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Sex chromatin and sex chromosome systems in nonditrysiian Lepidoptera (Insecta)

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Abstract

Eleven representatives of the superorder Amphiesmenoptera (Trichoptera + Lepidoptera) were examined for sex chromatin status. Three species represent stenopsychoid, limnephiloid and leptoceroid branches of the Trichoptera; eight species belong to the primitive, so-called nonditrysiian Lepidoptera and represent the infra-orders Zeugloptera, Dacnonypha, Exoporia, Incurvariina, Nepticulina and Tischeriina. The female-specific sex chromatin body was found in the interphase somatic nuclei of *Tischeria ekebladella* (Bjerkander 1795) (Lepidoptera, Tischeriina). The sex chromatin was absent in all investigated Trichoptera species as well as in all representatives of the nonditrysiian Lepidoptera except *Tischeria ekebladella*. The sex chromosome mechanism of *Limnephilus lunatus* Curtis 1834 (Trichoptera, Limnephilidae) is Z/ZZ. The sex chromosome mechanism of *Tischeria ekebladella* (Lepidoptera, Tischeriina) is ZW/ZZ including the W chromosome as the largest element in the chromosome set. The data obtained support the hypothesis that the Z/ZZ sex chromosome system, the female heterogamety and the absence of the sex chromatin body in interphase nuclei are ancestral traits in the superorder Amphiesmenoptera. These ancestral characters are probably kept constant in all the Trichoptera and in the most primitive Lepidoptera. The W sex chromosome and the sex chromatin evolved later in the nonditrysiian grade of the Lepidoptera. It is proposed that the sex chromatin is a synapomorphy of Tischeriina and Ditrysiia.

Key words: Amphiesmenoptera – Dacnonypha – Exoporia – Incurvariina – karyotype – Lepidoptera – Nepticulina – phylogeny – sex chromatin – sex chromosome – Tischeriidae – Tischeriina – Trichoptera – Zeugloptera

Introduction

The superorder Amphiesmenoptera includes two closely related orders: Trichoptera and Lepidoptera. The monophyly of the Amphiesmenoptera has been well documented (Kristensen 1984). The relationships of the major divisions within the Amphiesmenoptera, although much debated, still present some uncertainties (Kristensen 1997). The order Lepidoptera is usually divided into several suborders and infraorders (Fig. 1). Most of the Lepidoptera species belong to a monophyletic clade, Ditrysiia (Nielsen 1989) and any others belong to so-called nonditrysiian groups. The latter are more primitive in many morphological aspects and branched off earlier from the common evolutionary lineage of the Lepidoptera.

The most interesting cytogenetical peculiarity of the Amphiesmenoptera is the female heterogamety. Male heterogamety based on the sex chromosome mechanism ♀XX/♂XY (or on variants ♀XX/♂XY, ♂XX/♂XY₁Y₂ and some others) predominates in animals. Female heterogamety based on the sex chromosome mechanism ♀ZW/♂ZZ (or on variants ♀Z/♂ZZ, ♀ZW₁W₂/♂ZZ and some others) occurs mostly sporadically in different classes of animals. The conclusion about female heterogamety in Amphiesmenoptera is based on the study of several species of Trichoptera from the suborders Annulipalpia, Spicipalpia and Integripalpia (for a review see Marec and Novák 1998) and many species of Lepidoptera from the infra-order Ditrysiia. Among Ditrysiia female heterogamety has been demonstrated cytogenetically and in some cases also genetically in butterflies (Papilionoidea), in many bombycoid, noctuid and geometroid moths and in several so-called Microlepidoptera (Seiler 1914; Tazima 1964; Traut and Mosbacher 1968; Suomalainen 1969a, 1971; Robinson 1971; Bigger 1975; Ennis 1976; Clarke et al. 1977; Gupta and Narang 1981; Maeki 1981; Rishi and Rishi 1985; Kawazoe 1987a, b, c, d; Nilsson et al. 1988; Traut and Marec 1997). The available data on sex chromosome mechanisms in nonditrysiian Lepidoptera are very scanty. Female heterogamety and the sex mechanism Z/ZZ were found in *Micropterix calthella* (Zeugloptera, Micropterigidae) (Traut and Marec 1997). According to Kawazoe (1987a) *Endo-*

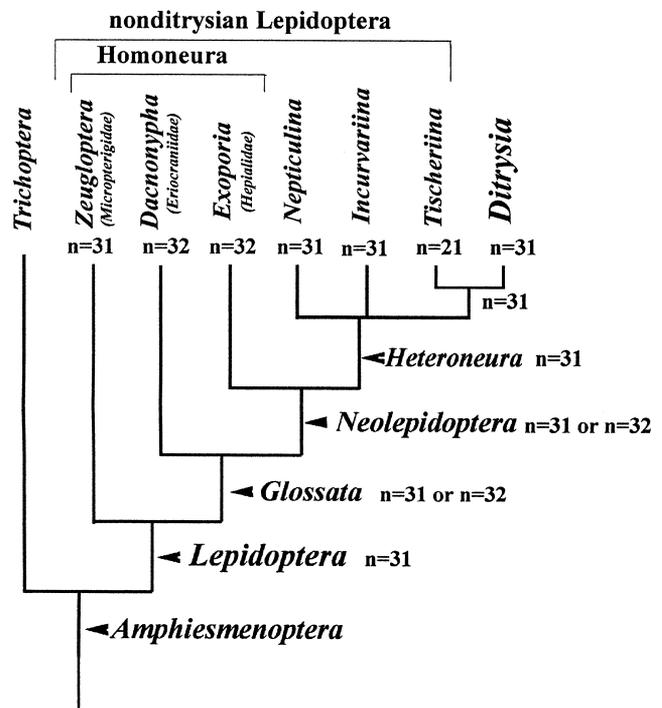


Fig. 1. Modal recent (on ends of branches) and probable ancestral (on bases of clades) haploid chromosome numbers in main clades of Lepidoptera. The phylogeny is based on the works of Kristensen (1984, 1997), Minet (1984) and Nielsen (1989). The Lepidopteran suborders and infra-orders which are not examined for chromosome number are not included in the cladogram. The ancestral chromosome numbers are deduced by Lukhtanov and Puplesiene (1996).

clita sinensis (Exoporia, Hepialidae) has a ZZ/ZW sex chromosome mechanism. There are no reports of male heterogamety in the superorder Amphiesmenoptera with the exception of two ghost moths, *Hepialus zhayuensis* Chu et Wang, 1985 and

Hepialus sp. (Hepialidae) from China (Fayao et al. 1988). In my opinion the latter requires verification.

All the Trichoptera studied possess the sex chromosome mechanism $\text{♀Z}/\text{♂ZZ}$ (for a review see Marec and Novák 1998). Different sex chromosome systems were found in Lepidoptera. In particular the mechanisms Z/ZZ , ZW/ZZ , $\text{ZW}_1\text{W}_2/\text{ZZ}$ (Suomalainen 1969a) and $\text{Z}_1\text{Z}_2\text{W}/\text{Z}_1\text{Z}_1\text{Z}_2\text{Z}_2$ (Traut 1986; Nilsson et al. 1988) are described.

A peculiarity of the ditrysian Lepidoptera with the mechanisms ZW/ZZ , $\text{ZW}_1\text{W}_2/\text{ZZ}$ and $\text{Z}_1\text{Z}_2\text{W}/\text{Z}_1\text{Z}_1\text{Z}_2\text{Z}_2$ is a presence of one or more female-specific heterochromatin bodies in somatic interphase nuclei (Traut and Marec 1996, 1997). This body is called sex chromatin. It is derived in interphase nuclei from the sex chromosome W (Ito 1977). Accordingly, the sex chromatin body is lacking in females with Z/ZZ sex chromosome mechanism and there are two bodies in females with ZW_1W_2 sex chromosome mechanism (Suomalainen 1969a). Apart from the advanced Lepidoptera female-specific interphase sex chromatin can be observed regularly in mammals. This similarity of Mammalia and ditrysian Lepidoptera is convergent because their sex chromatin arose independently in evolution and has a different cytogenetic basis (Traut and Marec 1996).

The sex chromatin was not found in several species of Trichoptera that were studied (Marec and Novák 1998) and nonditrysian Lepidoptera (Traut and Marec 1996). In Trichoptera and *Micropterix calthella* (Zeugloptera) the absence of the sex chromatin correlates with the lack of the sex chromosome W in females (Traut and Marec 1997; Marec and Novák 1998). On the basis of these findings, a hypothesis about the pattern and trends of the sex chromosome evolution in Amphipneoptera was recently formulated (Traut and Marec 1996, 1997; Marec and Novák 1998). According to this hypothesis:

- (1) the common ancestor of Trichoptera and Lepidoptera had a Z/ZZ sex chromosome mechanism;
- (2) the ancestor of Lepidoptera had the Z/ZZ sex chromosome mechanism like Trichoptera, the sister group of Lepidoptera;
- (3) this ancestral state (Z/ZZ) is kept constant in Trichoptera and nonditrysian Lepidoptera. It correlates with the lack of the sex chromatin in female somatic interphase nuclei;
- (4) the ZW/ZZ sex chromosome mechanism evolved later in the ditrysian branch of Lepidoptera. The appearance of the sex chromosome W leads to the formation of the sex chromatin in female somatic nuclei;
- (5) the ZW/ZZ mechanism evolved by fusion of the original Z with an autosome, resulting formally in a neo-Z/neo-W sex chromosome system;
- (6) secondary losses of the W chromosome can occur sporadically in Ditrysia. Loss of the W chromosome is accompanied by loss of the sex chromatin.

In order to test this hypothesis I have undertaken:

- (1) a study of additional species of Trichoptera for sex chromatin status and sex chromosome mechanism;
- (2) an examination of representatives of all Palaearctic nonditrysian moths groups for sex chromatin status;
- (3) a study of the sex chromosome mechanism in *Tischeria ekebladella*, a representative of the nonditrysian heteroneuran Lepidoptera. The nonditrysian Heteroneura occupy an intermediate position in the system of Lepidoptera between archaic homoneuran nonditrysian infraorders and more advanced Ditrysia (Fig. 1).

Materials and methods

Material

Last instar larvae of *Stenopsyche marmorata* (Trichoptera, Stenopsychidae) were collected by the author in the river Khamir near the city Zyryanovsk (Kazakhstan). Last instar larvae of *Molanna angustata* (Trichoptera, Molannidae) and of *Limnephilus lunatus* (Trichoptera, Limnephilidae) were collected by the author in the river Okhta (St. Petersburg, Russia). Adult females and males of *Micropterix shikotanica* (Lepidoptera, Micropterigidae) were collected by Dr M. Kozlov in Sakhalin (Russia). Adult females and males of *Eriocrania purpurella*, *Eriocrania semipurpurella* (Lepidoptera, Eriocraniidae) and *Hepialus humuli* (Lepidoptera, Hepialidae) were collected by A. V. Kupriyanov in the vicinity of St. Petersburg (Russia). Adult females and males of *Adela degeerella* (Lepidoptera, Adelidae) and *Hepialus sylvinus* (Lepidoptera, Hepialidae) were collected by Prof. Dr A. A. Stekolnikov in Borisovka (Belgorod district, Russia). Adult females and males of *Acalyptis* sp. (Lepidoptera, Nepticulidae) were collected by Dr J. Puplesiene in Iolotan (Turkmenistan). Last instar larvae of *Tischeria ekebladella* (Lepidoptera, Tischeriidae) were collected by the author in Borisovka (Belgorod district, Russia).

Chromosome and sex chromatin preparations

Chromosome preparations were made from ovaries and testes of last instar female and male larvae. The larvae were dissected under the microscope with $16\times$ and $32\times$ magnification in a drop of 1% sodium citrate solution. The ovaries and testes were removed using fine needles for micro-preparation with considerable caution in order to avoid damaging the gonad membranes. The dissected gonads were fixed in a freshly made mixture of glacial acetic acid (1 part) and ethanol (3 parts). Samples were stored in this mixture for 1–4 months at $+4^\circ\text{C}$. Then the gonads were stained in 2% acetic orcein for 7–14 days. The stained ovaries and testes were placed in a drop of 45% acetic acid on a slide, macerated and squashed. To determine the karyotypes and the sex chromosome systems, spermatogonial and oogonial mitotic metaphases as well as metaphases I, metaphases II and prometaphases of meiosis were studied, using a $100\times$ immersion objective. To determine the sex chromatin status, Malpighian tubules and other somatic tissues were dissected from both sexes. These tissues were stained with 2% acetic orcein and the polyploid interphase nuclei were inspected under a light microscope, using $40\times$ and $100\times$ immersion objectives.

Results

Karyotypes and sex chromosome mechanisms

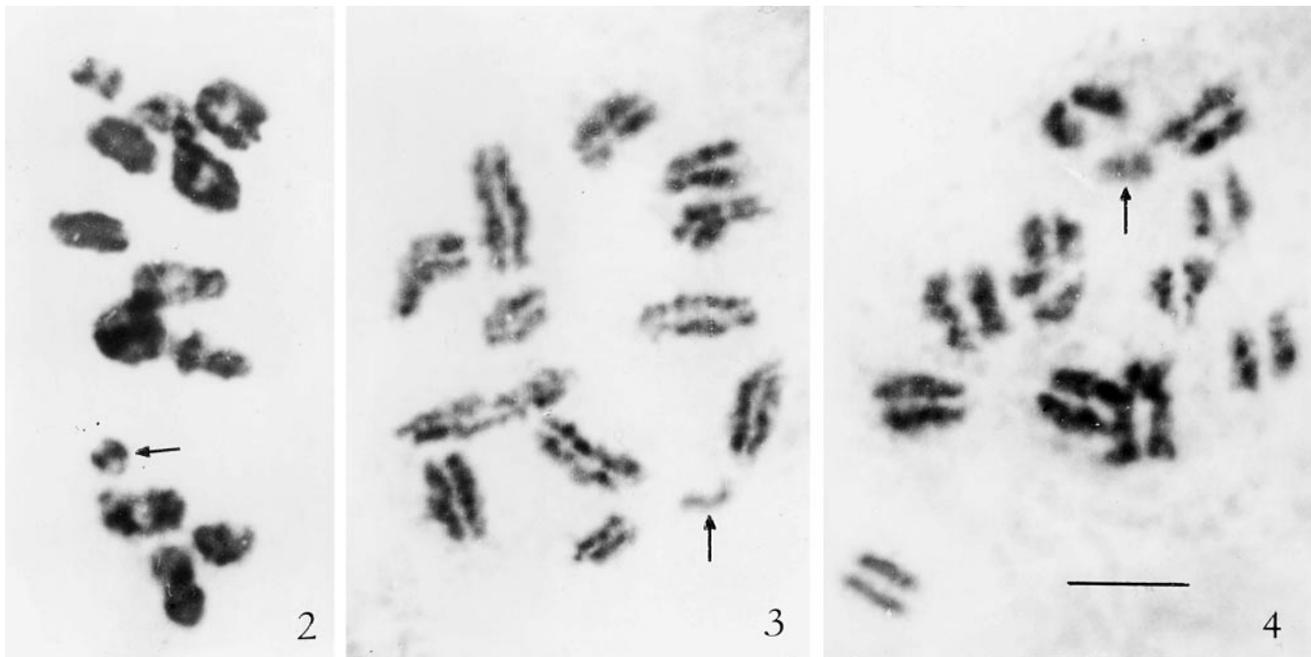
Limnephilus lunatus (Trichoptera, Limnephilidae)

Chromosomes were examined in preparations from four male and three female last instar larvae. In males the metaphase I complement showed 13 bivalents of different sizes and configurations (Fig. 2). The smallest bivalent was treated as the sex chromosome bivalent (ZZ). This statement is in accordance with the observation of the small Z chromosome univalent in females (Figs 3, 4). In males the bivalents were oriented parallel to the spindle fibres and were clearly chiasmatic. Thirteen chromosomes were found at metaphase II in all the cells studied. The same haploid chromosome number was detected earlier in males of *L. lunatus* by Pchakadze (1930).

During female meiosis, postpachytene oocytes (Fig. 3) and nurse cells in prophase I (Fig. 4) showed 12 bivalents of different sizes and a small weakly stained univalent. It was obvious that the univalent was the Z chromosome. Oogenesis in *L. lunatus* was achiasmatic. The bivalents consisted of two homologous chromosomes, oriented in parallel and well separated from one another. Hence, females of *L. lunatus* have $2n = 25$ and a ZO sex chromosome system. Males of *L. lunatus* have $2n = 26$ and a ZZ sex chromosome system.

Tischeria ekebladella (Lepidoptera, Tischeriidae)

Chromosomes were examined in preparations from three male and seven female last instar larvae. The male metaphase I comp-



Figs 2, 3, 4. Trichoptera: *Limnephilus lunatus*. 2: male metaphase I displaying 13 bivalents, the smallest bivalent consists of the two Z chromosomes (arrow); 3: postpachytene oocyte nucleus showing 12 achiasmatic autosome bivalents and a small weakly stained Z chromosome univalent (arrow); 4: prometaphase I complement of a nurse cell nucleus with 12 achiasmatic autosome bivalents and a small weakly stained Z chromosome univalent (arrow). Scale bar = 5 μ m

lement consisted of 23 bivalents of different sizes and configurations (Fig. 5). The same haploid chromosome number was detected in males of *T. ekebladella* earlier (Lukhtanov and Puplesiene 1990, 1996). Spermatogonial mitotic metaphases consisted of $2n = 46$ dot-like chromosomes. There are no especially large or especially small chromosomes in the diploid set (Fig. 6). The oogonial mitotic metaphase consisted of $2n = 46$ chromosomes including an especially long chromosome (Fig. 7) that was lacking in males. This longest chromosome was treated as the W sex chromosome. A Z chromosome could not be distinguished. Since the chromosome number was even in females, it may be assumed that females possess a ZW sex chromosome mechanism. The W chromosome was the largest element in the diploid set.

Hence, females of *T. ekebladella* have $2n = 46$ and a ZW sex chromosome system. Males of *T. ekebladella* have $2n = 46$ and a ZZ sex chromosome system.

Sex chromatin

The sex chromatin was found as a spherical, deeply stained body in females of *Tischeria ekebladella* (Fig. 9). It could be easily observed in all female interphase nuclei from different tissues (Fig. 9), whereas it could never be found in the males (Fig. 8). The sex chromatin of *T. ekebladella* is not a homogeneous mass, but consists of a relatively weakly stained kernel and a deeply stained cortex. Neither female nor male somatic cells displayed heterochromatin bodies in other studied species of Trichoptera and nonditrysian Lepidoptera. The compiled published and new data on the occurrence of heterochromatin sex bodies in somatic interphase nuclei of Trichoptera and nonditrysian Lepidoptera are presented in Table 1.

Discussion

Sex chromatin and sex chromosome mechanism in Trichoptera

No heteropycnotic body has been found in interphase nuclei either in males or in females in three examined species of Sten-

opsychidae, Limnephilidae and Molannidae. Thus, the sex chromatin is lacking in these species. Earlier, sex chromatin has been found to be absent from representatives of Hydropsychidae, Polycentropodidae, Rhyacophilidae and Limnephilidae (Marec and Novák 1998). Although only a few species have been studied for the sex chromatin status (Table 1), the data available are nevertheless quite representative. The species studied represent all the suborders, infra-orders and superfamilies of the Trichoptera in the system of Frania and Wiggins (1997: p. 55) except for the superfamily Sericostomatoidea.

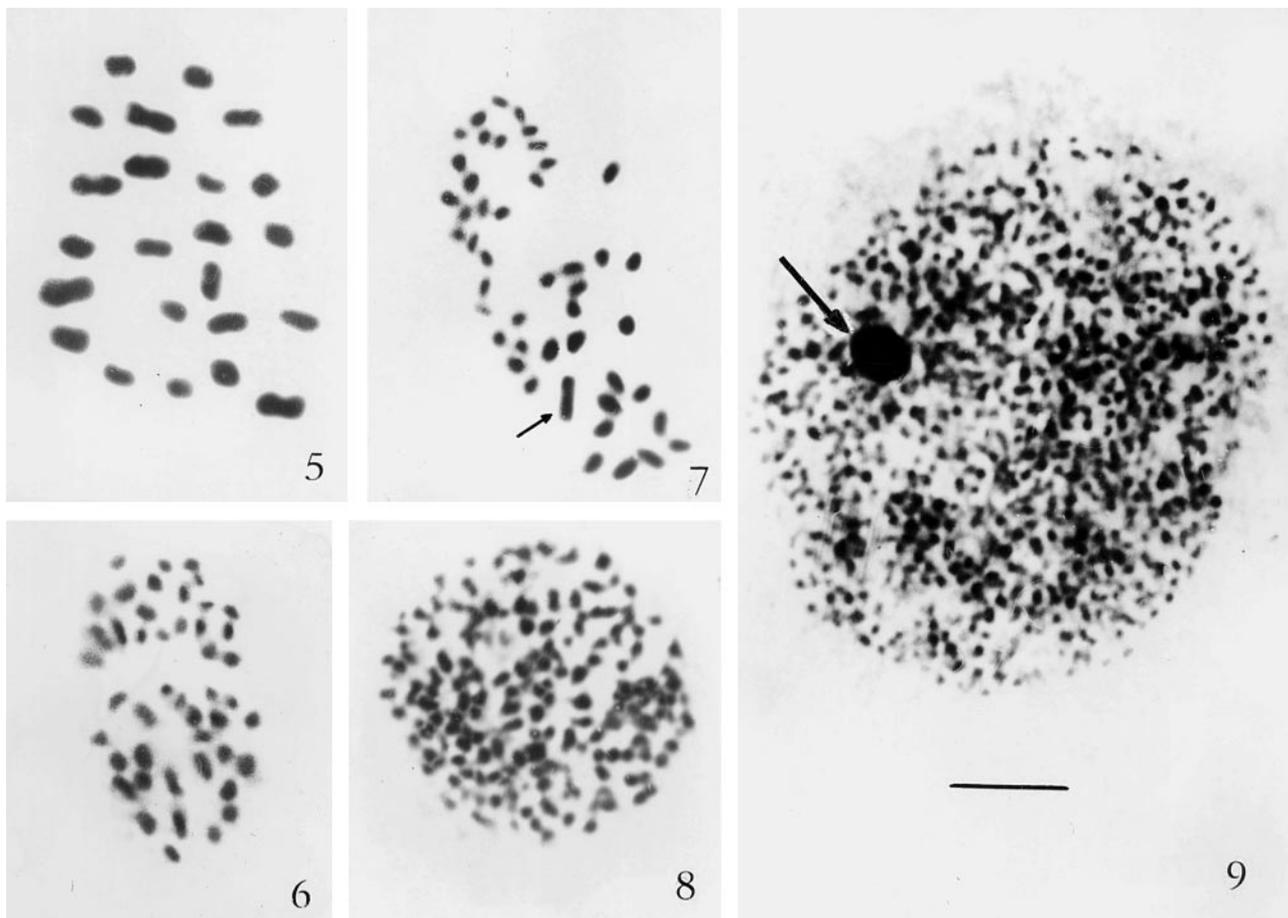
Limnephilus lunatus has a Z/ZZ sex chromosome system. Earlier, the same sex-determining mechanism was demonstrated in nine other Trichoptera species of the suborders Annulipalpia, Spicipalpia and Integripalpia (Klingstedt 1931; Suomalainen 1966; Kiauta and Lankhorst 1969; Kiauta and Kiauta 1979; Marec and Novák 1998). No other chromosome mechanism of sex determination is known for Trichoptera.

The sex chromosomes of *L. lunatus* ($n = 13$) (this article) and *L. decipiens* ($n = 10$) (Marec and Novák 1998) are similar. The Z chromosomes are the smallest ones in karyotypes of both species. In males the ZZ bivalent is much smaller than the others. The Z univalent in female meiosis of other Trichopteran families differs from the above in its structure, staining affinity and behaviour (Marec and Novák 1998). These variations argue that a differentiation of the sex chromosome system Z/ZZ occurred in the karyotype evolution of Trichoptera.

As a whole, the data obtained support the opinion of Traut and Marec (see introduction) that both the Z/ZZ chromosome mechanism and the lack of sex chromatin are characteristic of the order Trichoptera.

Sex chromatin and sex chromosome system in primitive nonditrysian homoneuran Lepidoptera

The Homoneura are a paraphyletic assemblage of nonditrysian Lepidoptera including the groups which are most 'primitive'



Figures 5, 6, 7, 8, 9. Lepidoptera (Tischeriina): *Tischeria ekebladella*. 5: male metaphase I displaying 23 bivalents; 6: diploid spermatogonial metaphase with $2n = 46$; 7: diploid oogonial metaphase with 46 chromosomes including a large W chromosome (arrow); 8: male larva: polyplod somatic interphase nucleus without any sex-specific heterochromatin; 9: female larva: highly polyploid somatic interphase nucleus with a sex chromatin body (arrow). Scale bar = $5 \mu\text{m}$

in respect of their morphology: the families Micropterigidae, Agathiphagidae, Heterobathmiidae, Eriocraniidae, Acanthopteroctetidae, Lophocoronidae, Neopseustidae, and the high-rank taxon Exoporia (Mnesarchaeoidea + Hepialoidea) (Nielsen and Kristensen 1996; Kristensen 1997). The data obtained confirm the lack of sex chromatin in Micropterigidae (Zeugloptera) and Hepialidae (Exoporia). These data also indicate the lack of sex chromatin in Eriocraniidae (Dacnonypha) (Table 1). The absence of sex chromatin is likely to be a common trait for the all homoneuran Lepidoptera.

The sex chromosome mechanism is Z/ZZ in *Micropterix calthella* (Micropterigidae, Zeugloptera) (Traut and Marec 1997) and ZW/ZZ in *Endoclita sinensis* (Hepialidae, Exoporia) (Kawazoe 1987a). Traut and Marec (1996, 1997) propose the Z/ZZ chromosome mechanism to be an original character for Lepidoptera that was inherited from the common ancestor of Lepidoptera and Trichoptera. This assumption needs further confirmation from an inquiry into the presence of sex chromatin and the kinds of chromosome mechanisms in additional primitive Lepidoptera, in particular in Agathiphagidae (Aglossata). The latter suborder as well as the Zeugloptera are both potential candidates for the role as the sister group of the remaining Lepidoptera (Kristensen 1984; Nielsen 1989; Ivanov 1994; Kristensen and Skalski 1998).

Sex chromatin and sex chromosome system in heteroneuran Lepidoptera

The monophyletic taxon Heteroneura includes the comparatively primitive nonditryisian superfamilies Nepticuloidea, Incurvarioidae, Palaephatoidea and Tischerioidea as well as numerous superfamilies of the more advanced enormous infra-order Ditryisia (Nielsen 1989; Kristensen and Skalski 1998).

A number of species within Ditryisia have been examined for sex chromatin status (for a review see Traut and Marec 1996) and sex chromosome mechanism (see Introduction). Sex chromatin has been found in the great majority of them. According to the well-argued assumption of Traut and Marec (1996), the ZW/ZZ sex chromosome mechanism and the female-specific sex chromatin are original characters in Ditryisia. Lack of sex chromatin is associated with secondary loss of the W chromosome and is a derived character in ditryisian Lepidoptera. The finding of sex chromatin in Tischeriina demonstrates that this character is not limited to Ditryisia. In the female of *Tischeria ekebladella* the possession of sex chromatin correlates with the presence of the W chromosome and the ZW/ZZ sex chromosome system. So, the Tischeriina are similar to Ditryisia in these characters. Sex chromatin can be regarded as a synapomorphy of Tischeriina and Ditryisia because in species of Nepticulina and Incurvariina studied up to now it was

Table 1. Presence (+) and absence (–) of heterochromatin bodies in somatic interphase nuclei of Trichoptera and nonditrysian Lepidoptera

Orders, suborders, families and species	Heterochromatin		Reference
	females	males	
Trichoptera			
Annulipalpia			
Hydropsychidae			
<i>Hydropsyche</i> sp.	–	–	Traut and Marec 1996; Marec and Novák 1998
Polycentropodidae			
<i>Polycentropus flavomaculatus</i> (Pictet 1834)	–	–	Traut and Marec 1996; Marec and Novák 1998
Stenopsychidae			
<i>Stenopsyche marmorata</i> Navas 1920	–	–	this article
Spicipalpia			
Rhyacophilidae			
<i>Rhyacophila</i> sp.	–	–	Marec and Novák 1998
Integripalpia			
Limnephilidae			
<i>Limnephilus decipiens</i> (Kolenaty 1848)	–	–	Traut and Marec 1996; Marec and Novák 1998
<i>Limnephilus lunatus</i> Curtis 1834	–	–	this article
<i>Anabolia furcata</i> Brauer 1857	–	–	Traut and Marec 1996;
Molannidae			
<i>Molanna angustata</i> Curtis 1834	–	–	this article
Lepidoptera			
Zeugloptera			
Micropterigidae			
<i>Micropterix aureatella</i> (Scopoli 1763)	–	–	Traut and Marec 1996
<i>Micropterix calthella</i> (Linnaeus 1761)	–	–	Traut and Marec 1996
<i>Micropterix aruncella</i> (Scopoli 1763)	–	–	Traut and Marec 1996
<i>Micropterix shikotanica</i> Kozlov 1988	–	–	this article
Dacnonypha			
Eriocraniidae			
<i>Eriocrania purpurella</i> Haworth 1828	–	–	this article
<i>Eriocrania semipurpurella</i> (Stephens 1835)	–	–	this article
Exoporia			
Hepialidae			
<i>Hepialus sylvinus</i> (Linnaeus 1761)	–	–	Traut and Marec 1996
<i>Hepialus sylvinus</i> (Linnaeus 1761)	–	–	this article
<i>Hepialus humuli</i> (Linnaeus 1758)	–	–	this article
Incurvariina			
Adelidae			
<i>Adela degeerella</i> (Linnaeus 1758)	–	–	this article
Incurvariidae			
<i>Paraclemensia acerifoliella</i> (Fitch 1856)	–	–	Ennis 1976
Nepticulina			
Nepticulidae			
<i>Acalyptis</i> sp.	–	–	this article
Tischeriina			
Tischeriidae			
<i>Tischeria ekebladella</i> (Bjerklander 1795)	+	–	this article

not found (Table 1); the state of this character remains unknown for Palaephatoidea. The revealing of this synapomorphy supports the idea of Nielsen (1983) and Minet (1984) that Tischeriidae is the sister group of Ditrysia. The data obtained fit well into the modern picture of Heteroneura phylogeny, viz., Nepticuloidea + (Incurvarioidea + (?Palaephatoidea + Tischerioidea + Ditrysia)) (Kristensen 1997; Kristensen and Skalski 1998). They contradict the older phylogenetic hypothesis (Common 1975; Kuznetsov and Stekolnikov 1978; Davis 1986; Kozlov 1987) on the monophyly of a group comprising the Tischeriidae + Nepticulidae.

In *Tischeria ekebladella* the W chromosome is the largest and

most darkly stained one. The lepidopteran W chromosome is usually equal to or lesser than the Z chromosome (Suomalainen 1969a; Kawazoe 1987a, b, c, d). The W chromosome as the largest element in the chromosome set was found in *Graphium sarpedon* (Linnaeus 1758) (Papilionidae) (Maeki 1981), *Ancylolomia japonica* (Zeller 1877) (Pyralidae) (Kawazoe 1987c) and *Galleria mellonella* (Linnaeus 1758) (Pyralidae) (Traut et al. 1999). The C-banding technique has showed that the large W chromosome in *Graphium sarpedon* consists of constitutive heterochromatin, whereas no heterochromatin or only a small amount has been revealed in other chromosomes (Maeki 1981: Fig. 4). The increase of the W chromosome size in *Tischeria*

ekebladella and *Graphium sarpedon* seems to be associated with an increase of the amount of heterochromatin.

Origin and evolution of the W chromosome in Lepidoptera

Although supporting the hypothesis of Traut and Marec (op. cit.) for the most part, the results obtained require some of its propositions to be revised. The data available allow a discussion of three alternative evolutionary scenarios describing probable trends and mechanisms of the sex chromosome evolution in Lepidoptera.

Scenario 1. The ZW/ZZ sex chromosome system evolved in the common ancestor of the branch Tischeriina + Ditrysia by fusion of the original Z with an autosome, resulting in a neo-Z/neo-W sex chromosome system

The scenario is very similar to the one of Traut and Marec (1996, 1997). The origin of the neo-W chromosome by this means necessarily results in a diminution of the autosome number by one pair as compared with that in the ancestral karyotype. However, the modal and probable ancestral chromosome number is the same ($n = 31$) in nonditryisian heteroneuran and primitive ditryisian Lepidoptera (Lukhtanov and Puplesiene 1996) (Fig. 1). Therefore, this scenario seems unlikely.

Scenario 2. The ZW/ZZ sex chromosome mechanism evolved in the common ancestor of the branch Nepticulina + Incurvariina + Tischeriina + Ditrysia by fusion of the original Z with an autosome, resulting in a neo-Z/neo-W sex chromosome system

The haploid chromosome number $n = 31$ and the sex chromosome system ZZ/Z were found in the primitive moths of the genus *Micropterix* (Zeugloptera) (Suomalainen 1969b; Traut and Marec 1997). If these characters are ancestral traits of the order Lepidoptera, it is conceivable that:

- (1) the karyotype $n = 32$ of *Dacnonypha* and *Exoporia* (Fig. 1) arose from the ancestral one by fission of one autosome pair;
- (2) the ZW/ZZ sex chromosome mechanism evolved later in the common ancestor of Heteroneura by fusion of the original Z with an autosome, resulting in a neo-Z/neo-W sex chromosome system. The origin of this system resulted in a diminution of the autosome number by one pair. Therefore the chromosome number $n = 31$, which is typical of *Nepticulina* and *Incurvariina* (Lukhtanov and Puplesiene 1996; Sruoga and Puplesiene 1998) appeared for the second time in Lepidoptera evolution.

Scenario 3. De novo formation of the W chromosome based on the B-chromosome

Recently, the possibility of *de novo* formation of sex chromosomes based on B-chromosomes has been argued (Kuznetsova et al. 1997). Accessory, or supernumerary B-chromosomes are known in different groups of animals including Lepidoptera (Robinson 1971). Examples of regular non-homologous pairing of a B-chromosome with a sex chromosome, resulting in a pseudobivalent formation in meiosis, have been described (Kuznetsova et al. 1997). In these cases the B-chromosome may be considered as a sex chromosome. The undifferentiated sex chromosome has initially no sex-determining genes and occurs optionally. This stage of the sex-chromosome evolution in Lepidoptera may be illustrated by *Solenobia triquetrella* (Hübner, [1813]) (Psychidae) in which the W chromosome is nonfunctional and two sex chromosome mechanisms, Z/ZZ and ZW/ZZ have been found in the form of

intraspecific variation (Seiler 1953). The possible next stage is the concentration of sex-determining genes in the W chromosome. This second stage may be illustrated by *Lymantria dispar* (Linnaeus 1758) (Lymantriidae) in which the Z chromosome carries male-determining genes, the W chromosome may carry female-determining genes, and the autosomes may carry both male and female genes (Winge 1937). The last stage of the sex-chromosome evolution in Lepidoptera may be illustrated by the silkworm, *Bombyx mori* (Linnaeus 1758) (Bombycidae) in which probably all or at least almost all sex-determining genes are located on the W chromosome (Tazima 1964).

The scenarios discussed above are testable hypothesis. To verify them, more extensive studies of sex chromosome mechanisms in nonditryisian Lepidoptera are necessary.

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Zusammenfassung

Geschlechtschromatin und Mechanismen der Geschlechtsbestimmung bei nichtditrysischen Lepidopteren (Insecta)

Elf Arten der Überordnung Amphiesmenoptera (Trichoptera + Lepidoptera) wurden auf die Existenz von geschlecht-spezifischem Heterochromatin in Kernen von Somazellen untersucht. Drei Arten repräsentieren die Überfamilien Philopotamoidea, Limnephiloidea und Leptoceroides der Ordnung Trichoptera. Acht andere Arten gehören zu den primitiven, sogenannten nichtditrysischen Lepidopteren und repräsentieren die Infraordnungen Zeugloptera, Dacnonypha, Exoporia, *Incurvariina*, *Nepticulina* und *Tischeriina*. Ein weibchen-spezifisches Heterochromatin wurde in somatischen Interphasenkernen von *Tischeria ekebladella* (Bjerkander 1795) (Lepidoptera, *Tischeriina*) gefunden. Ein geschlecht-spezifisches Heterochromatin fehlt in somatischen Interphasenkernen der untersuchten Trichopteren-Arten und in denen der Vertreter von Zeugloptera, Dacnonypha, Exoporia, *Incurvariina* und *Nepticulina*. *Limnephilus lunatus* Curtis 1834 (Trichoptera, Limnephilidae) hat den Z/ZZ-Typ der Geschlechtsbestimmung. Die Art *Tischeria ekebladella* (Lepidoptera, *Tischeriina*) zeigt den ZW/ZZ-Typ der Geschlechtsbestimmung. Das W-Chromosom ist das größte Element im Chromosomensatz von *Tischeria ekebladella*. Diese Befunden bestätigen die Hypothese, daß der Z/ZZ Typ der Geschlechtsbestimmung, die weibliche Heterogamete und das Fehlen des Geschlechtschromatin ursprünglich für die Überordnung Amphiesmenoptera sind. Diese ursprünglichen Merkmale bleiben wahrscheinlich bei allen Trichopteren und den primitivsten Lepidopteren unverändert. Das W-Chromosom und das Geschlechtschromatin entstanden auf einer späterer Evolutionsstufe der nichtditrysischen Lepidopteren. Es ist anzunehmen, daß das Geschlechtschromatin eine Synapomorphie von *Tischeriina* und *Ditrysia* ist.

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