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Absence of sex chromatin corresponds with a sex-chromosome univalent in females of Trichoptera

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Cytogenetics, Trichoptera, *Anabolia furcata*, *Hydropsyche* sp., *Limnephilus decipiens*, *Polycentropus flavomaculatus*, *Rhyacophila* sp., sex chromatin, sex chromosomes

Abstract. Five Trichoptera species, representing four different families of three suborders, have been examined for sex chromatin status in relation to their sex chromosome system. These were *Hydropsyche* sp., *Polycentropus flavomaculatus* (Pictet), *Rhyacophila* sp., *Anabolia furcata* Brauer and *Limnephilus decipiens* (Kolenatý). None of the species displayed sex-specific heterochromatin in highly polyploid nuclei of the Malpighian tubule cells. Such sex chromatin is a characteristic trait of the heterogametic female sex in the sister order Lepidoptera; it is derived from the heterologous sex chromosome W. Hence, the absence of sex chromatin in somatic nuclei of Trichoptera females indicated the lack of a W chromosome in their karyotype. Correspondingly, diploid chromosome sets of the females consisted of an odd chromosome number, two sets of autosomes and one sex chromosome Z. Thus, the Z/ZZ chromosome mechanism of sex determination has been confirmed. In pachytene and postpachytene oocytes, the Z chromosome having no pairing partner formed a univalent. In *Hydropsyche* sp., the Z-univalent was distinct as a compact, positively heteropycnotic element. Whereas, in two other caddis-flies, *P. flavomaculatus* and *L. decipiens*, it formed a negatively heteropycnotic thread. In postpachytene nuclei of nurse cells of *A. furcata*, two sister chromatids of the Z chromosome separated as a result of chromosome degeneration and formed a negatively heteropycnotic pseudobivalent. The species-specific differences in pycnosis may reflect a transcriptional activity/inactivity of the Z chromosome during meiotic prophase. The absence of sex chromatin and the sex chromosome system in Trichoptera are characters in common with the "primitive" Lepidoptera. This supports a hypothesis that the common ancestor of both orders had a Z/ZZ sex chromosome mechanism.

INTRODUCTION

The Trichoptera, or caddis-flies, are the most closely related to the Lepidoptera (e.g. Kobayashi & Ando, 1988; Neboiss, 1991), and it is accepted generally that the two insect orders constitute a monophyletic group, the superorder Amphiesmenoptera (Kristensen, 1991; Morse, 1997). Their close relationship also applies to basic cytogenetic characteristics.

Both Trichoptera and Lepidoptera exhibit a similar morphology and kinetic organization of chromosomes. The chromosomes are usually spherical, lack distinct primary constrictions (centromeres) and sister chromatids separate by parallel disjunction at mitotic metaphase (Suomalainen, 1966; Murakami & Imai, 1974). Due to these characteristics, both Trichoptera and Lepidoptera had been regarded as species with holokinetic chromosomes (meaning sensu stricto distribution of kinetic activity along the entire poleward

chromosome surface). However, recent findings in two caddis-flies, *Anabolia furcata* Brauer (Limnephilidae) (Wolf et al., 1992) and *Limnephilus decipiens* (Kolenatý) (Limnephilidae) (Wolf et al., 1997), confirmed an earlier finding of Gassner & Klemetson (1974) in a moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), that the chromosomes exhibit a localized kinetochore plate. The kinetochore plate, in contrast with that in the typical monokinetic chromosomes, covers a relatively large portion of the chromosomal surface. Thus, these findings favour a chromosome type intermediate between the traditional holokinetic and monokinetic chromosomes (see Wolf et al., 1992; Wolf, 1996). A polykinetic organization of chromosomes recently described in two lepidopteran species with reduced chromosome numbers, *Orgyia thyellina* Butler and *O. antiqua* (L.) (Lepidoptera: Lymantriidae), is the only exception known (Wolf et al., 1997).

A similarity of the commonest haploid chromosome numbers, n = 31 in the Lepidoptera and n = 30 in the Trichoptera, was interpreted as another trait in common (Suomalainen, 1966, 1969). Although there is a well-founded doubt about the n = 30 as a type chromosome number in the Trichoptera (Kiauta, 1971), relatively high chromosome numbers (n = 22–30) predominate in most studied families of the suborder Integripalpia. The low chromosome numbers (n = 13 and n = 15) in the other suborder, Annulipalpia, with only three species karyotyped (see Lankhorst, 1970), might have evolved as a derived character. Thus, it appears likely that the common ancestor of the Trichoptera and Lepidoptera had a high haploid chromosome number close to 30 (see discussion in Wolf et al., 1997).

Finally, the female heterogamety and achiasmatic female meiosis are the further synapomorphies corroborating a monophyletic origin of Trichoptera and Lepidoptera (Suomalainen, 1966; Rasmussen, 1977; Traut, 1977a; Nokkala, 1987). The mode of achiasmatic meiosis differs in many respects from that well known in *Drosophila* males. In females of Lepidoptera, oocytes undergo normal sequence of meiotic events until the end of pachytene stage; their chromosomes pair in bivalents and display fully-formed synaptonemal complexes (SCs). Beyond pachytene, no chiasmata occur. Instead the SCs are transformed into a modified form that later detaches from the bivalents and during chromosome segregation persists in the metaphase plate as the so-called "elimination chromatin" (for a review see Marec, 1996). Although we lack such detailed data in Trichoptera, one may assume a similar mode of achiasmatic meiosis. In a few studies on this topic, the absence of chiasmata and the "elimination chromatin" were reported for two caddis-flies, *Limnephilus decipiens* (Kolenatý) and *L. borealis* (Zetterstedt) (Suomalainen, 1966); achiasmatic oogenesis was also mentioned in other two limnephilid species, in *Glyphotaelius pellucidus* (Retzius) by Kiauta & Lankhorst (1969) and in *Allogamus auricollis* (Pictet) by Kiauta & Kiauta (1979).

In the majority of Lepidoptera species examined cytogenetically, the heterogametic female sex displayed another characteristic trait, sex-specific heterochromatin (= sex chromatin; for a review see Traut & Marec, 1996). The sex chromatin forms one or more heterochromatin bodies in somatic interphase nuclei. It is derived from the sex chromosome W, most Lepidoptera having a WZ/ZZ sex chromosome mechanism. All species, in which sex chromatin has been found, belong to the main clade of Lepidoptera, the Ditrysia. It has also been shown that secondary losses of the W chromosome, occurring sporadically in the Ditrysia, have resulted in a lack of the female-specific heterochromatin. However, sex chromatin has not been reported for any species of the "primitive", non-

ditrysian families indicating that they possess a Z/ZZ sex chromosome mechanism (Traut & Marec, 1996). The absence of a W chromosome in a "primitive" species *Micropterix calthella* (L.) (Micropterigidae) has been confirmed recently. Based on these findings, it has been proposed that the WZ sex-chromosome pair evolved later, in the ditrysian branch of Lepidoptera (Traut & Marec, 1997).

The Trichoptera are generally believed to possess a Z/ZZ chromosome system of sex determination. However, cytogenetic data on sex chromosomes in Trichoptera females are very poor, based on only few species investigated (Klingstedt, 1931; Suomalainen, 1966; Kiauta & Lankhorst, 1969; Lankhorst, 1970; Kiauta & Kiauta, 1979). In addition, no data have been published so far on the sex chromatin status as an indicator of the absence or presence of a W sex chromosome in Trichoptera. To fill this gap we have, in the present study, examined the sex chromatin status and sex chromosomes in selected representatives of all three Trichoptera suborders (i.e., Annulipalpia, Spicipalpia, and Integripalpia), each suborder supposedly constituting a monophyletic group (Morse, 1997). These were *Hydropsyche* sp. (Hydropsychidae) and *Polycentropus flavomaculatus* (Pictet) (Polycentropidae) representing the Annulipalpia, *Rhyacophila* sp. (Rhyacophilidae) from Spicipalpia, and *Anabolia furcata* Brauer and *Limnephilus decipiens* (Kolenatý) (both Limnephilidae) from Integripalpia.

MATERIAL AND METHODS

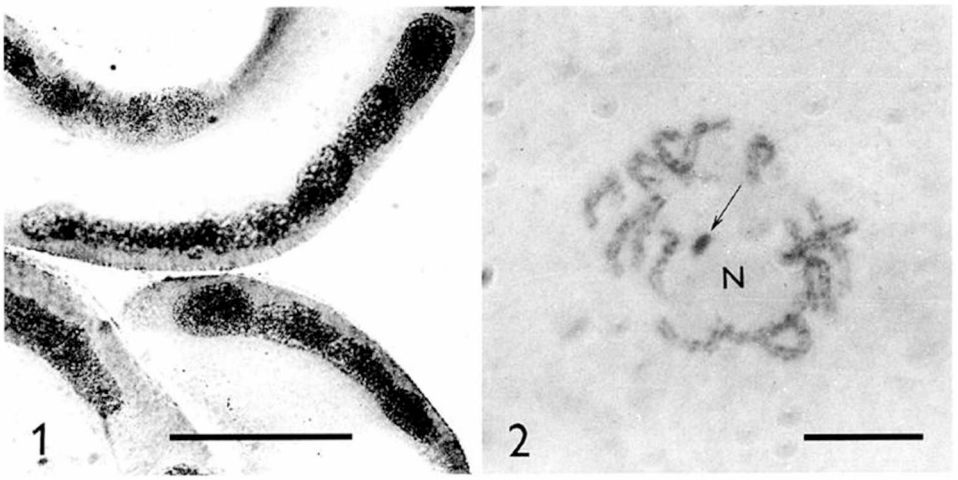
All samples of Trichoptera populations examined originated from localities in the South Bohemia (the Czech Republic). The last instar larvae of *Rhyacophila* sp. (Rhyacophilidae) were collected in the river Vltava near the settlement Františkov (the Šumava mountains) in August 1995. Theoretically, the larvae might belong, according to their morphology and adults flying in this locality, to three different species of the genus *Rhyacophila*: *R. obliterata* Mac Lachlan, *R. praemorsa* Mac Lachlan, and/or *R. fasciata* Hagen. Two other species, *Polycentropus flavomaculatus* (Pictet) (Polycentropidae) and *Hydropsyche* sp. (Hydropsychidae), were collected as fully grown or middle-aged larvae, respectively, in a stream flowing out from the pond Mrhal near České Budějovice in February 1995. Larvae of *Anabolia furcata* Brauer (Limnephilidae) were collected at the end of June 1995 in the river Smutná near the town Bechyně. Finally, larvae of *Limnephilus decipiens* (Kolenatý) (Limnephilidae) were collected in the pond Domin near České Budějovice in May 1994 and 1995. However, in these larvae, it was difficult to distinguish the sex. This species enters imaginal diapause after the emergence from pupae, and thus, larval testes and ovaries are developed poorly (Novák & Sehnal, 1963). Therefore, we reared the collected larvae of *L. decipiens* in an aquarium at laboratory conditions and fed them with reed leaves (Poaceae: *Phragmites communis*), lettuce leaves (Asteraceae: *Lactuca sativa*), and dead insect larvae. The larvae pupated within two weeks. For dissections, freshly emerged adults were used instead of larvae to be certain of their sex.

To determine the sex chromatin status of the caddis-flies, Malpighian tubules were dissected from both sexes. Highly polyploid nuclei of the tubule cells were stained with lactic acetic orcein (for details see Traut et al., 1986) and inspected under a light microscope at a magnification of 150–300 ×. Spread preparations of chromosomes from female ovaries were made essentially following the procedure described by Traut (1976) and by Schulz & Traut (1979) for pachytene mapping. Chromosomes were stained with lactic acetic orcein and examined in phase-contrast micrographs.

RESULTS

Hydropsyche sp.

A total of 11 female larvae and six male larvae were examined for the sex chromatin status in somatic cells of the Malpighian tubules. Highly polyploid nuclei of the cells were dissimilar to the branched or lobed nuclei frequently observed in moths and butterflies



Figs 1–2. *Hydropsyche* sp. female larvae. 1 – a part of the Malpighian tubules showing highly polyploid long nuclei without any sex-specific heterochromatin (scale bar – 100 μm); 2 – a pachytene oocyte nucleus with 14 autosomal bivalents and a small compact Z chromosome (arrow), a large grey spot in the central space represents a nucleolus (N) (scale bar – 10 μm).

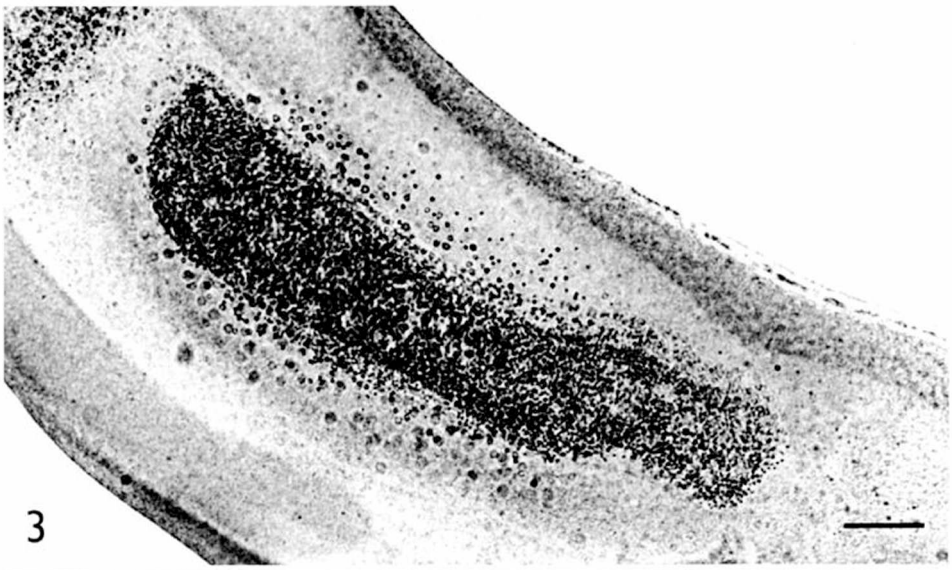
(see micrographs in Traut et al., 1986; Traut & Marec, 1996). Mostly, they were very long and wormiform, placed close and along walls of the corrugated tubules, with granular appearance of the chromatin. No deeply stained heterochromatin that would resemble to a sex-chromatin body was observed in either sex (Fig. 1).

Preparations of spread pachytene oocytes from two female larvae were screened. Most pachytene nuclei showed 14 autosomal bivalents consisting of two threads, each representing the two homologous chromosomes, with a typical chromomere pattern (Fig. 2). Normally, all bivalents could be seen lying on margins of the spread nucleus whose central space was filled in with a large grey spot representing a nucleolus. A short and thick positively heteropycnotic element that was always observed in the central space, lying on the nucleolus plate, was interpreted as a univalent of the sex chromosome Z. The characteristic co-location of the Z univalent and the nucleolus strongly suggests that the sex chromosome is a carrier of the nucleolus organizing region (NOR). Finally, based on chromosome counts in pachytene oocytes, it was observed that females of this species possess a diploid set of 29 chromosomes (14 autosomal pairs and one sex chromosome Z).

Polycentropus flavomaculatus

Preparations of Malpighian tubules were made from four female and two male larvae. In this species, the tubules were brown due to a large amount of small brown pigment

Figs 3–7. *Polycentropus flavomaculatus* (Pictet) female larvae. 3 – a highly polyploid oblong nucleus of the Malpighian tubule cell without any sex-specific heterochromatin, surrounded by small pigment granules; 4 – a diploid oogonial metaphase with 2n = 25 chromosomes; 5 – a pachytene complement of the oocyte nucleus with a ball of autosomal bivalents and a weakly stained thread of the Z-chromosome univalent (arrow); 6 – a pachytene oocyte nucleus with a ring-forming Z univalent (arrow) placed on the nucleolus (N); 7 – a pachytene oocyte nucleus showing the nucleolus plate (N) in the central space with the Z univalent (arrow) lying on it. Scale bars – 10 μm.



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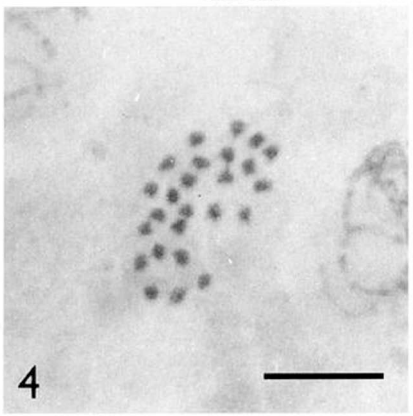

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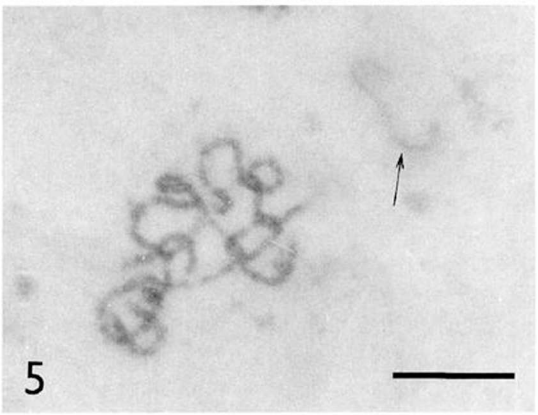

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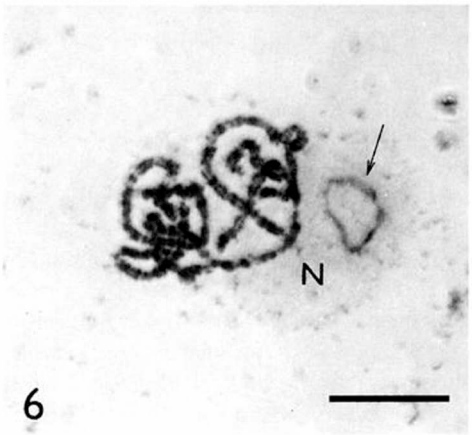

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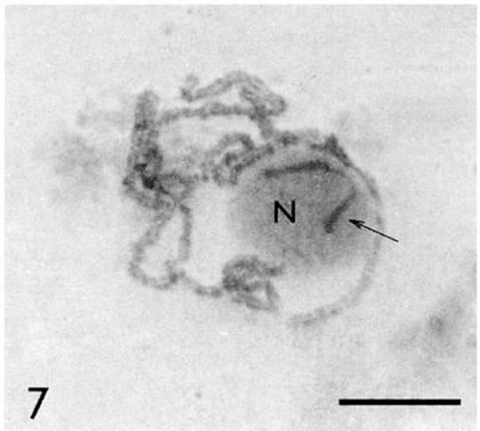

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granules and thus, visible through transparent cuticle. Highly polyploid nuclei were large and oblong, mostly showing a wurstlich shape or forming a thick rod, exceptionally rounded. In both sexes, chromatin of the nuclei displayed a coarse-grained texture, but without any sex-specific heterochromatin (Fig. 3).

For chromosome spreads, ovaries from two female larvae were used. In addition to early meiotic prophase I nuclei, the preparations also yielded many oogonial mitotic nuclei. Mitotic metaphase complements regularly showed 2n = 25 of rounded, dot-like chromosomes (Fig. 4). This implies that each chromosome set consisted of 24 autosomes (12 pairs) and one sex chromosome Z which was, however, indistinguishable from the autosomes. The presence of an odd chromosome was confirmed in pachytene nuclei where a thin thread of the presumed Z-chromosome univalent was observed in each spread nucleus, in addition to a ball of autosome bivalents. In most nuclei, the Z univalent could be easily found outside the nucleus as a thin, negatively heteropycnotic thread without distinct chromomere pattern (Fig. 5). In some nuclei, the Z univalent tended to form a ring with both telomere ends close each other (Fig. 6). Frequently, the Z univalent was seen lying on a large grey spot, the presumed nucleolus (Fig. 7). Since this was true also in cases, where it was separated from the rest of nuclei, it is concluded that the Z chromosome carries the NOR.

Rhyacophila sp.

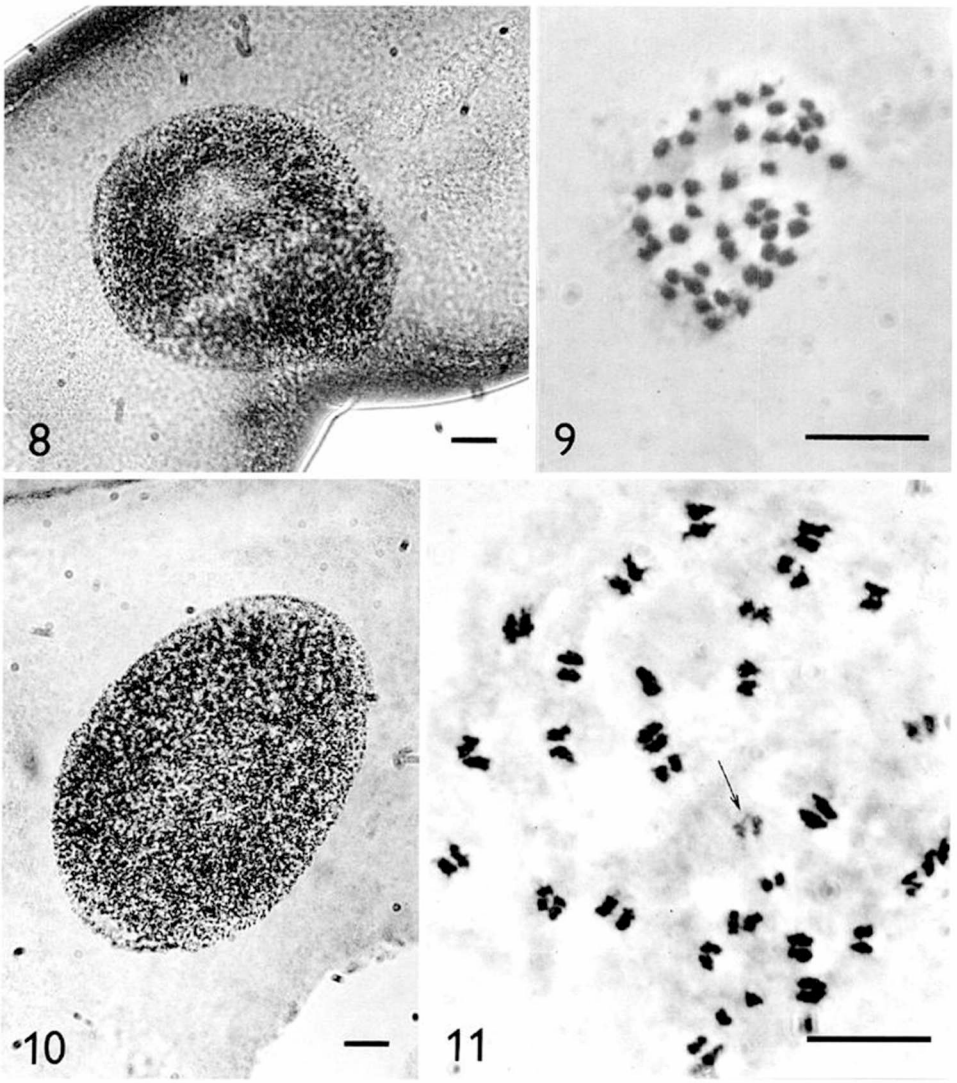
A total of five female and three male larvae were inspected for the sex chromatin status. Similar to the larvae of *P. flavomaculatus*, their Malpighian tubules were brown due to the content of small pigment granules. Highly polyploid nuclei were large, mostly spherical or ovoid, with fine-grained chromatin but without any sex chromatin body in either sex (Fig. 8).

Chromosome preparation was made from ovaries of one female larva. Oogonial mitotic metaphases consisted of 2n = 45 dot-like chromosomes (Fig. 9). Therefore, the diploid chromosome set was interpreted as 44 autosomes (22 pairs) and one Z chromosome that could not be distinguished due to the uniform appearance and equal size of the metaphase chromosomes. However, in this species we failed to obtain pachytene spreads of good quality. Pachytene bivalents were short, relatively thin, and some of them lacked a distinct chromomere pattern. This appeared to be caused by species-specific differences and probably did not result from preparation conditions. Thus, it was difficult to identify the presumed Z-chromosome univalent with certainty.

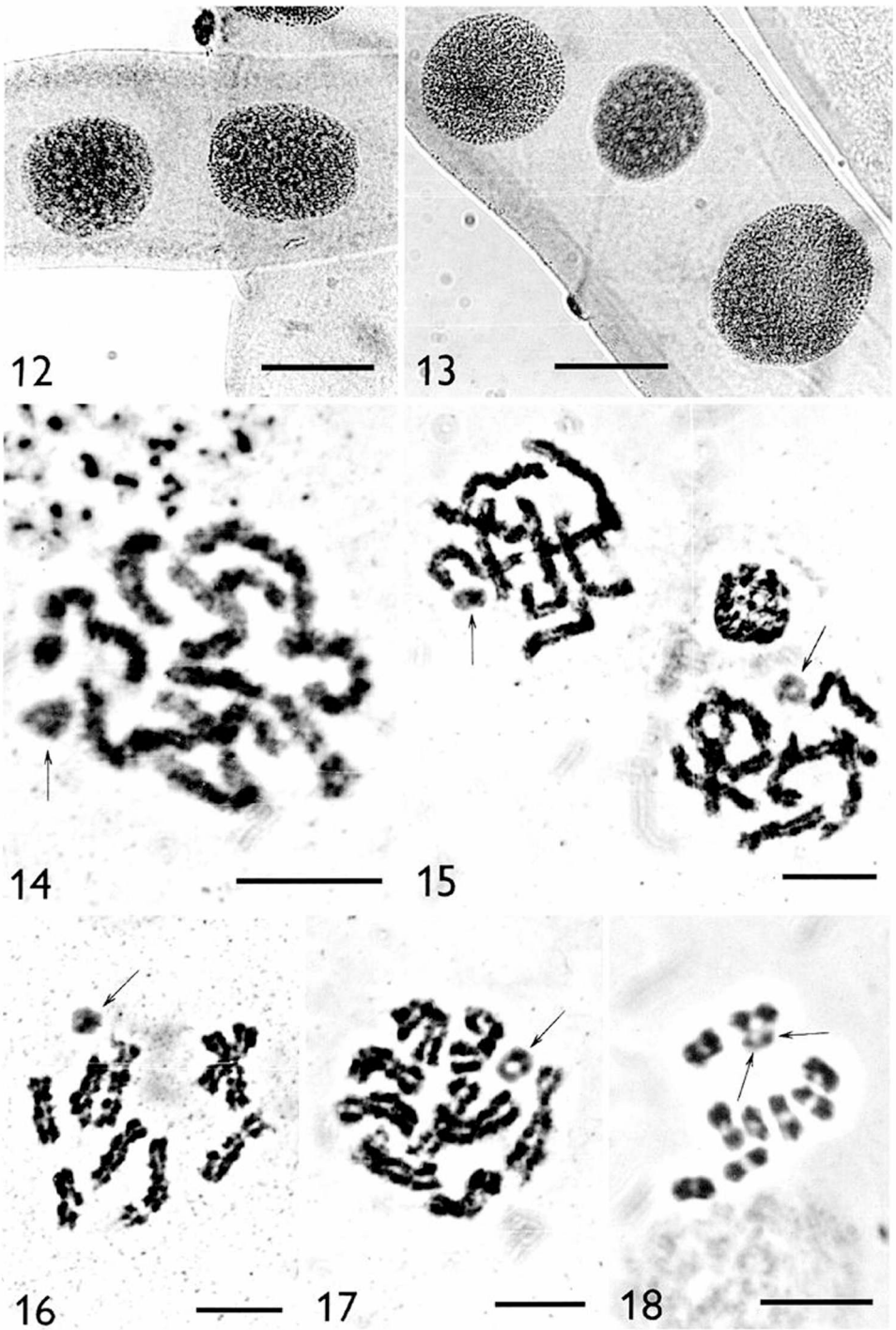
Anabolia furcata

In preparations of Malpighian tubules from six female and four male larvae, spherical or ovoid nuclei were exclusively observed. Their large size indicated, similar to the other species examined, a relatively high level of polyploidy. No sex chromatin body was found in either sex; the nuclei displayed uniform texture of dense, fine chromatin grains (Fig. 10).

Oocyte preparations from three female larvae yielded plenty of prometaphase I chromosome complements, representing, in all probability, diploid nuclei of nurse cells, in which postpachytene bivalents continuously shortened and degenerated. Complete and well spread chromosome sets consisted of 29 deeply stained autosome bivalents, each showing



Figs 8–11. 8–9: *Rhyacophila* sp. female larvae. 8 – a highly polyploid spherical nucleus of the Malpighian tubule cell without any sex-specific heterochromatin; 9 – a diploid oogonial metaphase with 2n = 45 chromosomes. Scale bars – 10 μm. 10–11: *Anabolia furcata* Brauer female larvae. 10 – a highly polyploid ovoid nucleus of the Malpighian tubule cell with uniform chromatin grains but without any sex-specific heterochromatin body; 11 – a prometaphase I chromosome complement of a nurse cell nucleus showing 29 deeply stained autosome bivalents and a weakly stained pseudobivalent of the presumed Z chromosome (arrow). Scale bars – 10 μm.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two parallelly aligned homologues separated by an unstained gap, and one weakly stained, negatively heteropycnotic pseudobivalent (Fig. 11). The pseudobivalent consisted of two small elements of about half size compared to most autosomes. It is suggested that this pseudobivalent represents two sister chromatids of the Z-chromosome univalent, and their separation is a consequence of chromosome degeneration in the nurse cells.

Limnephilus decipiens

Polyploid nuclei were inspected in Malpighian tubules from three female and three male adults. The nuclei were mostly spherical or oval, with uniform granular appearance of chromatin. Also in this species, no heterochromatin that would indicate the presence of a W chromosome was observed in either sex (Figs 12 and 13).

Chromosomes were examined in oocyte preparations from two freshly emerged adult females. For comparison, a preparation of testes from one male larva approaching pupation was examined. The female ovaries still contained oocytes in the stage of late pachytene but most spread nuclei already showed postpachytene (prometaphase I) bivalents. Late pachytene nuclei consisted of nine autosome bivalents with a disappearing chromomere pattern and a less stained, thinner and short thread of the Z-chromosome univalent. Both telomere ends of the Z univalent had a tendency to approach each other: in some nuclei, the Z univalent appeared like a horse shoe or U-shaped element (Fig. 14), in others formed a ring or even appeared selfpaired (Fig. 15). This tendency was also obvious in postpachytene nuclei (Fig. 16 and 17). In prometaphase I autosome bivalents, homologous chromosomes separated from each other and appeared consisting of large, deeply stained segments. In contrast, the considerably smaller Z-chromosome univalent was less and uniformly stained. In the preparation of male testes, most meiotic stages could be observed. In Fig. 18, metaphase I complement shows 10 bivalents, each with a typical gap between the two homologous chromosomes. Nine bivalents are similar in their size, whereas one is much smaller then the others. The small bivalent in spermatocytes was interpreted by Klingstedt (1931) as the sex chromosome bivalent (ZZ). This statement is in a good accord with our observation of the small Z-chromosome univalent in oocytes.

DISCUSSION

The world fauna of Trichoptera includes about 10,000 valid extant species (see review of Morse, 1997), on the basis of phylogeny of pupation, classified into three suborders: Annulipalpia (retreat makers), Spicipalpia (closed-cocoon makers), and Integripalpia (tube-case makers) (Wiggins & Wichard, 1989; Morse, 1997). Alternatively, some trichopterologists prefer the superfamily terms Hydropsychoidea, Rhyacophiloidea, and Limnephiloidea in the same sense as the subordinal names (see e.g. Neboiss, 1991). The present paper presents data on sex chromatin and sex chromosomes in five selected

Figs 12–18. *Limnephilus decipiens* (Kolenatý) adults. 12 (female) and 13 (male) – a part of the Malpighian tubules showing highly polyploid spherical nuclei without any sex-specific heterochromatin; 14 – a late pachytene oocyte nucleus with nine autosome bivalents and a U-shaped Z-chromosome univalent (arrow); 15 – two late pachytene oocyte nuclei, one with selfpaired and the other with a ring-forming Z univalent (arrows); 16 – a postpachytene oocyte nucleus showing a coiled up Z univalent (arrow); 17 – a postpachytene oocyte nucleus with a ring Z univalent; 18 – a spermatocyte nucleus displaying 10 metaphase I bivalents, the smallest bivalent consists of the two homologous chromosomes Z (arrows). Scale bars: 12–13 – 50 μm; 14–18 – 10 μm.

species. This is far from being sufficient, with respect to the overall number of species known, for making a general conclusion for the order Trichoptera. Nevertheless, the species examined here belong to four families, representing a cross-section through all three suborders, from the "primitive" family, Rhyacophilidae, to the most "advanced" family, Limnephilidae (see Kiauta, 1971; Wiggins & Wichard, 1989).

In Lepidoptera females, sex chromatin in somatic interphase nuclei has been proved useful as indirect evidence for the presence of W chromosome in the female genome (Traut & Marec, 1996, 1997). However, preparation of highly polyploid nuclei in either sex of the selected Trichoptera revealed no heterochromatin bodies that would resemble sex chromatin (this study). This finding strongly indicated the absence of a W chromosome, and favoured a Z/ZZ sex-chromosome system in the Trichoptera. The presence of one odd chromosome, presumably the Z chromosome, in the female karyotype of each species was than confirmed by chromosome analyses. Pachytene and postpachytene oocyte (or nurse cell) nuclei of *Hydropsyche* sp., *Polycentropus flavomaculatus*, and *Limnephilus decipiens* displayed a sex-chromosome univalent or, in the case of *Anabolia furcata*, nurse cell nuclei displayed a sex chromosome pseudobivalent. In *Rhyacophila* sp., the Z-chromosome univalent in pachytene oocytes was not identified. Nevertheless, diploid chromosome sets of mitotically-dividing oogonia consisted of the odd number of chromosomes (2n = 45, i.e., 22 autosome pairs and one Z chromosome); although the Z chromosomes could not be defined in the oogonial metaphases, it is concluded that this species also possesses the Z/ZZ chromosome mechanism of sex determination.

In the caddis-flies examined here, excepting *Rhyacophila* sp., the presumed Z chromosome was clearly distinct in nuclei of late prophase I oocytes. However, there were species-dependent differences in its structure, behaviour and staining affinity. Among pachytene autosomal bivalents, the Z chromosome was identified easily as the only unpaired element. In *Hydropsyche* sp., the Z-univalent appeared a short and thick, positively heteropycnotic element that was always found lying on the nucleolus plate in the central space of the nucleus. A similar heterochromatic Z-univalent was shown in oocyte preparations of *Tricholeiochiton* (= *Oxyethira*) *fagesii* (Guinadr) (Spicipalpia: Hydroptilidae) and three other caddis-flies from the Limnephilidae, *Chaetopteryx villosa* (Fabricius), *Allogamus auricollis* (Pictet), and *Glyphotaelius pellucidus* (Retzius) (Lankhorst, 1970; Kiauta & Kiauta, 1979; Kiauta & Lankhorst, 1969). In a closely related, "primitive" Lepidoptera *Micropterix calthella* (L.) (Micropterigidae), the Z-univalent presented itself in oocytes and young nurse cells as a straight or bent, positively heteropycnotic chromosome (Traut & Marec, 1997). Meiotic behaviour of the Z-univalent in *P. flavomaculatus* and *L. decipiens* females was different: in pachytene nuclei it formed a thin, negatively heteropycnotic or less stained thread that tended to form a circle. A weakly stained, negatively heteropycnotic Z chromosome was also found in postpachytene nuclei of nurse cells in *A. furcata* females. However, as a result of structural changes in nurse-cell chromosomes before subsequent polyploidization (Traut & Clarke, 1996) its two sister chromatids separated and formed a pseudobivalent. Finally, it should be noted that the Z chromosomes of *Hydropsyche* sp. and *P. flavomaculatus*, both representing Annulipalpia, appeared to carry the NOR. This might document their close relationship.

Pairing of the two homologous chromosomes via formation of the synaptonemal complex is a key event in meiosis I. It provides a mechanical framework for the precise course

of reciprocal crossing-over and indirectly ensures proper disjunction of homologous chromosomes (see Loidl, 1994). Pairing may also occur between two heterologous sex chromosomes in the heterogametic sex, depending on the degree of their homology and on the sex chromosome system (see Marec, 1996). However, in Trichoptera females, one can expect a peculiar meiotic behaviour of the Z-chromosome univalent due to the absence of a pairing partner. Our results suggest that there are two ways in which the Z univalent might overcome the pachytene stage. Either it forms a free euchromatic thread or a compact heterochromatic element. Both the ways appear species-specific, but there is insufficient data to state why the Z univalent follows one route in a certain species and the other elsewhere. Hypothetically, the different chromatin packaging may be related to transcriptional activity of the Z chromosome during meiotic prophase. A full transcriptional activity of pachytene chromosomes was demonstrated, for example, in young previtellogenic oocytes of a moth, *Ephestia kuehniella* (Traut, 1977b). Hence, a free thread of the Z-univalent might be preferred in the case of transcribed Z-linked genes. Conversely, compact packaging might reflect a transcriptionally inactive Z chromosome.

Among insects, the occurrence of a sex-chromosome univalent in the heterogametic sex is not limited to the Trichoptera and "primitive" Lepidoptera but is common in several insect orders (e.g. the X0 constitution in males of Odonata, Orthoptera, Psocoptera, many Homoptera and some Heteroptera; see discussion in Grozeva & Nokkala, 1996, and references therein). The X sex-chromosome of grasshoppers normally consists of facultative heterochromatin during spermatogenesis and thus, it appears positively heteropycnotic during meiotic prophase (e.g. Fossey, 1991). Antonio et al. (1993) studied the pycnotic cycle of the X-chromosome univalent during meiosis I in a grasshopper *Pyrgomorpha conica* Olivier (Orthoptera: Pyrgomorphidae). The X chromosome appeared positively heteropycnotic during prophase I and became negatively heteropycnotic during metaphase I as typical for grasshoppers. However, in contrast to other grasshoppers, the X chromosome showed alternating negatively and positively heteropycnotic zones during transition from diplotene to diakinesis. The authors attributed this reversion of pycnosis to differences in chromosome condensation, i.e., unrelated to euchromatinization that could finally disturb spermiogenesis by transcriptional activity. A pycnotic cycle of Z-chromosome univalents in females of some Trichoptera may, however, considerably differ from that of X-chromosome univalents in species with heterogametic male sex. In oogenesis, transcriptional activity of Z-linked genes might be required for the growth of oocytes (see Jablonka & Lamb, 1990).

So far, less than 40 Trichoptera species have been karyotyped (Lankhorst, 1970; Kiauta, 1971; Kiauta & Kiauta, 1979). Thus, it appears worthwhile to discuss briefly chromosome numbers observed, although the present study was not aimed at karyological data. The haploid chromosome number of n = 15 (14 A + Z), found in *Hydropsyche* sp., is identical to that reported for the only karyotyped member of the Hydropsychidae, *H. pellucidula* (Curtis). In *P. flavomaculatus*, which had never been cytogenetically examined, n = 13 (12 A + Z) was observed. The same haploid chromosome number was earlier found in another representative of the Polycentropidae, *Plectronemia conspersa* (Curtis). Also n = 23 (22 A + Z), observed in *Rhyacophila* sp., agrees well with two karyotyped species of the Rhyacophilidae, *R. nubila* (Zetterstedt) and *R. obtusidens* (Mac Lachlan) (reviewed by Lankhorst, 1970). In *A. furcata*, the haploid chromosome set was n = 30 (29 A + Z); this

high chromosome number, which is characteristic for the Limnephilidae (Kiauta, 1971), was also reported for a species of the same genus, *A. soror* (Mac Lachlan) (see Lankhorst, 1970). Finally, our study confirmed the karyological data of Klingstedt (1931) in *L. decipiens*. It is notable that the low chromosome number, n = 10 (9 A + Z) in this limnephilid species most probably represents a derived character, evolved by multiple chromosome fusions (Wolf et al., 1997). Recently, it has been shown that this mechanism was involved in karyotype evolution of species with low chromosome numbers in the moth genus *Orgyia* (Traut & Clarke, 1997).

In summary, the present results show that the lack of sex chromatin in somatic nuclei of Trichoptera females corresponds to the absence of a W chromosome in their genome. Consequently, the Z chromosome forms a univalent in prophase I oocytes. The Trichoptera share these characters with the "primitive" Lepidoptera. This implies that the common ancestor of Amphiesmenoptera had a Z/ZZ chromosome mechanism of sex determination and further supports the hypothesis of Traut & Marec (1996) that the WZ/ZZ sex chromosome system had evolved at the basis of the "advanced" Lepidoptera.

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