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Unusual sex chromosome inheritance in six species of small ermine moths (*Yponomeuta*, Yponomeutidae, Lepidoptera)

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First meiotic division in females and males of *Yponomeuta evonymellus* (L.), *Y. cagnagellus* (Hübner), *Y. malinellus* Zeller, *Y. padellus* (L), *Y. rorellus* (Hübner) and *Y. gigas* Rebel was studied in the light microscope. In the heterogametic females the sex chromosomes were found to be associated in a sex chromosome trivalent. The sex chromosome trivalent, written AA^WZ, consisted of a W-chromosome translocated to an autosome to form an A^W-chromosome, which was paired with the homologous autosome and the Z-chromosome. In the homogametic males the two Z-chromosomes were paired in a usual bivalent. The sex chromosome inheritance can be balanced in this way. The haploid chromosome number was n = 29 A + AA^WZ in females and n = 30 A + ZZ in males, for all species examined. It is of interest to notice that the chromosome number of *Y. rorellus* earlier has been differently determined to be n = 29 for males. Especially in females the bivalents were observed to be nonhomologously associated at the telomeres during meiotic prophase.

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The evolution of reproductive isolation in the European small ermine moth genus *Yponomeuta* was launched as a case study of speciation some ten years ago (WIEBES 1976). Since then several investigators have elucidated many aspects of taxonomy, genetics, reproductive biology and host plant specificity in this group of Lepidoptera.

Quite early the chromosome number was determined for several *Yponomeuta* species based on studies of male meiosis. The haploid number of chromosomes was found to be n = 31 for *Yponomeuta evonymellus, Y. malinellus, Y. padellus,* and *Y. vigintipunctatus* (THORPE 1929; BELIAJEFF 1930; REGNART 1933; SAITOH 1960; GERSHENZON 1967a), whereas *Y. rorellus* was reported to have a reduced number of chromosomes, n=29 (THORPE 1929; GERSHENZON 1967b). Such a rearrangement of the karyotype could provide support for the origin of *Y. rorellus* by a genetic revolution at a population bottleneck (LÖFSTEDT et al. 1986; MENKEN 1987).

To further substantiate this argument we decided to compare the karyotype of *Y. rorellus* with those of two close relatives, *Y. cagnagellus* and *Y. gigas*. *Y. gigas* is an endemic species of the Canary Islands but has many similarities with *Y. rorellus*, including a different host plant family and a simple pheromone compared to more ancestral ermine moths. In contrast *Y. cagnagellus* conforms to the majority of European small ermine moths with respect to these characteristics, but it is still morphologically and biochemically closely related to *Y. rorellus*.

Lepidopterous chromosomes have a marked tendency to contract to spherical or ovoid bodies in metaphase, making detailed investigations of the karyotype difficult (ROBINSON 1971). We found it important to reexamine the karyotypes of some of the above-mentioned small ermine moths with modern cytological methods, aimed at making the chromosomes more amenable to observation, and to include females as well as males in the study. Thus, to complete our study we also established the karyotypes of *Y. evonymellus, Y. malinellus* and *Y. padellus*. A detailed examination of first

Dedicated to Professor Åke Gustafsson on the occasion of his 80th birthday

meiotic division revealed an interesting sex chromosome trivalent in females of all species studied and a different chromosome number for *Y. rorellus* compared to earlier studies.

Material and methods

Small ermine moths were collected as late instar larvae from their host plants. *Yponomeuta evonymellus* (L.) and *Y. padellus* (L.) were collected in the vicinity of Lund, Sweden, from bird cherry (*Prunus padus*) and sloe (*Prunus spinosa*), respectively. *Y. cagnagellus* (Hübner), *Y. malinellus* Zeller, and *Y. rorellus* (Hübner) originated from the Netherlands, where they had been collected from spindle tree (*Euonymus europeus*), apple (*Malus sp.*), and willow (*Salix sp.*), respectively. *Y. gigas* Rebel was collected from *Populus alba,* on the island of Tenerife, the Canary Islands. Some of the larvae of each species were allowed to pupate and emerge under controlled environmental conditions, a 16 h light—8 h dark LD-cycle at 25°C constant temperature.

Gonads from male larvae and ovaries from adult females were prepared in Ringer's solution (7.5 g NaCl; 0.35 g KCl and 0.21 g $CaCl_2$ per litre). After hypotonic treatment in 0.075 M KCl solution for 2 min, gonads and ovaries were fixed in Carnoy II fixative (6:3:1) for 2 hours. The material was stored in new fixative at –18°C, up to several months.

Most slides were made according to the Feulgen-Giemsa staining method described by Puro and Nokkala (1977), which is especially useful for thin prophase chromosomes in oocytes of insects. Some slides were made according to the C-banding technique described by Camacho et al. (1985). After carrying ovaries to distilled water through a decreasing series of alcohols, dry squashes for C-banding were made by the dry ice method. All photomicrographs of slides were taken in a light microscope.

Results

Meiotic prophase in *Yponomeuta* females

Egg chambers from adult females of small ermine moths consist of the oocyte and seven nurse cells surrounded by a follicle cell monolayer. During egg maturation the oocyte enters first meiotic division, and in the mature egg the holokinetic chromosomes are fully condensed in a metaphase I plate.

Female meiosis was followed from early pachytene in medium-sized egg chambers, to metaphase I in mature eggs (Fig. 1–7). In all the examined stages the chromosomes were isopycnotic. During a period of pachytene the bivalents consist of homologous lampbrush chromosomes aligned in parallel and with structures interpreted as chromomeres and fuzzy loops (Fig. 1). In a few instances the pachytene lampbrush chromosomes were found to be connected by nonhomologous telomeric associations. In early pachytene two chromosome associated nucleoli were present in some preparations. In late pachytene the bivalents became more condensed and only one large, free nucleolus was found. After pachytene yolk production was intensified, the nucleolus disappeared and chromosome condensation proceeded without any diplotene and diakinesis stages, i.e., no chiasmata were present. At prometaphase bivalents could be observed as 1–5 μm long and 0.5–1 μm thick homologous chromosomes aligned in parallel and connected to each other by nonhomologous telomeric associations (Fig. 2). At metaphase I the chromosomes became even more condensed and the telomeric associations could form long bivalent chains (Fig. 3–5). When the material was treated at prolonged time in acetic acid before squashing, the bivalent chains tended to disappear (Fig. 6). At the end of egg maturation the spindle was visible and the bivalents were aligned in a metaphase I plane (Fig. 7).

Meiosis in *Yponomeuta* males

In male larvae of *Yponomeuta* the gonads consist of cysts with clusters of spermatocytes where meiosis occurs synchronously.

Meiosis was studied from pachytene to the end of the first meiotic division (Fig. 8–13). During pachytene homologues of chromosomes were tightly synapsed in bivalents (Fig. 8). After pachytene chromosome condensation proceeded into diplotene and diakinesis (Fig. 9). At metaphase I the bivalents became strongly compacted and could be connected by nonhomologous associations (Fig. 10–11). The bivalents were aligned in a non-radial metaphase I plate, which successively became tighter (Fig. 12).

After metaphase I homologous chromosomes separated regularly in anaphase I (Fig. 13), and meiosis was then completed by the second meiotic division.

Sex chromosome trivalent

In all the investigated *Yponomeuta* species a chromosome trivalent was found in female meiosis.

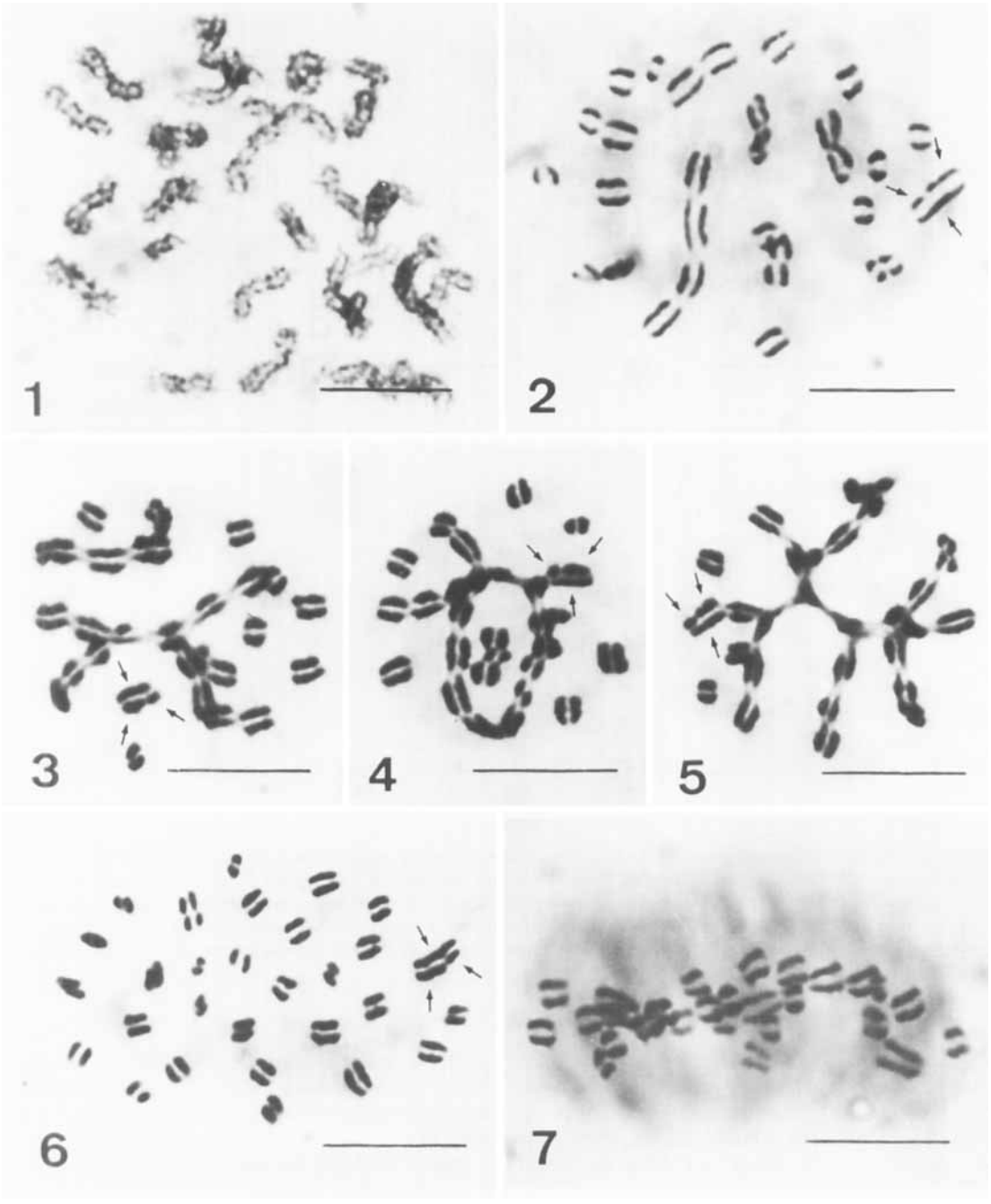


Fig. 1–7. Meiotic prophase in oocytes from adult *Yponomeuta* females. Feulgen-Giemsa method. **Fig. 1.** Pachytene lampbrush chromosomes in *Y. padellus*. **Fig. 2.** Nonhomologous telomeric associations at prometaphase in *Y. cagnagellus*. Arrows indicate the sex chromosome trivalent, n=29A+AA^{W}Z. **Fig. 3–5.** Nonhomologous telomeric associations at metaphase I in *Y. rorellus*. Arrows indicate the sex chromosome trivalent. Note that the trivalent could be associated to bivalents with telomeric associations in both ends (Fig. 4–5), n=29A+AA^{W}Z. **Fig. 6.** Only few nonhomologous telomeric associations at metaphase I in *Y. malinellus*. Arrows indicate the sex chromosome trivalent, n=29A+AA^{W}Z. **Fig. 7.** Spindle and metaphase I plane in *Y. evonymellus*. (Bars = 10 μm).

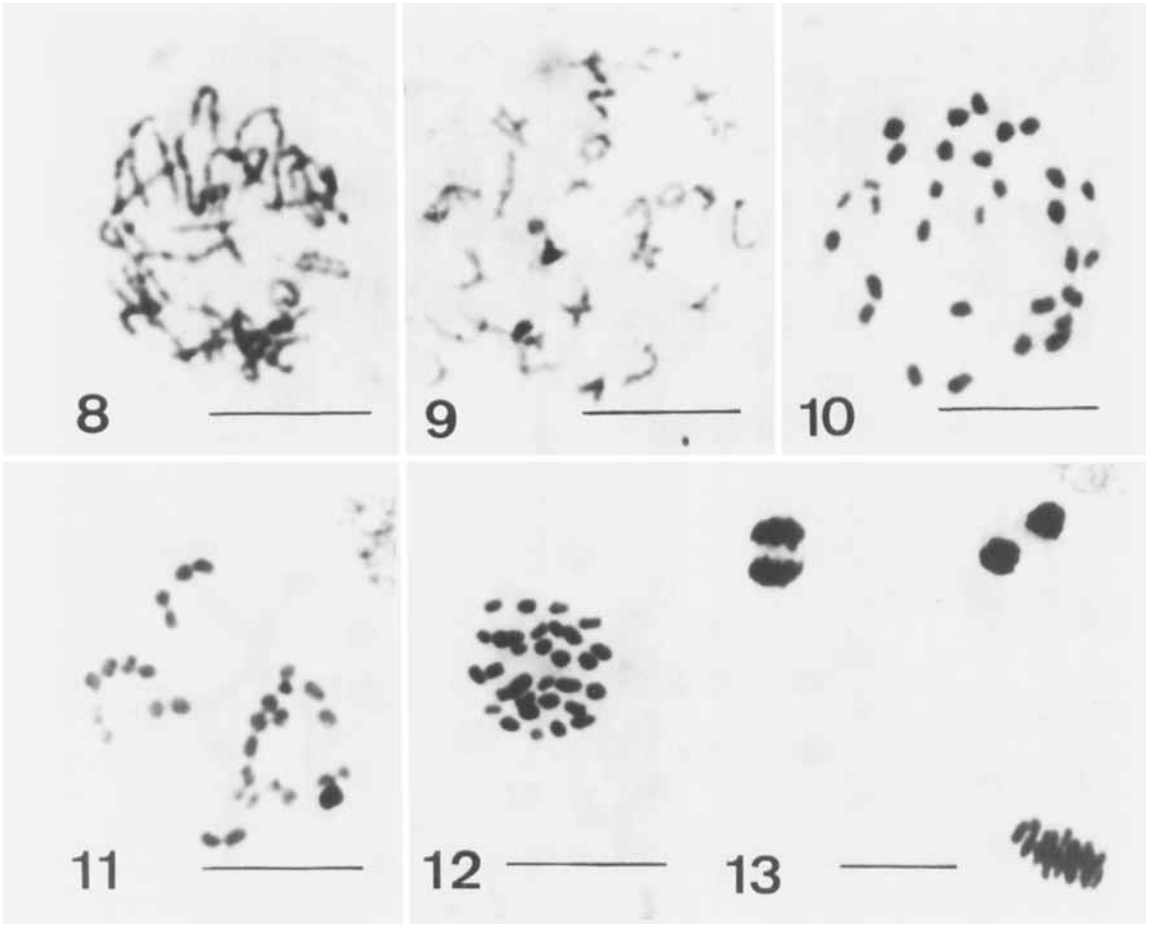


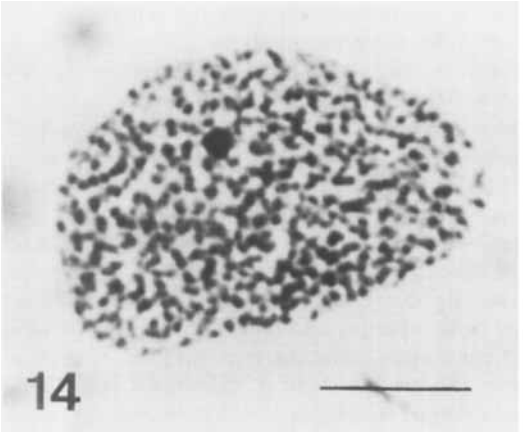
Fig. 8–13. Meiotic prophase in gonads from *Yponomeuta* male larvae. Feulgen-Giemsa method. **Fig. 8.** Pachytene with tightly synapsed bivalents in *Y. rorellus*. **Fig. 9.** Bivalents with chiasmata at diakinesis in *Y. rorellus*. **Fig. 10.** Bivalents entering metaphase I in *Y. rorellus*. Note the nonhomologous associations between bivalents, n=31. **Fig. 11.** Bivalents entering metaphase I in *Y. rorellus*. Nonhomologous associations between bivalents can be observed, n=31. **Fig. 12.** Metaphase I plate in *Y. rorellus*, n=31. **Fig. 13.** Metaphase I, anaphase I, and telophase I at first meiotic division in *Y. rorellus*. (Bars = 10 μm).

At prometaphase the trivalent consists of a short chromosome plus a chromosome of medium length, paired with a long chromosome (Fig. 2). At metaphase I the trivalent was more difficult to find, due to the chromosomes being maximally condensed (Fig. 3–6).

We suppose that the chromosome trivalent is a sex chromosome trivalent. If this is not the case, the chromosome number should vary among different females or males, which we did not observe.

In follicle cells from *Yponomeuta* females, but not in intercyst cells from *Yponomeuta* males, one distinct chromatic body was found and we interpreted it as a heteropycnotic W-chromosome (see TRAUT and MOSBACHER 1968) (Fig. 14). This means that the heterogametic female sex is of ZW type and not ZO.

After C-banding the longest chromosome in the sex chromosome trivalent was found to be more darkly stained in the part paired with the chromosome of medium length in the trivalent. (Fig. 15). For this reason the longest chromosome was interpreted as a W-chromosome (intensely C-banded) with an autosomal chromosome translocated to it. The chromosome of medium length was thus interpreted as a Z-chromosome, and the smallest



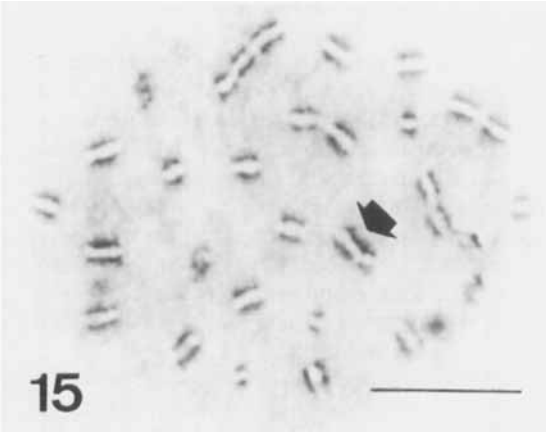


Fig. 14–15. Sex chromatin in *Yponomeuta* females. **Fig. 14.** One heteropycnotic body (sex chromatin) in an interphase nucleus of a follicle cell from *Y. gigas*. Feulgen-Giemsa method. **Fig. 15.** C-banded meiotic chromosomes in *Y. evonymellus*. Arrow indicates the longest chromosome in the sex chromosome trivalent. Note that it is stained more darkly in the part of the chromosome that corresponds to the medium sized chromosome in the trivalent. The C-banded part of the trivalent is interpreted as the W-chromosome translocated to an autosome, and the trivalent could therefore be written $AA^{W}Z$. (Bars = 10 μm).

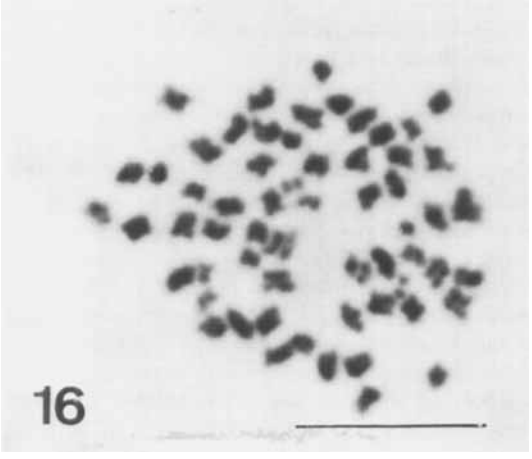


Fig. 16. Mitotic chromosomes in oogonia from *Yponomeuta gigas*, 2n=61. (Bar = 10 μm).

chromosome should be the autosome homologous to the translocated one. Consequently, the trivalent could be written $AA^{W}Z$. C-bands were also found in the bivalents and especially at the telomeres (Fig. 15).

Chromosome numbers in *Yponomeuta*

The chromosome number was constant in all the examined *Yponomeuta* species (Table 1). In female meiosis the number of bivalents was found to be 29 plus one sex chromosome trivalent, i.e., $n=29A+AA^{W}Z$ (Fig. 2–6). In mitosis in oogonia of *Y. gigas* (Fig. 16) and in follicle cell mitosis in egg chambers of *Y. rorellus* the diploid chromosome number was determined to be 2n=61.

In male meiosis the number of bivalents was observed to be 31, which then ought to be written as n=30A+ZZ (Fig. 10–12).

Discussion

From the present study it is clear that female meiosis of *Yponomeuta* is achiasmatic, while male meiosis has chiasma formation. During prophase in females the chromosomes are aligned in parallel in the bivalents and the trivalent and chromosome condensation proceeds without any chiasma formation (Fig. 1–7). In males, on the other hand, both diplotene and diakinesis are present in the meiotic prophase (Fig. 8–13). Achiasmatic meiosis in female but not in male Lepidoptera is common (see NOKKALA 1987).

Nonhomologous telomeric assocations between bivalents were observed in all *Yponomeuta* females studied. According to NOKKALA (1987), who described the same phenomenon in *Sphinx ligustri* females, the telomeric associations disappear more or less at metaphase I. In *Yponomeuta* females, however, we could also see long bivalent chains at metaphase I (Fig. 3–5), but as the length of the bivalent chains is reduced at prolonged time in acetic acid before squashing, it is hard to say anything about the pattern by which such associations occur. The affinity between telomeres must be strong since it is difficult to squash connected bivalents apart. However, it is not clear to us whether the telomeric associations are specific or not. The two smallest chromosomes in the sex chromosome trivalent are always associated at the telomeres, but it is not likely that the trivalent or any bivalent in other cases is always connected with the same neighbour since the bivalent chain could have different appearance (Fig. 3–5). A possibility, suggested by NOKKALA (1987), is that maternal telomeres are preferentially connected with other maternal telomeres and paternal telomeres, with other paternal ones. This would result in maternal chromosomes and paternal chromosomes separated in different pseudo-linkage groups, as far as the telomeric associations are preserved throughout metaphase I.

Bivalent associations were also observed in *Yponomeuta* male meiosis (Fig. 10–11). NOKKALA (1987), however, did not report any associations in males of another Lepidoptera species, *Sphinx ligustri*. Whether the associations are telomeric or not could not be established due to the dotlike shape of the bivalents in male metaphase I. However, it is probable that bivalent connections have a crucial role for a normal chromosomal segregation, at least in females of *Yponomeuta*.

All *Yponomeuta* species examined by us had a special sex chromosome inheritance, not described before in insects as far as we know. It seems, however, to be comparable with the inheritance described for some mammals by FREDGA (1970), although in these species the chromosomes are not holokinetic as in Lepidoptera. In the homogametic (male) sex of *Yponomeuta* the two Z-chromosomes are paired and separated as usual. However, in the heterogametic female sex the W-chromosome is translocated to an autosome and then paired with the Z-chromosome and the autosome homologue. The sex chromosome trivalent in females could consequently be written $AA^{W}Z$. Females must produce two types of eggs; one type with the translocated A^{W}-chromosome and one type with the Z-chromosome and the autosome A. Combined with the Z-chromosome-bearing sperms one would get males with AA and ZZ and females with $AA^{W}Z$. The sex chromosome inheritance is thus balanced in this way.

Sex chromosome trivalents have previously been described in some Lepidopteran females (SUOMALAINEN 1969), but these trivalents were interpreted as a Z-chromosome aligned with two shorter W-chromosomes, which had arisen from a chromosome break in one original W-chromosome. The reason for this interpretation was that SUOMALAINEN (1969) found two heteropycnotic bodies in interphase nuclei, which would correspond to the two W-chromosomes. However, in one case, in somatic cells of a female of *Bactra furfurana*, he found only one sex chromatin body. Our suggestion is that this could be due to the same sex chromosome inheritance as we have observed in six *Yponomeuta* species.

SUOMALAINEN (1971) has pointed out that sex chromosome pairs, unequal in length, are unusual in the Lepidoptera, but gives an example in the moth *Lozotaenia fosterana* where he interpreted the smallest of the two sex chromosomes as a W-chromosome arisen from a deletion in an original W-chromosome of the same length as the Z. From C-banding of the sex chromosome trivalent in *Yponomeuta* females (Fig. 15) the sex chromosomes seem to be somewhat unequal in length, but with the Z-chromosome being the smallest. This indicates that the mechanism by which unequal sex chromosome pairs arise does not necessarily follow the pattern suggested by SUOMALAINEN (1971).

The most common haploid chromosome number in Lepidoptera is n=31 (ROBINSON 1971), which equals the basic haploid chromosome number we have found in *Yponomeuta* (Table 1). This number was established in males of the six species studied. In females, on the other hand, two of the chromosomes in the basic karyotype have been fused to

Table 1. Haploid chromosome numbers among different *Yponomeuta* species determined in female and male meiosis

Species	Female meiosis	Reference	Male meiosis	Reference
Y. evonymellus	n=29A+AA^{W}Z	This study	n=30A+ZZ	BELIAJEFF 1930 REGNART 1933
Y. cagnagellus	n=29A+AA^{W}Z	This study	n=30A+ZZ	This study
Y. malinellus	n=29A+AA^{W}Z	This study	n=30A+ZZ	This study SAITOH 1960 GERSHENZON 1967a
Y. padellus	n=29A+AA^{W}Z	This study	n=30A+ZZ	This study GERSHENZON 1967a
Y. rorellus	n=29A+AA^{W}Z	This study	n=30A+ZZ n=29	This study THORPE 1929 GERSHENZON 1967b
Y. gigas	n=29A+AA^{W}Z	This study	n=30A+ZZ	This study

Note that the chromosome number found in *Y. rorellus* males (n=31) differs from earlier investigations

each other to form an A^W-chromosome, which shows up in the sex chromosome trivalent. Thus, the diploid chromosome number in females is only 61.

The haploid chromosome number (n=31) for *Y. rorellus* males found by us does not agree with the reports of THORPE (1929) and GERSHENZON (1967b), which claimed n=29 for this species. The explanation for this discrepancy is not clear to us, but in our population the chromosome number of *Y. rorellus* is not different from those of other European small ermine moths. Thus, in contrast to the earlier literature, our recent study of ermine moth chromosome complement does not give any support for the hypothesis about a "genetic revolution" at a population bottleneck in the early history of *Y. rorellus* (LÖFSTEDT et al. 1986; MENKEN 1987). The hypothesis can of course still be true, but is now harder to confirm.

The sex chromosome trivalent in *Yponomeuta* is of considerable interest as it shows how reduction in the basic chromosome set can occur in Lepidoptera through successive fusions of holokinetic chromosomes. In order to follow such alterations it would be of interest to study the chromosome numbers in additional *Yponomeuta* species and in genera related to *Yponomeuta*.

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