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Polyploidy in bisexual Lepidoptera species (Insecta: Lepidoptera): old hypotheses and new data

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Abstract. A wide range of interspecific variation of the haploid chromosome number ranging from n=28 (*C. serratella*) to n = 57 (*C. lutipennella* and *C. spinella*) is found in the genus *Coleophora*. All high chromosome number species (n=57, n = 52 and n = 40) examined at the stage of maximum chromosome condensation have bivalents of a similar size as species with low chromosome numbers (n = 28 and n = 29). The increase of the chromosome number, maintaining the original size of bivalents, is related to the extension of the area of metaphase plates and correspondingly to the areas of sex cells. The comparative analysis of chromosome numbers in the genus *Coleophora* and in related groups of Lepidoptera indicates that karyotypes with low chromosome numbers (n = 28 and n = 29) are plesiomorphic, whereas karyotypes with substantially higher chromosome numbers may reliably be considered to be of secondary origin. These new findings are contrary to the well-known observation that "the greater the chromosome number, the smaller the size of the chromosomes and vice versa, the smaller the number the larger the chromosomes" (Lorković 1990). Also, new data from *Coleophora* allow to reopen the discussion on the role of polyploidy and fragmentation in the evolution of Lepidoptera karyotypes. A hypothesis for the allopolyploid origin of high chromosome numbers in the genus *Coleophora* is presented.

Key word. Allopolyploidy, bisexual species, chromosome number, *Coleophora*, karyotype, Lepidoptera, polyploidy.

Introduction

Polyploidy is a frequent phenomenon within parthenogenetic animal species. It is particularly common amongst parthenogenetic moths. Due to the classical works of Seiler (1923, 1936, 1961, 1963) and Astaurov (1940, 1977), in the order Lepidoptera polyploidy is known from tetraploid parthenogenetic species and from tetraploid and triploid parthenogenetic laboratory races. The cytogenetic origin and the reproduction of parthenogenetic polyploid forms has been deciphered.

Within bisexual animals polyploidy does not occur very often and has so far not reliably been established in the order Lepidoptera. According to a currently accepted view, the appearance of high chromosome numbers in karyotypes of bisexual Lepidoptera is the result of fragmentation, but not of polyploidy (Suomalainen 1969; Robinson 1971; White 1973). This was confirmed by the circumstance that so far in the Lepidoptera high chromosome numbers are correlated with small chromosome size, while the species of low chromosome numbers possess large-sized chromosomes (de Lesse 1960; White 1973; Werner 1975; Suomalainen & Brown 1984; Lukhtanov 1991). Moreover, this correlation has even been formulated as a specific rule: "The greater the chromosome number, the smaller the size of the chromosomes, and vice versa, the smaller the number the larger the chromosomes" (Lorković 1990).

During the examination of *Coleophora* (Lepidoptera: Coleophoridae) karyotypes, the junior author discovered a phenomenon contradicting Lorkovićs rule: at the stage of the first metaphase of the meiosis (i.e. at the stage of maximum chromosome condensation) species with high chromosome numbers (n = 57, n = 52 and n = 40) on average possess bivalents of the same size as species with low chromosome numbers (n = 28 and n = 29) (Puplesiene 1993). Additional studies presented below have confirmed this regularity. The increase in bivalent numbers keeping their original sizes is followed by a striking enlargement of the metaphase plate area and, consequently, spermatocyte space. These facts do not coincide with the concept of simple fragmentations, which at first sight are likely to occur in Lepidoptera, because Lepidoptera chromosomes lack localised centromeres and the chromosome fragments posses kinetic activity (Suomalainen 1969; Murakami & Imai 1974; Maeki 1980a, 1980b; 1981). These results give reason to reconsider the role of polyploidy in the origin of high chromosome number karyotypes in the Lepidoptera.

Material and methods

For this work Larvae were collected in Lithuania, in the region of St. Petersburg (Russia), in Tadzhikistan and in Turkmenistan. Only males were used for the karyological analysis. Larvae were dissected in a drop of 1 % sodium citrate or physiological solution at 28x and 56x magnifications. In order to avoid damaging the testes membrane the latter were removed carefully using fine needles for micro preparation. Parts of the larval material was reared to imago for precise identification.

The testes were fixed in a freshly made mixture of acetic acid (1 part) and ethanol (3 parts). Samples were stored in this mixture for 1—4 months at +4 °C. They were then stained in 2 % acid orcein for 1—7 days. The stained testes were placed in a drop of 45 % acetic acid on a slide, macerated and squashed. The karyotypes were studied at the stage of metaphase I (MI) or metaphase II (MII) using a 100x immersion objective.

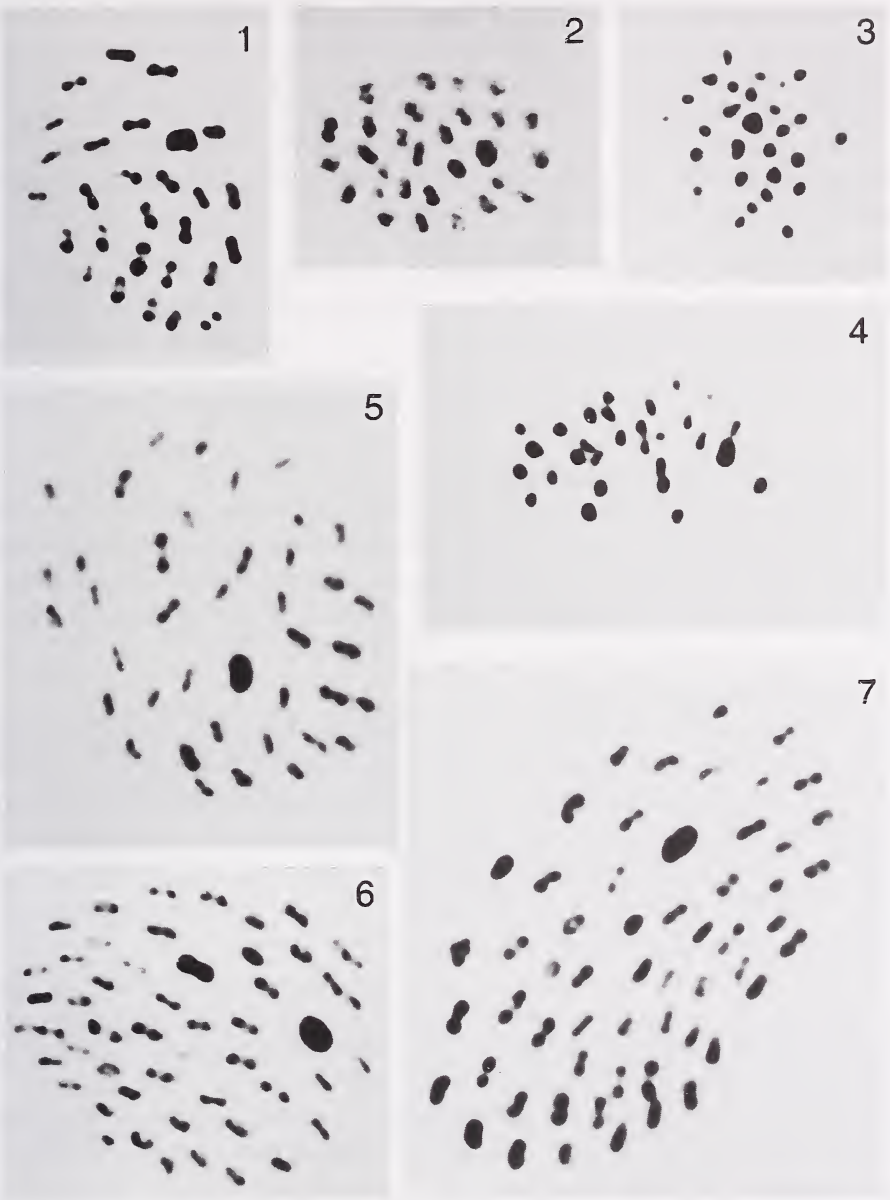
The size of the metaphase plates (MI and MII) has been measured on photographs. Only cells with native metaphase plate structures and undamaged cell membranes were used for measurements.

Results

In total, 9 *Coleophora* species have been examined.

1. *Coleophora serratella* (Linnaeus, 1761) (fig. 1)

Larvae were collected in Taurage, Lithuania, May 9th, 1990 from the host plant *Alnus incana*. Maturation divisions of sex cells were found at the end of the last larval instar prior to pupation. The haploid chromosome number n = 28 was counted in 13 metaphase plates (MI) from eight individuals. One large bivalent (approximately 1.6 times as large as the second in a descending size series) clearly stands out in the complement. This large bivalent is distinguished by a delayed termination of chiasmata. Therefore this bivalent ist still ring-shaped in the prometaphase, while the other ones are already typically dumbbell-shaped. The remaining bivalents have sizes similar to other *Coleophora* species and show a smooth graduation of size diminution.



Figs 1—7: The first (MI) or second (MII) meiotic metaphase of spermatogenesis in *Coleophora* species: 1) *C. serratella* (Linnaeus, 1761), MI, n = 28; 2) *C. binderella* (Kollar, 1832), MI, n = 29; 3) *C. vitisella* Gregson, 1856, MII, n = 29; 4) *C. glitzella* Hofmann, 1869, MII, n = 29; 5) *C. follicularis* (Vallot, 1802), MI, n = 40; 6) *C. tadzhikiella* Danilevskiy, 1955, MI, n = 52; 7) *C. spinella* (Shrank, 1802), MI, n = 57. Bar represents 10 um.

2. *Coleophora binderella* (Kollar, 1832) (fig. 2)
Larvae were collected in Taurage, Lithuania, May 9th, 1990 from *Alnus incana*. Maturation divisions of sex cells were found in the third and fourth larval instars. The haploid chromosome number n = 29 was established from 6 metaphase plates (MI) from three individuals. One large bivalent (approximately 1.5 times as large as the second in a descending size series) stands out in the complement. The other bivalents have similar sizes as in other *Coleophora* species and show a smooth graduation of size diminution.

3. *Coleophora vitisella* Gregson, 1856 (fig. 3)
Larvae were collected in the vicinity of St. Petersburg, Russia, in early June, 1992 from *Vaccinium vitus idae*. Maturation divisions of sex cells were found in larvae of middle and last instars. The haploid chromosome number n = 29 was counted in 9 metaphase plates (MII) from one individual. One chromosome was larger than the rest.

4. *Coleophora glitzella* Hofmann, 1869 (fig. 4)
Larvae were collected in the vicinity of St. Petersburg, Russia, in early June, 1992 from *Vaccinium vitus-idae*. The maturation divisions of sex cells were found in larvae of medial instars. The haploid chromosome number n = 29 was established in 6 metaphase plates (MII) from one individual. One distinctly large element clearly stands out in the complement.

5. *Coleophora sibiricella* Falkovitsh, 1972
Larvae were collected in the vicinity of St. Petersburg, Russia, in early June, 1992 from *Betula* sp. The maturation divisions of sex cells were found in larvae of medial instars. The haploid chromosome number n = 29 was found in 6 metaphase (MI) and prometaphase plates from five individuals. One large bivalent stands out in the complement. The remaining bivalents have similar sizes as other *Coleophora* species and show a smooth graduation of size diminution.

6. *Coleophora follicularis* (Vallot, 1802) (fig, 5)
Larvae were collected in the vicinity of Kara Kala, Turkmenistan, April 1st, 1990 from *Inula* sp. The maturation divisions of sex cells were found in the last instar larvae. The haploid chromosome number n = 40 was counted in 8 metaphase plates (MI) from two individuals. One distinctly large bivalent (approximately twice as large as the second in a descending size series) clearly stands out in the complement. The other bivalents have similar sizes as other *Coleophora* species and show a smooth graduation of size diminution.

7. *Coleophora tadzhikiella* Danilevskiy, 1955 (fig. 6)
Larvae were collected in the Varzob canyon, Tadzhikistan, June 23rd, 1991 from *Malus domestica* and *Malus sieversii*. The maturation divisions of sex cells were found at the end of the last larvae instar. The haploid chromosome number n = 52 was established in 7 metaphase plates (MI) from three individuals. Two large bivalents clearly stand out in the complement. One bivalent is approximately 1.4—1.5

times as large as the other one. The first bivalent is distinguished by delayed condensation of the homologous. In metaphase I this bivalent is oval, while the other ones are dumbbell-shaped. The second bivalent is approximately 1.5—1.8 times as large as the next in a descending size series. All remaining bivalents have similar sizes as other *Coleophora* species and show a smooth graduation of size diminution.

8. *Coleophora lutipennella* (Zeller, 1838)
Larvae were collected in the vicinity of Taurage, Lithuania, May 11th, 1990 from *Quercus robur*. The maturation divisions of sex cells were found in larvae of medial and late instars. The haploid chromosome number n = 57 was counted in 4 metaphase plates (MI) from three individuals. One large bivalent (approximately twice as large as the second in a descending size series) clearly stands out in the complement. The rest of the bivalents have similar sizes as other *Coleophora* species and show a smooth graduation of size diminution.

9. *Coleophora spinella* (Shrank, 1802) (fig. 7)
Larvae were collected in the vicinity of Taurage, Lithuania, May 26th, 1991 from *Malus domestica* and in Vilnius on May 22nd, 1992 from *Sorbus aucuparia*. The maturation divisions of sex cells were found at the end of the last larval instar. The haploid chromosome number n = 57 was found in 16 metaphase plates (MI) from five individuals. One large bivalent (approximately twice as large as the second in a descending size series) clearly stands out in the complement. The remaining bivalents have similar sizes as other *Coleophora* species and show a smooth graduation of size diminution.

In summary, n = 28 was determined in one, n = 29 in four, n = 40 in one, n = 52 in one and n = 57 in two species. The karyotypes of all species studied are characterised by the presence of one large bivalent, which is considerably larger (from 1.4 to 2 times, varying between species) than the next in a descending size series. At the stage of maximum chromosome condensation species with high chromosome numbers (n = 57, n = 52 and n = 40) possess bivalents of approximately equal size when compared to species with low chromosome numbers (n = 28 and n = 29). In high chromosome number species the area of metaphase plates (MI) significantly

Table 1: The chromosome numbers and the areas of metaphase plates of *Coleophora* species.

Species	Chromosome number	Number of plates with measured areas	Stage of measured metaphase plates	Mean area of measured metaphase plates (μm^2) standard error
C. serratella	n = 28	5	MI	130 + 2.4
C. binderella	n = 29	5	MI	90 + 2.9
C. sibiricella	n = 29	5	MI	111 + 6.3
C. vitisella	n = 29	5	MII	64 + 5.4
C. follicularis	n = 40	5	MI	322 + 1.4
C. tadzhikiella	n = 52	5	MI	259 + 6.3
C. lutipennella	n = 57	4	MI	294 + 26.7

exceeds this area in low chromosome number species (table 1, figs 1—2, 5—7). Correspondingly, spermatocytes of high chromosome number species are also of large size.

Discussion

Ancestral karyotype in the genus *Coleophora*

In order to determine the ancestral state of a karyotype, an analysis of the mechanisms and directions of its transformation is indispensable. Thus, if high chromosome number karyotypes are considered to be primitive when compared to low ones, then polyploidy as a cytological mechanism of karyotype evolution is out of question. In order to reconstruct the primitive state of any biological feature, the

Table 2: Chromosome numbers of moths in superfamilies of the gelechioid complex (Oecophoroidea, Coleophoroidea, Elachistoidea and Gelechioidea.

Superfamily, family, species	Haploid chromosome number	Reference
Superfam. Oecophoroidea		
Fam. Oecophoridae		
Depressaria groteella Rob.	29	Ennis 1976
D. nervosa Denn. et Schiff.	30	Regnart 1933
Psilocorsis quercicella Clem.	27	Ennis 1976
Tonica niviferana Walk.	30	Kaur 1988
Fam. Xyloryctidae		
Metathrinca tsugensis Kearfott	30	Kawazoe 1987a
Superfam. Coleophoroidea		
Fam. Coleophoridae — see table 1		
Superfam. Elachistoidea		
Fam. Elachistidae		
Elachista monosemiella Rössler	29	Lukhtanov & Puplesiene 1996 (as *Elachista cerusella* Hbn.)
Elachista adscitella Stt.	29	Puplesiene 1993 (as *Elachista revinctella* Z.)
Perittia weberella Whit.	30	Lukhtanov & Puplesiene 1996
Superfam. Gelechioidea		
Fam. Gelechiidae		
Anacampsis disquei Meess	29	Lukhtanov & Kuznetsova 1988
A. innocuella Zell.	29	Ennis 1976
Phthorimaea operculella Z.	29	Bedo 1984
Eucordylea resinosae Free	30	Ennis 1976
Eucordylea sp.	29	Ennis 1976
Exoteleia dodecella L.	12	Ennis 1976
E. nepheos Free	12	Ennis 1976
E. pinifoliella Cham.	11	Ennis 1976
Pulicalvaria piceaella Kft.	30	Ennis 1976
Sitotroga cerealella Olivier	30	Lukhtanov & Kuznetsova 1988
Tachyptilia populella Cl.	29	Beliajeff 1930

comparative morphological method, corroborated by data of palaeontology and by studies on the development of this feature, is widely used in embryogenesis. Since the latter two approaches cannot be applied in cytogenetic studies, the phylogenetic interpretation of karyotype transformations can only be based on comparative morphological studies. Despite this restriction and with reference to the experience of morphologists, we are convinced that this method of reconstructing ancestral karyotypes can be applied under the following conditions: 1) karyotypes of the largest possible number of species and forms, including the primitive representatives in the group under research, and 2) karyotypes of sister groups ought to be included in the analysis.

Consequently, we have also analysed some related taxa, including the large group of Microlepidoptera families usually called the "gelechioid complex". The taxonomy and phylogeny of the gelechioid complex has been worked out by Kuznetzov & Stekolnikov (1984) and essentially added to and changed by Sinev (1992) (fig. 8). Despite some differences in the interpretation of the size, shape and position of some superfamilies, the authors of both systems agree on the presence of two main evolutionary lineages, e.g. the gelechioid and the oecophoro-coleophoroid one. The data available (see table 2) clearly indicate that the evolution of the gelechioid karyotype was initiated from the number n = 30 (see also: Lukhtanov & Puplesiene 1996), which is the most common one within all families of the gelechioid (families Gelechiidae and Elachistidae) and oecophoro-coleophoroid (families Oecophoridae and Xyloryctidae) lineages studied so far, with the exception of the family Coleophoridae (fig. 8).

n = 29 occurs in different gelechioid families and, as a rule, is accompanied by the presence of one large chromosome (Lukhtanov & Puplesiene 1996). In the Gelechiidae and Elachistidae n = 29 is found to be as common as n = 30.

In the Coleophoridae, n = 30 has not been observed at all. *Coleophora* karyotypes are characterised by the high diversity of chromosome numbers ranging from n = 28 to n = 57. Nevertheless, a modal chromosome number of n = 29 is evident, with one very large bivalent in the complement. This observation and the fact that such karyotypes are most usual in the gelechioid lineage and also occur in the sister superfamily Oecophoroidea, suggest that in *Coleophora* it reflects the initial evoultionary situation. In this genus, the high-n karyotypes are evidently of secondary origin.

Are high-n karyotypes in the Coleophoridae the result of polyploidy or fragmentation?

The origin of high-n *Coleophora* karyotypes from the initial n = 29 may be due to either chromosome fragmentation or polyploidy. A hypothesis of the origin of high chromosome numbers in Lepidoptera via polyploidy was promoted by Lorković (1941, 1949), who found a number of butterfly karyotypes, where a sequence of multiples of a basic number could be deduced in closely related species. However, due to the disagreement of karyotype peculiarities in high-n species with the structure expected in polyploids, this concept was rejected by subsequent researchers (Suomalainen 1969; White 1973), including Lorković himself (Lorković 1990). If a high-n karyotype appears as a result of polyploidy the chromosomes in such a

complement should be of approximately equal size to those in species with the initial number. In fact, in all cases known so far in the Lepidoptera, the increase of the chromosome number if compared to the initial karyotype is followed by a reduction of chromosome size, whereas the decrease of chromosome numbers correlates to the enlargement of chromosome size (de Lesse 1960; White 1973; Werner 1975; Suomalainen & Brown 1984; Lukhtanov 1991). The chromosome volume of related species with low and high numbers is approximately identical. It is evident that this correlation conforms far better to the concept that chromosome numbers vary due to fragmentation and fusion rather than to polyploidy.

Two further arguments against the polyploidy concept can be found in the literature, although in our opinion they do not apply to this concept.

1) As a strong argument against polyploidy experiments on cytophotometric measurements of DNA content in the meiotic cells of moths of the genus *Cidaria* (Suomalainen 1965) have frequently been cited. According to the experimental evidence, the DNA content in species with low chromosome numbers (n = 13, n = 17) does not differ significantly from species with ordinary chromosome numbers (n = 30 and 32). However, species with n = 30 and 32 are not polyploid, because these or very close numbers are typical for *Cidaria* spp. [=species] (Geometridae) and also for the Lepidoptera as a whole. They represent the initial state of karyotype evolution in the groups mentioned above. In this case n = 13 and 17 are reliably of secondary origin, i.e. reduced numbers. Therefore, these experiments do not really refer to the problem of polyploidy.

2) The haploid complement of high-n species is nearly always characterised by the presence of one single large chromosome, but not of two large identical ones, as could be expected of the ploidic (White 1973). Beyond doubt the occurrence of two identical, very large chromosomes in the complement might be expected only as the result of autopolyploidy, but not of allopolyploidy. And just the allopolyploidy is the most probable origin of polyploidy in bisexual species (see Results).

Despite the remarks mentioned already, it may be considered in general that in all cases found hitherto the appearance of high chromosome numbers may be more easily explained by fragmentation processes.

An absolutely different picture is found in the genus *Coleophora*, where the origin of high-n value karyotypes corresponds better to the hypothesis of polyploidy than to the concept of fragmentation. At the stage of maximum DNA condensation, all high-n species (n = 57, n = 52 and n = 40) possess bivalents of on average equal size if compared to low-n species of the same genus (n = 28 and n = 29). The increase of the bivalent number, though keeping the original size of the genome, is followed by the enlargement of the metaphase plates area, consequently the area of spermatocytes. As follows from the experiments on DNA quantity in *Cidaria* species (Suomalainen 1965) and from observations on sex cell sizes in diploid and polyploid races of *Bombyx mori* (Astaurov 1977), both the chromosome and gametocyt sizes correspond to their DNA content in the Lepidoptera. Therefore, these facts suggest not only the duplication of the ancestral chromosome number (n = 29) in *C. spinella* and *C. lutipenella* (n = 57), but also the approximate duplication of the amount of DNA during this process. It is easy enough to imagine how, resulting from the polyploidy of the supposed initial stage for the genus, n = 29, the number is almost

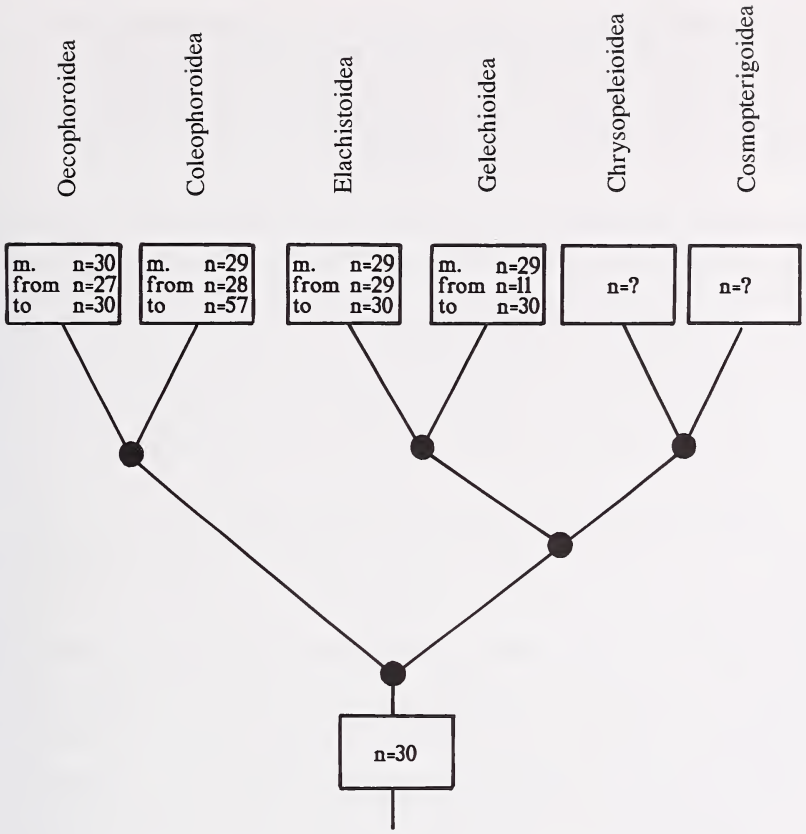


Fig. 8: Scheme of phylogenetic relations (after Sinev 1992) and chromosome number distribution in gelechioid moths. n — haploid number of chromosomes; m. n. — modal number of chromosomes; from n... to n... — range of interspecific variability of chromosome number.

exactly doubled (2n−1=57). Therefore, a decrease of bivalent sizes does not take place. The decrease in bivalent numbers if compared to the theoretical 2n = 58 is easy to explain by the allopolyploid origin of high-n species (see Discussion: Hypothetical scheme).

The polyploidy hypothesis is also in agreement with the similarity of the karyotype structure in two high-n species: *C. spinella* and *C. lutipenella* (n = 57). In the complements of both species on large bivalent distinctively stands out at the stage of the first metaphase of spermatogenesis, approximately twice as large as the second one, the other bivalents forming a smoothly descending size series. These two species are not closely related. In the system of Căpuşe (1973) they have even been referred to two different genera. Therefore, both karyotypes have probably evolved independently from low chromosome numbers, typical for *Coleophora*. The probability of independent evolution of two similar karyotypes resulting from coincidence of a great number of similar fragmentations is very low. This process is much better explained by assuming polyploidy.

The karyotype of *C. tadzhikiella* (n = 52) possibly arose by the autosome fusion in a hypothetical tetraploid ancestor. This process might lead to the appearance of the second large bivalent, not homologous to the first one. In a similar way, the high chromosome number karyotype of *C. folicullaris* (n = 40) could arise as a result of polyploidy and further chromosome fusion.

Barriers against the evolution of polyploidy in bisexual species of Lepidoptera

1) General complications and constraints in the evolution of autopolyploidy

The disruption of the chromosome sex determination mechanism in the polyploid structure is often assumed to be one of the main factors restricting the occurrence of polyploidy in animals (Muller 1925; Robinson 1971). However, as revealed by Astaurov (1972), this is not essential, if the Y- or W-chromosome determines the characters of the heterogametic sex even under the chromosome constitutions XXXY (normal male) and ZZZW (normal female). Just this mechanism of sex determination is known in the Lepidoptera and thoroughly examined in the silkworm *Bombyx mori*, though it is not unique within this order (Tazima 1964).

Bisexuality itself creates serious barriers for the occurrence of tetraploid species. In diploid species, the spontaneous appearance of tetraploid individuals is an extremely rare phenomenon. The coincidence of its appearance in one population and the copulation of two tetraploid individuals of different sex necessary for the origin of a tetraploid line is highly unlikely. Theoretically, the occurrence of tetraploidy may be achieved by other means; e. g. as the result of the fusion of two diploid gametes. However, the formation of diploid gametes is also rare. Thus, during many years of investigations on the spermatogenesis in Lepidoptera, in the course of which millions of cells in dozens of species have been observed thoroughly, we only once succeeded in observing the presence of three diploid spermatids together with normal ones in a single bundle (fig. 9, Lukhtanov, unpublished). The probability that this individual will fertilise a female also containing diploid egg nuclei, and that the fusion of just these diploid gametes will occur, is even immeasurably lower.

In diploid females reproducing via ameiotic parthenogenesis or in tetraploid females reproducing through meiotic parthenogenesis, the formation of diploid gametes is a normal phenomenon. That is why the probability of the occurrence of autotetraploidy increases in the contact zone of parthenogenetic and bisexual lines of the same species.

However, the appearance of an autotetraploid individual is insufficient for the origin of a tetraploid line. It is at least necessary to repeat the combination of all processes described above once more in order to have a second tetraploid individual occurring in the population. Moreover, it must mate with the first one. All this seems to be an actually inconceivable case.

The main argument against the possible occurrence of bisexual autotetraploid races in Lepidoptera cannot be derived from the arguments described above, but from the discovery of autotetraploid male sterility contrary to the autotetraploid female fertility in *Bombyx mori* (Astaurov 1940, 1974). These differences between sexes are due to peculiarities of the gametogenesis characteristic of the Lepidoptera,

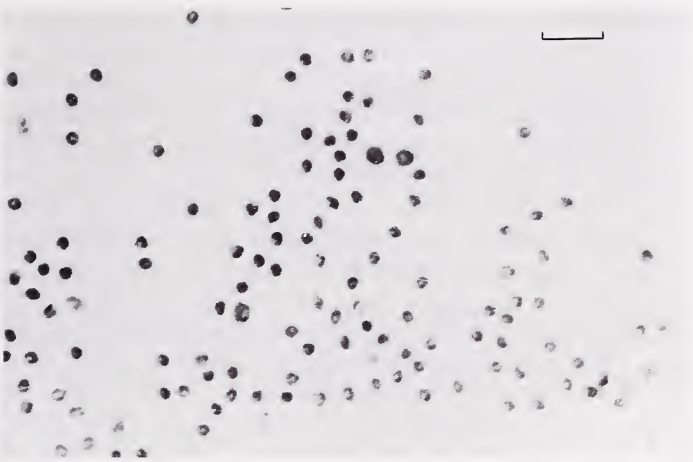


Fig. 9: Three diploid spermatids in a brundle together with normal haploid ones in male of *Zygaena fillipendulae* (Lepidoptera, Zygaenidae). Bar represents 10 um.

namely achiasmatic oogenesis and chiasmatic spermatogenesis. The absence of chiasmata in autotetraploid females leads to the formation of a normal number of common or doubled bivalents during meiosis and, as a consequence, to the regular disjunction of chromosomes. In autotetraploid males the polyvalent conjugation of homologues is complicated by the formation of chiasmata. It leads to the disturbance of chromsome disjunction in the course of the reduction division. This leads to the conclusion that tetraploid bisexual species could only become allopolyploid by the way of hybridisation of species whose homologous chromosomes lost the capacity to conjugate entirely or to a greater extent. Thus, polyploidy in bisexual Lepidoptera cannot be achieved without hybridisation.

2) Obstacles on the way to allopolyploidy in the Lepidoptera

Theoretically, an allotetraploid organism may occur due to the fusion of a diploid spermatozoid of one species with a diploid ovule of another species. However, all the obstacles and barriers existing on this way have already been described in the preceding paragraph. Moreover, as frequently described in the literature, interspecific hybridisation in Lepidoptera is of low frequency in nature.

A much more realistic way to obtain an allotetraploid line might be found in the hybridisation of triploid parthenogenetic females of one species with diploid males of another species. Triploid parthenogenetic races are not a rarity amongst polyploid animals (Astaurov 1977). Within triploid females the reduction division is absent in the meiosis, therefore these females can produce triploid gametes, the conjunction of which with the normal haploid spermatozoids may lead to the occurrence of tetraploid organisms. Though the evolution of allotetraploidy via this way demands the coincidence of a number of circumstances (the presence of a contact zone between triploid and a related diploid species and their interspecific hybridization), it is not unlikely and was discovered in a number of plants and animals (Astaurov

1974). Theoretically, in the Lepidoptera an additional difficulty occurs not found in other species with heterogametic females (Vasiliev 1985). In the case of female heterogamy (male ZZ; female ZW) the chromosome constitution of the triploid female and, correspondingly, of the triploid ovule will be either ZZW or ZWW. Due to the fusion of this ovule with a spermatozoid (always Z), only females will appear, with a chromosome constitution of ZZZW or ZZWW. (If the W-chromosome unambiguously determines the female sex, the appearance of males is impossible). But the experiments of Astaurov (1974) on *Bombyx mori* have shown that in triploids somatic polyploidy is not rare. It leads to the occurrence of mixoploids (3n + 6n) and as a consequence to hexaploid oocytes. Meiosis in 6n-oocytes includes both maturation divisions resulting in the possible appearance of egg nuclei (ZZZ) and correspondingly, after the fertilisation, tetraploid males (ZZZZ).

A hypothetical scheme for the evolution of the high-n karyotype in the genus *Coleophora*

The evolution of bisexual allotetraploid species in the Lepidoptera as well as in other animals (Astaurov 1972, 1977) must include the stages of parthenogenesis, interspecific hybridisation and polyploidy. The reconstruction of the course of events which could result in allotetraploid species within the genus *Coleophora* is very difficult, since parthenogenesis is not known within this group at present. High-n karyotypes are extraordinarily stable in this genus. In our studies no case of intraspecific chromosome polymorphism has been found. During meiosis the chromosome conjugation is always regular, without the formation of any uni- and polyvalents or other discruptions which could be expected in interspecific hybrids. In our opinion, this implies that, if the studied species are tetraploids, they must have gone through an extended evolutionary process. Thus, attempts to find initial tetraploid forms among recent species via hybridisation are most likely to be unsuccessful.

It is interesting to note that in the high-n *Coleophora* species the initial number of $n = 29$ is not exactly duplicated ($2n = 58$) but that one bivalent is found less ($2n-1 = 57$). The appearance of $n = 57$ might easily be explained by the hybridisation of species A ($n = 29$) with species B ($n = 28$). Hybrid karyotypes with $n = 57$, obtained as the result of this process, however, should have two large bivalents as low chromosome species of *Coleophora* already have one large bivalent. It is, of course, possible to imagine that one of the large bivalents could disappear due to structural rearrangements. However, we suggest another origin of the karyotype $n = 57$ as described below.

In the initial karyotype ($n = 29$) there is one large bivalent. A rather large number of examples in the Lepidoptera demonstrates that the large bivalent, if present, frequently is the sexual one (Suomalainen 1969; Kawazoe 1987a, 1987b, 1987c). In *C. lutipennella* and *C. spinella* only one very large bivalent is present. It might therefore be assumed that during the polyploidisation of the initial karyotypes all chromosomes except the sex ones were doubled. Such an assumption may be admitted for the Lepidoptera because in the silk-worm sex determination in the tetraploid form is possible by two mechanisms: ZW (female), ZZ (male) (one sex bivalent in a set of the tetraploid structure) and also ZZWW (female), ZZZZ (male)

(two sex bivalents in a set of the tetraploid structure) (Tazima 1964). The first mechanism is even more advantageous as it provides the normal (1 : 1) sex ratio. The loss of two sex chromosomes in the process of hybridisation and polyploidy is possible if the first initial species has the sex determination mechanism AAZ0female/AAZZmale. The second initial species should then have the sex determination mechanism BBZWfemale/BBZZmale. Sex determatination by the mechanisms ZWfemale/ZZmale or ZOfemale/ZZmale are commonly found in the order Lepidoptera (Traut et al. 1986). Within the gelechioid moths, of which the Coleophoridae form part, both variants may meet even within a single genus (Ennis 1976).

The aim of the scheme given below is not to reconstruct the evolutionary origin of polyploidy in *Coleophora*, but only to demonstrate its principal possibility. The scheme of the experimental production of an allotetraploid bisexual race in silkworms (Astaurov 1972) forms the basis of our assumption. The difficulties and limitations during the process of the evoultion of bisexual polyploids in Lepidoptera, as discussed above, and the peculiarities of high-n karyotypes of the supposedly polyploid species of *C. lutipennella* and *C. spinella* and the peculiarities of an ancestral karyotype of *Coleophora*, reconstructed under (1), have been taken into account.

As a first step the initial bisexual species (28AAZA0female/28AAZAZA male, n = 29) should turn to ameiotic parthenogenesis. As a result a diploid parthenogenetic race 28AAZA0 female would arise. Somatic polyploidy will also initiate the appearance of mixoploid females (28AAZA0 + 56AAAAZAZA00), thus developing two types of gametes: unreduced (28AAZA0) and reduced ones (28AAZA0, 28AAZAZA and 28AA00).

As a second step triploid individuals should arise due to the fertilization of parthenogenetic females of species AA by the males of another bisexual diploid species 28BBZBZB (gametes 28BZB). These will possess the following chromosome constitutions: 28AAZA028BZB female, 28AAZAZA28BZB male, 28AA0028BZB female. The females can reproduce through ameiotic parthenogenesis while the males are sterile.

In a third step hexaploid oocytes 56AA000056BZBZB would occur within the triploid females 28AA0028BZB due to somatic polyploidy. In such oocytes the meiosis includes both maturation divisions resulting in the occurrence of the following types of gametes: 28AA28B000, 28AA28B00ZB and 28AA28B0ZBZB. Due to the fusion of different types of allotriploid female gametes with the male gametes of the species 28BBZBZB (gametes 28BZB), allotetraploid females and males will arise: 28AA28BB000ZB, n = 57 (female); 28AA28BB00ZBZB, n = 57 (male) and 28AA28BB0ZBZBZB, n = 58 (probably intersex). Individuals of the first variant have the sex determination mechanism ZB0 and are females while individuals of the second variant have the sex determination mechanism ZBZB and must be males.

Conclusions

Despite the probable assumption of polyploidy in *Coleophora*, at the present stage of investigation an alternative hypothesis cannot be ruled out completely. It would

assume that high-n karyotypes of Coleophoridae species arose as the result of fragmentation with a subsequent enlargement of chromosome size. Processes of chromosome size enlargement without a change of chromosome numbers have been described in the Lepidoptera (Werner 1975; Lukhtanov & Kuznetsova 1988). They can either be explained by polytenization of the chromosomes or by their duplication (Werner 1975). The latter explanation seems to be the most probable. Duplications may be required after fragmentations as well as for the rebuilding of the telomeric segments in places of chromosome breaks, providing the integrity and individuality of the chromosome (White 1973).

We would finally like to note that both hypotheses (polyploidy and fragmentation) remain speculative in the application to the present case. However, it appears to be important that for the first time a group of bisexual species has been found in the Lepidoptera, where the probability of high chromosome number formation due to polyploidy appears to be rather high. In order to substantiate the polyploidy hypothesis it will be necessary to measure the amount of DNA contained in the nuclei of species with n = 57 and n = 52 as well as in species with n = 28 and n = 29. More interesting results may be obtained by studying the C- and G-banding of *Coleophora* low-n and high-n species, by the electrophoretic analysis of the share of duplicated loci in potentially tetraploid species, and also by the karyological study of additional species closely related to *C. lutipennella* and *C. spinella*.

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Zusammenfassung

In der Gattung *Coleophora* sind die Chromosomen bei den Arten mit höheren Chromosomenzahlen (von n = 52 bis n = 57) genau so groß wie bei denen mit niedrigen Zahlen (n = 28 und n = 29). Die Vermehrung der Chromosomen bei gleichzeitigem Erhalt ihrer Größe führt zu starker Vergrößerung der Spermatocyten. Diese Angaben stehen im Widerspruch zu der Regel von Lorković (1990) „Je höher die Chromosomenzahl, desto geringer die Chromosomengröße." Sie unterstützen die Hypothese, die Polyploidie als Mechanismus der Chromosomenzahl-Evolution anzusehen.

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