-	12	11+neoXY	PETITPIERRE <i>et al.</i> 1990
<i>O. cribrata</i>	?	24/-	12	11+neoXY	PETITPIERRE <i>et al.</i> 1988
	New York State	-/-	12	11+neoXY	PETITPIERRE <i>et al.</i> 1990
	New York State	24/-	(12)	?	PETITPIERRE <i>et al.</i> 1993
<i>O. notata</i>	?	-/-	18	17+XY	PETITPIERRE <i>et al.</i> 1988
	New York State	-/-	18	17+XY	PETITPIERRE <i>et al.</i> 1990
<i>O. pilosa</i>	?	-/-	17	16+XY	PETITPIERRE <i>et al.</i> 1988
	New York State	-/-	17	16+XY	PETITPIERRE <i>et al.</i> 1990
<i>O. americana</i>		-/-	-	-	Chromosomes not studied

¹ Species were arranged according to a mtDNA phylogeny (FUNK *et al.* 1995). Positions of two species (*O. californiana* and *O. americana*) not included in the phylogeny were judged by reference to LESAGE (1986).

² Numbers in parentheses were inferred from the diploid numbers.

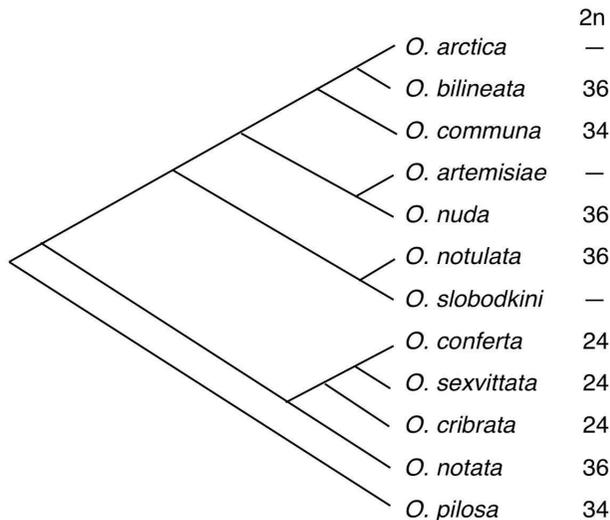


Fig. 5. Molecular phylogeny of *Ophraella* estimated by FUNK *et al.* (1995) with distribution of the known chromosome numbers (2n).

of *O. communis* and detailed karyotypes based on the spermatogonial metaphase plates are still unavailable for the other species of *Ophraella*. Thus, it is premature to draw any conclusion for the evolutionary process in the change of chromosome number in the genus.

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