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## KARYOTYPE STRUCTURE IN HIGHER LEPIDOPTERA, (PAPILIONOMORPHA)\*

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Karyotype characteristics are widely used in taxonomy. In insects, they proved to be of great value in many cases, both in explaining the relationship of closely related species (Blackman, 1985), and in solving problems concerning phylogeny in higher taxa (Kuznetsova, 1985). Chromosome analysis was applied with good results in determining species independence of a number of forms classified as *Erebia tyn-darus* Esp. (Satyridae) and in the genus *Agrodiaetus* (Lycaenidae) (Lesse, 1960). At the same time, presently available information on chromosomes is of less significance toward understanding the macrosystem of the Lepidoptera, because most of the butterflies studied are characterized by similar karyotypes. Beliajeff (1930), after analysis of chromosomes number in 94 species from 22 families concluded that a greater part of the butterflies, irrespective of their taxonomic group, is characterized by similar values. He assumed  $n = 30$  or  $n = 31$  as the initial number of chromosomes in butterfly evolution. This was confirmed later after analyzing a large number of species, approximately a thousand, in the 60s (Robinson, 1971), amounting to 0.7% of the order. A chromosome number of 31, or close to it, is the modal number for most of the higher Lepidoptera investigated (Robinson, 1971). Analogous karyotypes were found in all members of the most primitive families: (Micropterygidae, Eriocraniidae, Hepialidae and Incurvariidae) analyzed so far, although those studied are very few (Suomalainen, 1969a).

Karyotype evolution may also occur without a change in chromosome number, as a result of structural rearrangements. This is why data important for taxonomic purposes may be obtained by a more detailed karyotype analysis, including chromosome structure analysis. Unfortunately, information on karyotype structure in butterflies is scarce. A number of authors recorded that chromosomes or groups of chromosomes different in size (Beliajeff, 1930; Lesse, 1960; Suomalainen, 1965; Maeki and Ae, 1968a, b; Werner, 1975) may be distinguished, only a few of them, however, used the structural karyotype characteristics to compare closely related species (Maeki and Ae, 1968a) or species from different families (Goodpasture, 1976). Only in individual cases, differences or similarities in karyotype structure between species were confirmed by metric chromosome analysis (Bigger, 1975, 1976; Goodpasture, 1976; Gupta and Narang, 1981).

It is generally believed that karyotype analysis in butterflies is unusually laborious (Robinson, 1971). This is mainly because of the small size and large number of chromosomes. Species having more than 380-440 chromosomes in a diploid set are known. Besides that, butterfly chromosomes lack localized centromers, making application of the centrometric coefficient, a generally accepted value in karyology, impossible and makes identification of chromosomes difficult.\*\*

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\*\*Information on chromosomes of this type (holokinetic) in insects can be found in a paper by Kuznetsova (1979).

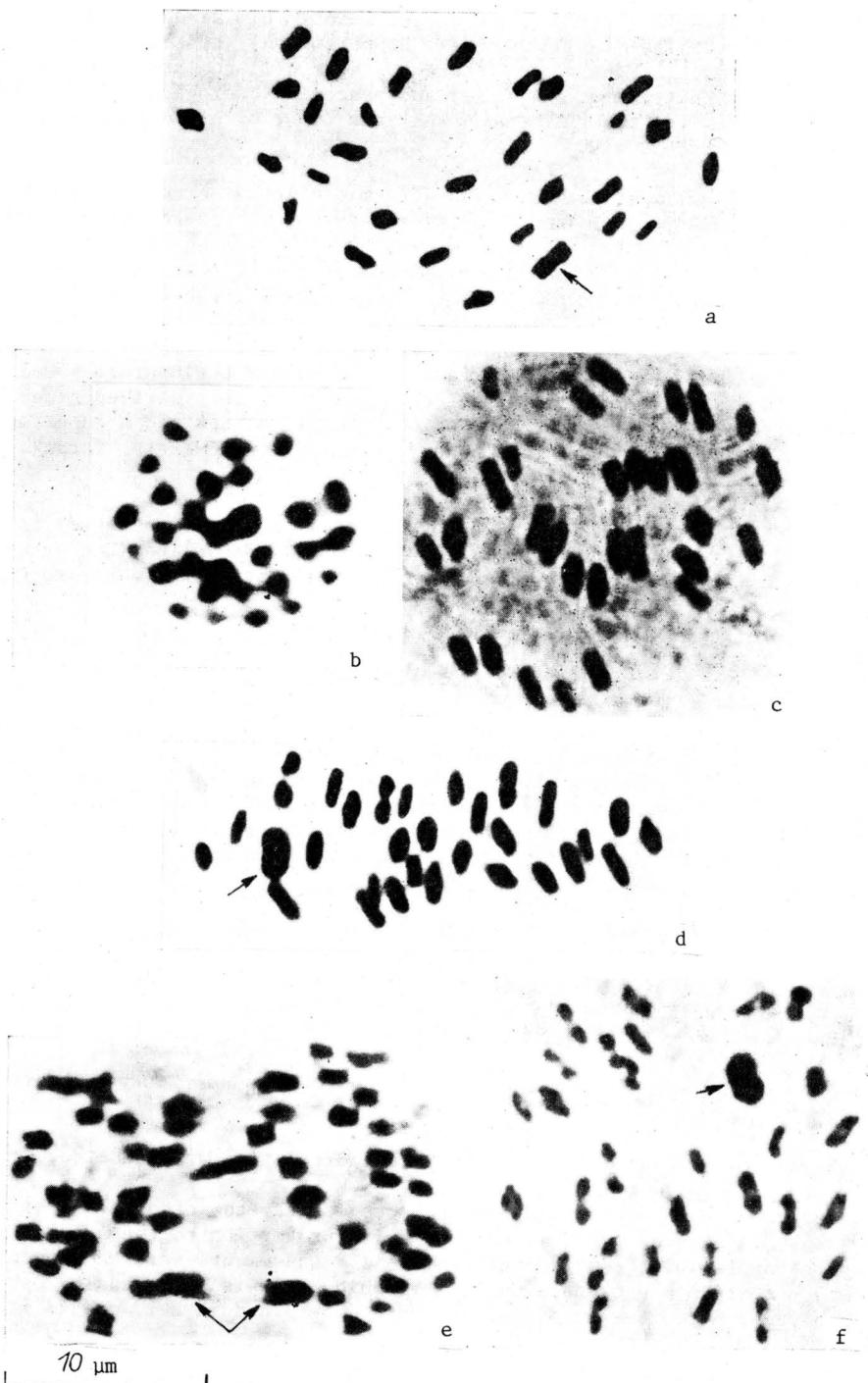


Fig. 1. Chromosomes in metaphase I of spermatogenesis in Gelechioidea, Yponomeutoidea and Tortricoidea. a) *S. ceraella* Ol.,  $n = 30$ ; b) *A. disqueut* M.,  $n = 29$ ; c) *Y. malinellus* Z.,  $n = 31$ ; d) *A. crataegana* H.,  $n = 30$ ; e) *A. crataegana*; f) *T. viridana* L.,  $n = 30$ . Arrows indicate the large bivalent. In Fig. 1f - delayed homologue separation in the large bivalent.

There is one more circumstance which makes karyotype analysis considerably more difficult in insects. In the adult stage, meiotic divisions regularly occur only in diurnal butterflies (Papillonoidea, Hesperioidae) (Lesse, 1960). In all other Lepidoptera, meiosis occurs in different stages of late larval, pronymphal or pupal development, depending on species and preceding ecological conditions. The stages most convenient for chromosome analysis (most of all metaphase I) are short, not longer than 3-5 days.

In this study, chromosome number was determined in 17 butterfly species, in 15 of which karyotype structure was investigated (absolute and relative chromosome size). The species studied belong to 14 genera, 11 families and 9 superfamilies of higher Lepidoptera of the infraorder Papilionomorpha according to Kuznetsov and Stekol'nikov's system (1978). This infraorder includes a large group of so-called Ditrysia, the basic evolutionary trends and taxonomy of which are presented in papers by Brock (Brock, 1971) and Kuznetsov and Stekol'nikov (1986). Species studied belong to relatively primitive superfamilies of higher Lepidoptera - Gelechioidea, Yponomeutoidea, Tortricoidea, as well as a number of more derived groups - Pyraloidea, Geometroidea, Noctuoidea, Bombycoidea, Papilionoidea and a superfamily - Zyganoidea the position of which in taxonomy is still controversial (Brock, 1971; Kunetsov and Stekol'nikov (1986).

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#### MATERIALS AND METHODS

Only males were used in analysis of karyotype. The caterpillars were, in most cases, collected in the field and reared in the laboratory until they reached the appropriate developmental stage. Part of the material was always reared to adult stage and identified. Information on material source, development stage of species studied, and in some cases, on host plants is presented below, in the section 'Karyotype characteristics'. Most of the material, except that described separately was identified by V. A. Lukhtanov.

Testes\* removed from the abdomen or whole insects were preserved in an ethanol-acetate mixture (3:1) and stored in a fixative for 1 to 8 months. Prepared testes were stained with 2% lactoacetorceine for 1-10 days. Temporary smears were prepared in 45% acetic acid and or 40% lactic acid. Karyotypes were studied in metaphase I (MI) and in some cases also in metaphase II(MII) of spermatogenesis.

Microphotographs were made with an MFN-II photo-attachment and an Amplival microscope.

Chromosome number and size, both absolute and relative were studied.

In order to determine the relative chromosome size, the area of one of the homologues in both bivalents was measured and its proportion to the area of the genome on the metaphase plate was calculated. All measurements were made on microphotographs, prepared in one scale using a graphic analysis apparatus by Opton (West Germany). Seven hundred seventy-six sizes were determined in all, on 275 metaphase plates.

The differences in karyotype structure in species with the same chromosome number were tested by Student's t-test, and for this purpose, successive comparisons of pairs of relative areas of the appropriate chromosomes in different species were made, or two-factor dispersal analysis was used. Dispersal analysis enables comparison of whole karyotype structures in different species without comparing

\*The testes are found in the fifth segment of the abdomen on the dorsal side, below or beside the dorsal vessel.

individual chromosomes. This is especially valuable when the karyotypes compared consist of a large number of small chromosomes. In such cases determining homologous pairs of the metaphase plates is difficult. We assumed that chromosome size is determined mainly by two factors: A - species, B - the number of chromosomes in the size sequence\*. In the relative size comparison the effect of factor A was 0, as the total relative area of chromosomes in the karyotype of any species is equal to 1. The effect of factor B was always significant, but is of little value as it does not permit comparison of different species. Interaction of factors A and B is of the greatest importance and its significance shows that change in the sequence of relative chromosome sizes in the two species compared does not occur collaterally. This means that the karyotypes differ in structure. The scheme presented corresponds to two factor dispersal analysis, used to determine the absence of parallelism of the two processes (Plokkinskiy, 1975).

Statistic data processing was done with EWM "Electronika-60".

#### KARYOTYPE CHARACTERISTICS OF SPECIES STUDIED

An asterisk indicates that the karyotype is here described for the first time.

#### GELECHIOIDEA

##### GELECHIIDAE

\**Sitotroga cerealella* Olivier (Fig. 1a). Laboratory culture. Leningrad, WIZR: caterpillars at end of last larval stage,  $n = 30$ . The first bivalent clearly larger than others, latter forming more or less gradually decreasing size sequence.

\**Anacampsis disquei* Meess (Fig. 1b). Maritime Terr. in vic. Ussurijsk, Gornotaezhnoe; M. V. Kozlov; imago. Analysis confirmed by M. M. Omellko,  $n = 29$ . Only adult forms were present in our material, in which, as previously noted, meiosis does not usually occur. In one ♂, however, we were able to find a meiotic division, which made it possible to determine the number of chromosomes in the genome.

#### YPONOMEUTOIDEA

##### YPONOMEUTIDAE

*Yponomeuta malinellus* Z. (Fig. 1c). N Caucasus, Plyatigorsk, V. A. Lukhtanov, caterpillars at end of last larval stage, collected on apple trees,  $n = 31$ ; bivalents forming gradually decreasing size sequence. The same number of chromosomes in this species was also recorded by other authors (Gershenson, 1967; Robinson, 1971).

#### TORTRICOIDEA

##### TORTRICIDAE

\**Archips crataegana* Hbn. (Fig. 1d). N Caucasus, Plyatigorsk, Beshtau Mt., V. A. Lukhtanov, caterpillars at end of last larval stage, collected on maple and ash. Analysis by V. I. Kuznetsov,  $n = 30$ . In this karyotype, one large bivalent is distinctive, more than 1.5 times as large as next one in sequence. Anaphase separation in this bivalent occurs later than in others (Fig. 1c). The remaining form a gradually decreasing size sequence.

\*Males of Lepidoptera are homogametic; this is why homologous chromosomes are equal in size.

*Tortrix viridana* (Fig. 1f). N Caucasus, Pyatigorsk, Mashuk Mt.; V. A. Lukhatov, caterpillars at end of last larval stage, collected on oak,  $n = 30$ . Like the preceding, this karyotype is characterized by a large bivalent, approximately twice the size of the next in the sequence, the remaining forming gradually decreasing size sequence. The first has a number of distinctive features during meiosis: at the end of prophase, contraction by coiling is slower; in prometaphase, when all other bivalents are already in the characteristic "dumbbells" transport form, this one remains in the circular; in anaphase, a delayed homologue separation is observed.

The same number and a similar karyotype is characteristic of individuals of this species from Spain Cortiz and Templado (1976).

#### ZYGAENIDEA

##### ZYGAENIDAE

\**Zygaena dorycnii* Ochs. (Fig. 2a). N Caucasus, Pyatigorsk, Beshtau Mt.; V. A. Lukhatov, caterpillars in second half of last larval stage,  $n = 30$ ; bivalents forming gradually decreasing size sequence. Among them, the small 29th and extremely small 30th bivalent is more distinctive.

*Zygaena carniolica* Sc. (Fig. 2b). N Caucasus, Pyatigorsk, Beshtau Mt.; V. A. Lukhatov, caterpillars in second half of last larval stage,  $n = 30$ ; bivalents forming gradually decreasing size sequence. One small bivalent is quite distinctive.

According to information from the literature, a subspecies of this species - *Z. c. onobrychis* has 31 chromosomes (Burgeff and Haupt, 1976), two others - *Z. c. illiterata* (Larsen, 1976) - 30 chromosomes in a haploid set.

*Zygaena filipendulae* L. (Fig. 2c). N Caucasus, Pyatigorsk, Beshtau Mt.; V. A. Lukhatov, caterpillars in second half of last larval stage,  $n = 30$ ; bivalents forming gradually decreasing size sequence. One small bivalent is quite distinctive.

The same chromosome number was found in *Z. f. pulchrior*, *Z. f. gigantea* (Burgeff and Haupt. 1967), and *Z. f. syriaca* (Lassen, 1976).

#### PYRALOIDEA

##### PYRALIDAE

*Galleria mellonella* L. (Fig. 2d). Laboratory culture, Leningrad, WIZR, protonymphs,  $n = 30$ : all bivalents forming gradually decreasing size sequence. Other authors also record  $n = 30$  as the chromosome number in this species (Robinson, 1971).

#### PHYCITIDAE

\**Plodia interpunctella* Hbn. (Fig. 2e). Leningrad, V. A. Lukhtanov, caterpillars at end of last larval stage. Analysis by A. L. Lvov,  $n = 31$ ; bivalents forming gradually decreasing size sequence. One small one is distinctive.

#### NOCTUOIDEA

##### NOCTUIDAE

*Mamestra brassicae* L. (Fig. 3a). Laboratory cultured from a Lower Rhine population of France, University of Leningrad,  $n = 31$ . One large bivalent is characteristic; the others forming gradually decreasing size sequence. One small one is quite distinctive. The same number of chromosomes is observed in individuals of this species from Japan (Saitoh, 1959) and West Germany (Werner, 1975).



Fig. 2. Chromosomes in metaphase I of spermatogenesis in Zygaenoidea and Pyraloidea. a) *Z. doricni* O.,  $n = 30$ ; b) *Z. carniolica* Sc.,  $n = 30$ ; c) *Z. filipendulae* L.,  $n = 30$ ; d) *G. mellonella* L.,  $n = 30$ ; e) *P. interpunctella* H.,  $n = 31$ .



Fig. 3. Chromosomes in metaphase I of spermatogenesis in Noctuidae and Geometridae. a) *M. brassicae* L.,  $n = 31$ ; b) *A. ruminis* L.,  $n = 31$ ; c) *O. fuscifasciata caucasica* S.,  $n = 16$ ; d) Geometridae sp.,  $n = 14$ .

*Acronicta rumicis* L. (Fig. 3b). Laboratory cultured from specimens collected in Borisovka, Belgorod Province, University of Leningrad, pronymphs,  $n = 31$ ; the bivalents forming gradually decreasing size sequence. One small one is quite distinctive.

$n = 31$  was also observed in a specimen from West Germany.

## LYMANTRIIDAE

\**Olene fascelina caucasica* Sheljuzhko (Fig. 3c). N Caucasus, Pyatigorsk, Beshtau Mt.; V. A. Lukhtan, caterpillars at end of last larval stage,  $n = 16$ . The chromosomes form a more or less gradually decreasing size sequence, although on some metaphase plates 4 groups can be distinguished: very large (2 bivalents), large (3), medium (5), and small (6). The chromosomes can be grouped into similar categories according to their area.

## GEOMETROIDEA

### GEOMETRIDAE

*Geometridae* sp. (Fig. 3d), N Caucasus, Pyatigorsk, Beshtau Mt., V. A. Lukhtanov pronymphs, caterpillars collected on oak,  $n = 14$ ; all bivalents forming gradually decreasing size sequence.

## BOMBYCOIDEA

### BOMBYCIDAE

*Bombyx mori* L. (Fig. 4a). Hybrid of "Kaukaz 1" and "Kaukaz 2", Zheleznovodsk R., Stawropol' Terr.), caterpillars in 5th day of 5th larval stage,  $n = 28$ ; bivalents forming gradually decreasing size sequence. According to many authors (Robinson, 1971), all populations of this species investigated so far have,  $n = 28$ .

## PAPILLIONOIDEA

### PIERIDAE

*Pieris brassicae* L. (Fig. 4b), Leningrad, V. A. Lukhtanov, pupae,  $n = 15$ . There seem to be 2-3 extremely small bivalents in the karyotype of this species according to our observations. Two size groups can be distinguished based on data obtained by statistical analysis (Table 2), of 11 and 4 chromosomes respectively. Other authors also tried to categorize the karyotype according to size. Some recognize 3 groups: 8 large, 4 medium, and 3 small chromosomes (Rishi and Rishi, 1977), others point to the presence of 2 extremely small bivalents (Bauer, 1967). Doncaster (1912) distinguished 3-4 small bivalents, confirmed in our material.

## NYMPHALIDAE

*Melitaea didyma*. Esp. (Fig. 4b). N Caucasus Pyatigorsk, Beshtau Mt., V. A. Lukhtanov, pupae. Caterpillars were collected on *Linaria genistifolia* (L.) Mill. (Scrophulariaceae) and fed with this plant,  $n = 27$ . One large bivalent is distinctive in the karyotype. The others form a gradually decreasing size sequence. The large bivalent stands out in meiosis (it lags behind in the division cycle). A different number of chromosomes, namely  $n = 28$ , was observed in this species from Italy (4 specimen) and Iran (1 specimen) (Lesse, 1960).

## CHROMOSOME NUMBER

Chromosome number in the groups studied varies in a wide range: from  $n = 14$  in Geometridae sp. to  $n = 31$  in species belonging to the superfamilies Yponomeutoidea, Pyraloidea, and Noctuoidea, in most of the species (in 14 out of 17), the chromosomes number is approximately the same and fluctuates between 27 and 31. It is known that Lepidoptera in general are characterized by a stable chromosome number. The majority of species analyzed so far for karyotype have  $n = 28, 29, 30, 31$ , the last two values, however, being the most common. As can be seen, information on

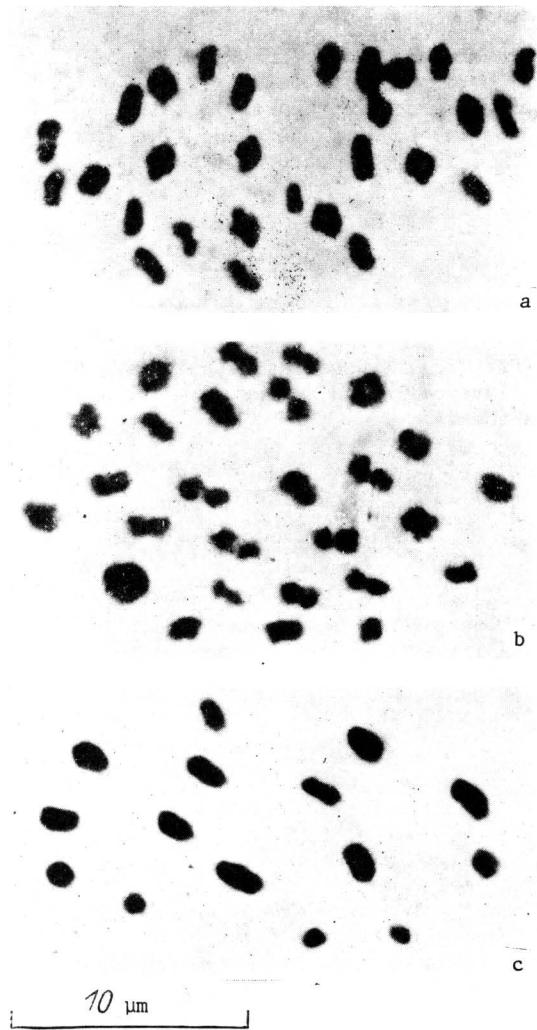


Fig. 4. Chromosomes in metaphase I of spermatogenesis in Bombycoidea and Papilionoidea. a) *B. mori* L.,  $n = 28$ ; b) *M. didyma* E.,  $n = 27$ ; c) *P. brassicae* L.,  $n = 15$ .

chromosome number cannot be used to solve problems of phylogeny in Lepidoptera, as no correlation between the level of taxon divergence and chromosome number is observed.

At the same time, the high stability of chromosome number as a species characteristic has to be emphasized. In the analysis of 43 individuals from 17 species, we did not encounter any variation within the populations. Comparison of our data with that in literature shows that individuals within the range of the species generally do not differ in chromosome number. Therefore, variation of this feature within a population can be treated as an indication of the necessity of a more detailed analysis of the taxonomic chromosome pattern position. For example, subspecies *Zygaena carniolica onobrychis* has  $n = 31$  (Burgeff and Haupt, 1967), while other geographic forms of this species are characterized by  $n = 30$ .

Table 1

## Absolute chromosome sizes in Lepidoptera

Species	Chromosome number	Average area of one homologue $\mu\text{m}^2$		Average total genome area in state of maximal spiralization of chromosomes, $\mu\text{m}^2$	
		min	max	N	$\bar{x} \pm S.E.$
<b>Gelechioidea</b>					
1. <i>Sitotroga cerealella</i>	30	0.29 $\pm$ 0.017	0.50 $\pm$ 0.028	9	10.0 $\pm$ 0.20
<b>Yponomeutoidea</b>					
2. <i>Yponomeuta malinellus</i>	31	0.42 $\pm$ 0.026	0.69 $\pm$ 0.029	8	15.3 $\pm$ 0.34
<b>Tortricoidea</b>					
3. <i>Archips crataegana</i>	30	0.40 $\pm$ 0.036	1.06 $\pm$ 0.069	8	13.2 $\pm$ 0.42
4. <i>Tortrix viridana</i>	30	0.34 $\pm$ 0.21	0.61 $\pm$ 0.053	7	12.1 $\pm$ 0.53
<b>Zygaenoidea</b>					
5. <i>Zygaena dorycnii</i>	30	0.32 $\pm$ 0.017	0.61 $\pm$ 0.030	6	10.6 $\pm$ 0.33
6. <i>Zygaena carniolica</i>	30	0.33 $\pm$ 0.028	0.46 $\pm$ 0.020	12	11.2 $\pm$ 0.33
7. <i>Zygaena filipendulae</i>	30	0.30 $\pm$ 0.027	0.39 $\pm$ 0.015	12	9.9 $\pm$ 0.18
<b>Pyraloidea</b>					
8. <i>Galleria mellonella</i>	30	0.54 $\pm$ 0.024	1.01 $\pm$ 0.046	5	18.7 $\pm$ 0.80
9. <i>Plodia interpunctella</i>	31	0.51 $\pm$ 0.028	0.95 $\pm$ 0.059	6	18.6 $\pm$ 0.91
<b>Noctuoidea</b>					
10. <i>Mamestra brassicae</i>	31	0.34 $\pm$ 0.024	0.85 $\pm$ 0.038	10	16.9 $\pm$ 1.24
11. <i>Acronicta rumicis</i>	31	0.25 $\pm$ 0.016	0.65 $\pm$ 0.032	14	11.5 $\pm$ 0.44
12. <i>Olene fusceline caucasica</i>	16	0.78 $\pm$ 0.065	1.29 $\pm$ 0.162	11	14.3 $\pm$ 0.31
<b>Bombycoidea</b>					
13. <i>Bombyx mori</i>	28	0.36 $\pm$ 0.016	0.54 $\pm$ 0.023	13	11.7 $\pm$ 0.34
<b>Papilionoidea</b>					
14. <i>Pieris brassicae</i>	15	0.48 $\pm$ 0.040	0.98 $\pm$ 0.067	6	10.0 $\pm$ 0.58
15. <i>Melitaea didyma</i>	27	0.59 $\pm$ 0.027	1.06 $\pm$ 0.058	—	—

n.b. N - number of metaphase plates measured

## ABSOLUTE CHROMOSOME SIZE

As is known, considerable variation in chromosome size exists among species *G. mellonella* and *P. interpunctella* (Pyraloidea), for example, have larger chromosomes than all other species we studied (diag. 2d, e). Size differences observed visually are confirmed by total area measurements of chromosomes in the karyotype (Table 1). We have tried to analyze how total chromosome area changes in the species we investigated. Generally, mean area total varies in quite a wide range -- from 9.9 to 18.7  $\mu\text{m}^2$  (Table 1). Variability of this characteristic within superfamilies does not, however, illustrate the actual relationship between them. Therefore, the group of species having similar chromosome size includes members of Gelechioidea, Zygaenoidea and Papilionoidea (Table 1), of which the first is one of the most primitive, and the last one of the most derived in the infraorder Papilionomorpha.

Average chromosome area totals in some groups of Lepidoptera varies insignificantly, being sufficiently stable in various taxonomic levels. For example, in the two species of Pyraloidea we investigated, area totals are very similar (Table 1). According to Goodpasture (1976) in 3 moth species (Noctuidae) total length of metaphase chromosomes (during mitosis) was 554-567  $\mu\text{m}$  and in 3 Hesperiidae species was different but also varies insignificantly (647-658  $\mu\text{m}$ ).\* From the data obtained

\*The order of magnitude given by the author is questionable (Goodpasture, 1976). Since the average length of metaphase chromosomes was approximately 2  $\mu\text{m}$  (Goodpasture, 1976: 770, Fig. 22), total chromosome area should be smaller by an order.

by Werner (1975), however, it follows that various Noctuidae, even if in one genus, differ significantly in absolute the chromosome size. The author investigated this trait in 20 moth species and found that average metaphase chromosome length totals in their karyotypes varies from 0.66 to 1.00  $\mu\text{m}$ , which corresponds to approximately 5-fold differences in chromosome volume. Our data also shows that two species from two different genera - *Mamestra brassicae* and *Acronicia rumicis* - clearly differ in absolute chromosome area (Table 1, Fig. 3a and b). It must be noted, however, that genera *Mamestra* and *Acronycta* belong to different Noctuidae subfamilies.

The existing information is, therefore, contradictory. Generally, there are few species in which the absolute chromosome size is known, therefore, no conclusions can be drawn.

We scrupulously investigated the possibility of using the character "total chromosome size" in taxonomy of closely related forms. This character, it was found, varies between species of one genus - *Zygaena carniolica* and *Z. filipendulae* and the differences are statistically significant ( $P < 0.01$ ). This data as well as that cited from Werner (1975) shows that absolute chromosome size changes during the evolution of lepidopterous species. Natural mechanisms of chromosome size modification without change in their number are not known. They may be associated with different DNA configurations or with changes in the quantity of DNA in cells. Werner (1975) believes that the last may be the result of polyteny or local duplications in chromosomes. Considerable changes in the quantity of DNA in the genome, it seems, must have occurred in evolution. The fact that according to data obtained by reassociation kinetics, the quantity of DNA in *Barathra brassicae* (Noctuidae) and *Adoxophyes orana* (Tortricidae) genomes differ twofold (Jurkovicova & Touw, 1979) speaks for this. It is not known, however, if this process is accompanied by change in chromosome size.

Although there is little information on absolute chromosome size, that found in the literature and obtained in our studies, permit the assumption that change in chromosome size, without change in the number, is one of the evolutionary trends in karyotype in Lepidoptera. It is possible that changes in the genome size (and the quantity of DNA in a haploid set of chromosomes) accompany these mentioned above.

#### RELATIVE CHROMOSOME SIZE

Relative chromosome size, unlike absolute chromosome size, should not undergo modifications during coiling in prophase to late metaphase. However, data on other groups exist which show that the rate of coiling may not be identical in different chromosomes, and is then reflected in their relative size (Blackman, 1985). In order to explain the change in relative chromosome size in the division cycle in Lepidoptera we measured the area of chromosomes in various stages of coiling and determined their percentage ratio to the total area of the genome in *Archips crataegana*. In the first case prometaphase and early metaphase chromosomes were measured. When the total area of a haploid set of chromosomes was 22.2  $\mu\text{m}$ . In both cases, relative area of corresponding chromosomes was found to be very similar (Table 2 and 3). Slight differences were only observed in two pairs out of 30, and in one case only, the difference was statistically significant ( $P < 0.01$ ). On the basis of the obtained results, it can be assumed that relative chromosome size in Lepidoptera is quite a stable characteristic and may be used in comparative karyological species description.

As was earlier demonstrated, species having identical chromosome number may differ in karyotype structure. In the majority of species studied, all chromosomes form a gradually diminishing size sequence (Table 2). It has to be emphasized that in visual analysis, also in this karyotype, which we will from now call "conservative", 2-3 pairs of chromosomes which stand out from the sequences may be distinguished. Most often, these are very small chromosomes, both in *Zygaena* species, and in *P. interpunctella* and *A. rumicis*.

The karyotype with one pair of large chromosomes distinctive in the sequence is quite common. In our material, such karyotype was observed in *S. ceraella*, *A.*

Table 2

## Relative chromosome size (1-31) in Lepidoptera studied

Species	Volume of mate- rial studied			Chromo- some number	Average relative chromosome area and its standard deviation, $X \pm S.E.$ , %	
	A	B	C		1	2
<b>Superfam. Gelechioidea</b>						
1. <i>Sitotroga cereella</i>	4	37	16	30	$6.57 \pm 0.169$	$0.480 \pm 0.06$
<b>Superfam. Yponomeutoidea</b>						
2. <i>Yponomeuta malinellus</i>	3	20	20	31	$4.77 \pm 0.109$	$4.44 \pm 0.073$
<b>Superfam. Tortricoidea</b>						
3. <i>Archips crataegana</i>	I II	3 4	23 15	22 30	$7.45 \pm 0.229$ $8.21 \pm 0.392$	$4.89 \pm 0.150$ $4.80 \pm 0.091$
4. <i>Tortrix viridana</i>	2	24	22	30	$10.06 \pm 0.295$	$4.97 \pm 0.312$
<b>Superfam. Zygaenoidea</b>						
5. <i>Zygaena doricnii</i>	2	22	11	30	$5.49 \pm 0.185$	$4.92 \pm 0.129$
6. <i>Zygaena carniolica</i>	2	22	17	30	$5.24 \pm 0.159$	$4.70 \pm 0.056$
7. <i>Zygaena filipendulae</i>	2	39	14	30	$5.10 \pm 0.202$	$4.53 \pm 0.057$
<b>Superfam. Pyraloidea</b>						
8. <i>Galleria melonella</i>	2	17	15	30	$5.29 \pm 0.242$	$4.80 \pm 0.161$
9. <i>Plodia interpunctella</i>	2	26	15	31	$5.76 \pm 0.283$	$5.03 \pm 0.155$
<b>Superfam. Noctuoidea</b>						
10. <i>Mamestra brassicae</i>	2	54	15	31	$6.31 \pm 0.299$	$5.11 \pm 0.122$
11. <i>Acronycta rumicis</i>	2	28	18	31	$5.25 \pm 0.243$	$4.51 \pm 0.085$
12. <i>Olene fascelina caucasica</i>	3	61	15	16	$10.73 \pm 0.434$	$9.37 \pm 0.158$
<b>Superfam. Bombycoidea</b>						
13. <i>Bombyx mori</i>	2	46	14	28	$5.05 \pm 0.075$	$4.76 \pm 0.034$
<b>Superfam. Papilionoidea</b>						
14. <i>Pieris brassicae</i>	4	60	15	15	$9.86 \pm 0.193$	$8.76 \pm 0.132$
15. <i>Melitaea didyma</i>	2	13	15	27	$8.09 \pm 0.258$	$5.30 \pm 0.279$
<b>Chromosomes</b>						
Species	3	4	5	6	7	8
1	$4.51 \pm 0.067$	$4.35 \pm 0.054$	$4.19 \pm 0.047$	$4.06 \pm 0.039$	$3.94 \pm 0.030$	$3.86 \pm 0.029$
2	$4.20 \pm 0.041$	$4.10 \pm 0.040$	$3.99 \pm 0.036$	$3.89 \pm 0.030$	$3.83 \pm 0.027$	$3.73 \pm 0.027$
3 (I)	$4.42 \pm 0.059$	$4.23 \pm 0.046$	$4.13 \pm 0.039$	$3.99 \pm 0.036$	$3.87 \pm 0.031$	$3.79 \pm 0.025$
3 (II)	$4.56 \pm 0.09$	$4.29 \pm 0.031$	$4.17 \pm 0.036$	$4.06 \pm 0.034$	$3.95 \pm 0.039$	$3.77 \pm 0.040$
4	$4.23 \pm 0.093$	$4.04 \pm 0.052$	$3.88 \pm 0.046$	$3.78 \pm 0.038$	$3.65 \pm 0.035$	$3.59 \pm 0.034$
5	$4.68 \pm 0.097$	$4.46 \pm 0.055$	$4.33 \pm 0.051$	$4.22 \pm 0.057$	$4.14 \pm 0.055$	$3.99 \pm 0.041$
6	$4.55 \pm 0.055$	$4.33 \pm 0.053$	$4.18 \pm 0.049$	$4.06 \pm 0.037$	$3.97 \pm 0.039$	$3.91 \pm 0.039$
7	$4.35 \pm 0.029$	$4.25 \pm 0.038$	$4.13 \pm 0.035$	$4.01 \pm 0.036$	$3.91 \pm 0.039$	$3.85 \pm 0.042$
8	$4.47 \pm 0.067$	$4.28 \pm 0.046$	$4.15 \pm 0.029$	$4.02 \pm 0.031$	$3.92 \pm 0.022$	$3.82 \pm 0.028$
9	$4.76 \pm 0.116$	$4.40 \pm 0.069$	$4.23 \pm 0.066$	$4.08 \pm 0.063$	$3.91 \pm 0.052$	$3.82 \pm 0.042$
10	$4.71 \pm 0.107$	$4.48 \pm 0.083$	$4.15 \pm 0.072$	$4.02 \pm 0.069$	$3.83 \pm 0.047$	$3.74 \pm 0.048$
11	$4.31 \pm 0.062$	$4.18 \pm 0.044$	$4.02 \pm 0.048$	$3.95 \pm 0.050$	$3.85 \pm 0.041$	$3.79 \pm 0.044$
12	$8.34 \pm 0.127$	$7.78 \pm 0.095$	$7.30 \pm 0.100$	$6.74 \pm 0.106$	$6.27 \pm 0.079$	$5.86 \pm 0.069$
13	$4.65 \pm 0.039$	$4.52 \pm 0.031$	$4.40 \pm 0.039$	$4.30 \pm 0.051$	$4.18 \pm 0.047$	$4.08 \pm 0.039$
14	$8.21 \pm 0.107$	$7.84 \pm 0.082$	$7.61 \pm 0.069$	$7.35 \pm 0.084$	$7.07 \pm 0.088$	$6.87 \pm 0.070$
15	$4.50 \pm 0.061$	$4.36 \pm 0.070$	$4.26 \pm 0.061$	$4.15 \pm 0.041$	$4.08 \pm 0.042$	$3.99 \pm 0.049$
<b>Chromosomes</b>						
Species	9	10	11	12	13	14
1	$3.76 \pm 0.036$	$3.69 \pm 0.039$	$3.61 \pm 0.045$	$3.52 \pm 0.034$	$3.44 \pm 0.030$	$3.39 \pm 0.030$
2	$3.66 \pm 0.026$	$3.61 \pm 0.024$	$3.55 \pm 0.023$	$3.52 \pm 0.022$	$3.45 \pm 0.022$	$3.40 \pm 0.028$
3 (I)	$3.72 \pm 0.028$	$3.61 \pm 0.023$	$3.52 \pm 0.021$	$3.48 \pm 0.017$	$3.40 \pm 0.024$	$3.33 \pm 0.021$
3 (II)	$3.71 \pm 0.035$	$3.63 \pm 0.040$	$3.55 \pm 0.041$	$3.47 \pm 0.042$	$3.43 \pm 0.037$	$3.35 \pm 0.035$
4	$3.51 \pm 0.031$	$3.42 \pm 0.034$	$3.35 \pm 0.027$	$3.26 \pm 0.030$	$3.24 \pm 0.030$	$3.17 \pm 0.027$

*crataegana*, *T. viridana* with  $n = 30$ , in *M. brassicae* with  $n = 31$ , and in *M. didyma* with  $n = 27$ . The size of all remaining chromosomes in the species mentioned gradually decreases.

Even in species having a constant chromosome number and a seemingly similar

Table 2 (continued)

Species	Chromosomes					
	9	10	11	12	13	14
5	3.87 ± 0.057	3.75 ± 0.051	3.69 ± 0.058	3.55 ± 0.060	3.50 ± 0.058	3.43 ± 0.056
6	3.83 ± 0.037	3.70 ± 0.031	3.62 ± 0.020	3.54 ± 0.023	3.50 ± 0.025	3.48 ± 0.022
7	3.76 ± 0.041	3.70 ± 0.041	3.63 ± 0.035	3.58 ± 0.035	3.50 ± 0.031	3.44 ± 0.030
8	3.76 ± 0.027	3.71 ± 0.023	3.63 ± 0.025	3.55 ± 0.027	3.49 ± 0.030	3.43 ± 0.027
9	3.75 ± 0.042	3.69 ± 0.042	3.55 ± 0.043	3.47 ± 0.043	3.40 ± 0.043	3.34 ± 0.046
10	3.62 ± 0.037	3.53 ± 0.038	3.45 ± 0.034	3.34 ± 0.028	3.24 ± 0.029	3.21 ± 0.033
11	3.73 ± 0.041	3.66 ± 0.041	3.61 ± 0.032	3.49 ± 0.024	3.43 ± 0.028	3.38 ± 0.031
12	5.57 ± 0.078	5.32 ± 0.066	4.98 ± 0.078	4.72 ± 0.063	4.54 ± 0.075	4.33 ± 0.083
13	4.01 ± 0.046	3.92 ± 0.043	3.84 ± 0.043	3.73 ± 0.048	3.66 ± 0.039	3.55 ± 0.033
14	6.56 ± 0.094	6.20 ± 0.093	5.87 ± 0.092	5.14 ± 0.154	4.79 ± 0.154	4.33 ± 0.139
15	3.94 ± 0.045	3.88 ± 0.032	3.80 ± 0.028	3.75 ± 0.033	3.63 ± 0.036	3.56 ± 0.032

Species	Chromosomes					
	15	16	17	18	19	20
1	3.32 ± 0.034	3.24 ± 0.032	3.17 ± 0.025	3.12 ± 0.027	3.02 ± 0.031	2.92 ± 0.029
2	3.34 ± 0.023	3.26 ± 0.027	3.22 ± 0.020	3.15 ± 0.025	3.10 ± 0.024	3.05 ± 0.029
3 (I)	3.25 ± 0.024	3.17 ± 0.024	3.09 ± 0.021	3.03 ± 0.025	2.97 ± 0.026	2.88 ± 0.031
3 (II)	3.27 ± 0.033	3.17 ± 0.033	3.11 ± 0.030	2.96 ± 0.038	2.91 ± 0.036	2.80 ± 0.034
4	3.10 ± 0.028	3.05 ± 0.028	2.99 ± 0.032	2.91 ± 0.034	2.83 ± 0.031	2.78 ± 0.027
5	3.32 ± 0.048	3.25 ± 0.034	3.16 ± 0.030	3.09 ± 0.033	2.99 ± 0.042	2.92 ± 0.034
6	3.37 ± 0.024	3.30 ± 0.022	3.23 ± 0.020	3.16 ± 0.027	3.10 ± 0.028	3.05 ± 0.033
7	3.36 ± 0.031	3.30 ± 0.026	3.23 ± 0.029	3.17 ± 0.030	3.10 ± 0.029	3.02 ± 0.024
8	3.36 ± 0.029	3.30 ± 0.026	3.25 ± 0.029	3.19 ± 0.026	3.09 ± 0.029	2.98 ± 0.031
9	3.27 ± 0.047	3.19 ± 0.047	3.08 ± 0.040	3.02 ± 0.040	2.91 ± 0.043	2.84 ± 0.048
10	3.17 ± 0.028	3.09 ± 0.029	3.03 ± 0.024	3.00 ± 0.030	2.94 ± 0.027	2.88 ± 0.026
11	3.30 ± 0.022	3.27 ± 0.018	3.18 ± 0.026	3.09 ± 0.022	3.01 ± 0.025	2.94 ± 0.034
12	4.17 ± 0.072	3.78 ± 0.186	—	—	—	—
13	3.50 ± 0.028	3.38 ± 0.032	3.32 ± 0.025	3.26 ± 0.025	3.20 ± 0.020	3.14 ± 0.023
14	3.46 ± 0.152	—	—	—	—	—
15	3.49 ± 0.032	3.41 ± 0.034	3.29 ± 0.038	3.25 ± 0.039	3.16 ± 0.029	3.12 ± 0.026

Species	Chromosomes					
	21	22	23	24	25	26
1	2.86 ± 0.022	2.75 ± 0.033	2.69 ± 0.030	2.61 ± 0.029	2.51 ± 0.038	2.37 ± 0.047
2	2.97 ± 0.025	2.89 ± 0.023	2.83 ± 0.038	2.69 ± 0.038	2.59 ± 0.039	2.45 ± 0.038
3 (I)	2.83 ± 0.027	2.75 ± 0.032	2.69 ± 0.034	2.60 ± 0.036	2.50 ± 0.035	2.39 ± 0.037
3 (II)	2.73 ± 0.036	2.66 ± 0.039	2.54 ± 0.043	2.49 ± 0.041	2.40 ± 0.047	2.26 ± 0.057
4	2.73 ± 0.026	2.65 ± 0.026	2.59 ± 0.026	2.54 ± 0.028	2.44 ± 0.035	2.34 ± 0.036
5	2.83 ± 0.046	2.77 ± 0.043	2.87 ± 0.045	2.55 ± 0.067	2.46 ± 0.059	2.36 ± 0.064
6	2.95 ± 0.042	2.85 ± 0.039	2.74 ± 0.037	2.65 ± 0.036	2.56 ± 0.043	2.42 ± 0.041
7	2.95 ± 0.017	2.90 ± 0.021	2.82 ± 0.031	2.71 ± 0.030	2.65 ± 0.035	2.55 ± 0.036
8	2.91 ± 0.031	2.88 ± 0.026	2.80 ± 0.035	2.69 ± 0.025	2.57 ± 0.040	2.47 ± 0.035
9	2.77 ± 0.045	2.70 ± 0.049	2.59 ± 0.045	2.50 ± 0.039	2.39 ± 0.042	2.27 ± 0.046
10	2.83 ± 0.030	2.72 ± 0.029	2.64 ± 0.035	2.56 ± 0.039	2.48 ± 0.043	2.38 ± 0.046
11	2.84 ± 0.035	2.75 ± 0.033	2.67 ± 0.029	2.58 ± 0.042	2.48 ± 0.041	2.40 ± 0.039
12	—	—	—	—	—	—
13	3.04 ± 0.040	2.95 ± 0.044	2.84 ± 0.054	2.65 ± 0.054	2.48 ± 0.067	2.36 ± 0.066
14	3.08 ± 0.027	2.93 ± 0.034	2.90 ± 0.054	2.78 ± 0.042	2.67 ± 0.047	2.53 ± 0.057
15	3.02 ± 0.040	2.95 ± 0.039	2.86 ± 0.037	2.76 ± 0.044	2.62 ± 0.038	2.50 ± 0.044

Species	Chromosomes					
	27	28	29	30	31	
1	2.20 ± 0.060	1.95 ± 0.056	1.76 ± 0.066	1.40 ± 0.122	—	
2	2.32 ± 0.036	2.20 ± 0.036	2.05 ± 0.034	1.88 ± 0.045	1.64 ± 0.040	
3 (I)	2.28 ± 0.031	2.14 ± 0.036	2.00 ± 0.039	1.75 ± 0.061	—	
3 (II)	2.17 ± 0.061	2.05 ± 0.067	1.88 ± 0.071	1.57 ± 0.082	—	

karyotype structure, differences can be demonstrated with the help of special methods. For this purpose, we compared relative chromosome area of pairs occupying specific position in the size sequence for each pair of karyotypes compared: A1-A1, A2-A2, A3-A3 etc. Until now, for example, it was thought that the majority of species studied of the genus *Zygaena* have the same karyotype ( $n = 30$ ) (Larsen, 1976; Burgeff, Haupt, 1967). It was found, however, that karyotypes of 3 species

Table 2 (continued)

Species	Chromosomes				
	27	28	29	30	31
4	2.28 ± 0.038	2.16 ± 0.040	2.02 ± 0.043	1.70 ± 0.055	—
5	2.28 ± 0.064	2.13 ± 0.076	1.77 ± 0.095	1.46 ± 0.097	—
6	2.29 ± 0.047	2.12 ± 0.048	1.90 ± 0.039	1.55 ± 0.084	—
7	2.18 ± 0.048	1.94 ± 0.055	1.70 ± 0.074	1.25 ± 0.122	—
8	2.37 ± 0.034	2.23 ± 0.037	2.07 ± 0.051	1.88 ± 0.048	—
9	2.12 ± 0.042	1.98 ± 0.037	1.88 ± 0.054	1.67 ± 0.054	1.43 ± 0.063
10	2.29 ± 0.053	2.18 ± 0.048	1.94 ± 0.055	1.70 ± 0.074	1.25 ± 0.122
11	2.30 ± 0.044	2.20 ± 0.047	2.05 ± 0.053	1.93 ± 0.075	1.69 ± 0.098
12	—	—	—	—	—
13	2.06 ± 0.099	—	—	—	—
14	2.33 ± 0.069	2.11 ± 0.097	—	—	—
15	2.15 ± 0.153	—	—	—	—

n.b. 1. In the column "volume of material studied": A - number of individuals; B - number of metaphase plates on which chromosome area was measured; C - chromosome number. 2. In *Archips crataegana*, in line I - data on area of weakly spiraled chromosomes, in line II - data on area of strongly spiraled chromosomes. 3. In the column "average relative area", numbers 1, 2, 3, etc. denote the number of the chromosomes in the sequence.

of this genus observed differed considerably. As the difference between *Z. filipendulae* and *Z. carniolica* is minimal (in 4 pairs of chromosomes), between *Z. filipendulae* and *Z. doricinii* it concerns 17 pairs and is considerable.

It can be expected that higher taxa (subgenera, genera, families) will differ by a larger number of structural changes than closely related species do. As a matter of fact, more significant differences were found in comparison of species belonging to different genera in one family (*Tortrix* and *Archips*, *Mamestra* and *Acronicta*) (Table 3). The same conclusion can be drawn from Goodpasture's data (Goodpasture, 1976), according to which, 3 species of Noctuidae and 3 of Hesperiidae have karyotype similar in structure within each of the families, but differing between them. Correlation between the level of taxon divergence and variability of relative chromosome area, however, cannot be observed at all times. Between *G. mellonella* and *Z. filipendulae*, belonging to rather distant families of Pyraloidea and Zygaenoidea, there are no differences in the characteristics discussed. The karyotypic similarity in these species is clearly convergent and does not illustrate the actual level of differentiation, because various chromosome rearrangements could lead to similar karyotypes.

Another example of inconsistency in differences of relative chromosome size and the degree of taxon proximity was found in the genus *Zygaena*. Regarding this characteristic, *Z. filipendulae* is closer in relation to *Z. carniolica* than to *Z. doricinii*, although *Z. filipendulae* and *Z. doricinii* belong to one subgroup of *Zygaena*, and *Z. carniolica* - to another - *Argumena* (Neumann and Trenewan, 1984). It is therefore, difficult to speak of structural karyotype characteristics as a feature of a higher taxon.

The method of comparative karyological analysis is used to reveal chromosome differences between species and estimate minimal chromosome number by which the species differ. The actual number of structural changes which occurred in evolution was, it seems, considerably higher. Unfortunately, very small chromosomes in Lepidoptera are inconvenient for differentiation staining, which could demonstrate the number and kind of structural changes.

The species having "conservative" karyotype in our material differed from each other by 0-17 chromosome pairs (Table 3). Variability in species with one large pair of chromosomes in their karyotype is more pronounced (by 4-19 pairs). Greatest variation was observed (in 23-28 chromosome pairs) between species characterized by conservative karyotype in *Tortrix viridana*, having the largest first pair of chromosomes among species of this karyotype. Statistically significant differences ( $P <$

Table 3  
The number of chromosomes which significantly differentiate species ( $P < 0.05$ , in brackets  $P < 0.01$ ) in the comparison of pairs of relative chromosome areas

Species	$n = 30$												$n = 31$			
	1	2	3	4	5	6	7	8	9	10	11	12				
1. <i>Sitotroga cerealella</i>	—	5 (2)	8 (4) 2 (1)	25 (21) 21 (16)	4 (2) 12 (7)	12 (9) 16 (9)	12 (9) 19 (14)	9 (5)								
2. <i>Archips crataegana</i> I	8 (4)	2 (1)	—	16 (15) 11 (3)	16 (14) 23 (19)	16 (14) 25 (21)	16 (13) 25 (25)	17 (13)								
3. <i>Archips crataegana</i> II	25 (21)	21 (16)	—	—	—	25 (21)	25 (25)	26 (22)								
4. <i>Tortrix viridana</i>	4 (2)	12 (7)	11 (3)	23 (19)	—	17 (6)	10 (6)									
5. <i>Zigaena loriensis</i>	4 (2)	16 (9)	16 (14)	25 (21)	5 (0)	4 (1)	2 (1)									
6. <i>Zigaena carniolica</i>	12 (9)	19 (14)	16 (13)	25 (25)	17 (6)	0 (0)	—									
7. <i>Zigaena filipendulae</i>	9 (5)	15 (11)	17 (3)	26 (22)	10 (6)	—	—									
8. <i>Galleria mellonella</i>									20 (19)	20 (19)	21 (17)	6 (3)	6 (2)	6 (2)	6 (3)	
9. <i>Yponomeuta malinellus</i>									—	—	21 (17)	6 (3)	5 (11)	15 (11)	—	
10. <i>Plodia interpunctella</i>																
11. <i>Mamestra brassicae</i>																
12. <i>Acroneuria rumicis</i>																

n.b. — data which differentiates species in dispersal analysis is printed in semi-bold type.

0.01) in karyotype structure between these species and species of the genus *Zygaena*, as well as *G. mellonella* (conservative karyotype), were found by dispersal analysis (Table 3).

In analyzing this data, it must be noted that variations in relative chromosome size does not always reflect the actual variation in karyotype. The most important inconsistency may result in comparison with species of different karyotype structure -- conservative and that with one distinctive large pair of chromosomes. The increment in size of the pair without the change in chromosome number may result from two phenomena: 1) transposition of genetic material from other chromosomes and 2) the increased quantity of DNA in this pair as a result of duplication, for example. Both processes lead to size modification of all chromosomes in the karyotype, although in the first case these changes are significant in several chromosomes and in the latter in only one pair.

In order to explain the causes of variation in relative chromosome size between species, the largest pair can be excluded from consideration, and the relative area of remaining chromosomes may be calculated. In the case of transposition of genetic material, the newly determined size will differ in the karyotypes analyzed, while in the second case, it will not. We actually performed such a calculation in species with  $n = 30$  and found that the difference between *T. viridana* and 6 other species, highly significant in the previous analysis, now was significant only in 2-18 chromosomes out of the 29 remaining. The results do not exclude duplications as a factor modifying the relative chromosome structure. At the same time, it is obvious that the differences demonstrated, are to a large extent associated with numerous interchromosomal rearrangements. The results therefore enable us to conclude that the species compared differ in many chromosomes.

#### THE ROLE OF CHROMOSOME REARRANGEMENTS IN KARYOTYPE EVOLUTION IN LEPIDOPTERA

As mentioned earlier, chromosome number is stable in Lepidoptera; karyotype structure, however, varies. This means that numerous rearrangements had occurred during evolution of the various groups, generally leading to no change in chromosome number. The Gelechiidae are characterized by  $n = 29-30$ , Tortricidae  $n = 30$ , Yponomeutidae  $n = 30-31$ , Noctuoidea  $n = 31$  (Burgeff, Haupt, 1967; Gershenson, 1967; Robinson, 1971; Werner, 1975; Larsen, 1976; Ennis, 1976; Mohanty, Nayak, 1983; Bedo, 1984; and our results). In the groups mentioned, only a few species have chromosome numbers different from the modal characterizing the order, equal to 28, 29, 30, 31 in a genome. This data enables us to assume that among the changes in karyotype, various types of intra- and interchromosomal rearrangements occurred and not chromosome breakages or unions. Variations in karyotype structure of closely related species in the genus *Zygaena* probably resulted from such mutations (Table 3). Bigger (1976) drew analogous conclusions earlier after comparing two species of the genus *Pieris* (Pieridae). Staining showed that only 8 chromosome pairs out of 25 were identical in *P. napi* and *P. rapae* karyotypes, the others differed by one or several characteristics. Fontana (1976) found heteromorphic bivalents and round chromosome pairing in meiosis. The author also concluded that the karyotypes in parental forms differ considerably, although chromosome number is constant. This means that divergence (of ancestral forms) in these species was accompanied by chromosomal structural changes, including reciprocal translocation. A greater part of rearrangements occurring during evolution is probably associated with the holokinetic nature of chromosomes. It is known that in organisms with monokinetic chromosomes numerous rearrangements, including reciprocal translocations may lead to acentric and bicentric chromosome formation and sterility of a part of the gametes during gametogenesis. In organisms with holokinetic chromosomes, as was demonstrated by Bauer (1967) in experiments with *Pieris brassicae*, reciprocal translocations do not lead to such an abrupt increase in the number of sterile gametes. Probably this is why the fixation of chromosomal rearrangements in Lepidoptera may occur quite quickly.

Three basic tendencies which lead to karyotype change can be observed in Lepidoptera. One of them is associated with change in chromosome number. Lepidoptera

are characterized by the longest numerical series among insect chromosome numbers from  $n = 5$  in *Agathymus aryxna* of the family Megathymidae (Freeman, 1969) to  $n = 217-223$  in *Lysandra atlantica* of the family Lycaenidae (Lesse, 1970). Chromosome breakage and union are the processes which permit such changes in number during evolution. The two other tendencies are more sharply marked, however, and do not lead to change in the number of chromosomes. Either, the stable, "conservative" as we called it, karyotype structure, in which all chromosomes form a gradually and uniformly decreasing size sequence remains, or distinctive in size, sometimes very large pair appearing in the karyotype. Both variants are often found in Lepidoptera. The first variant was already noted by Suomalainen (1965). The second, with the large pair, was recorded several times in various groups of lower Lepidoptera (Belajeff, 1930; Lesse, 1960; Suomalainen, 1969b; Maeki and Ae, 1968a, b; Werner, 1975; results of other authors and ours). Some assumptions can be made as to the factors which favor the formation of the second type of karyotype, without change in the chromosome number. Translocation of genetic material from several chromosomes to one, which results in the enlargement of the latter, leads to the formation of a gigantic linkage group. The frequency of the recombination of genes translocated to this chromosome decreases what may have consequences where the preservation of a set of favorable properties is concerned. The high chromosome number which remains ensures the high level of recombinant variation in other properties, which may be important in sustaining the adaptability of species.

Suomalaines (1969b, 1971) assumed that the large pair of chromosomes constitutes the sex chromosomes. Our observation of the behavior of the largest bivalent in *Tortrix viridana*, *Archips crataegana* (Tortricidae) and *Melitaea didyma* (Nymphalidae) in meiosis (weak coiling in metaphase, delayed homolog separation in anaphase) also enable us to infer that this bivalent is a sex bivalent. It must be emphasized, however, that the sex bivalent is not always the largest in the genome in Lepidoptera (Traut and Rathjens, 1973; Bigger, 1975, 1976; Gupta and Narang, 1981).

The three tendencies mentioned, observed in the process of karyotype change, are differently marked in the various taxa. The "conservative" karyotype structure, it seems, is characteristic of a majority of groups.

The tendencies towards the enlargement of one pair in the karyotype is clearly marked in Tortricidae. In all members of this family with  $n = 30$  analyzed until now for karyotype, the large pair of chromosomes is present (Suomalainen, 1971; Ennis, 1976; Ortiz and Templado, 1976; our data). Only Tortricidae with a different chromosome number (less than 30) do not have the enlarged chromosome pair (Suomalainen, 1969b; Ennis, 1976; Ortiz and Templado, 1976).

The trend towards change in chromosome number is most clearly developed in the Lycaenidae and Satyridae (Robinson, 1971). In *Erebia* (Satyridae), for example, the numbers recorded are from  $n = 7$  (*E. aethiopellus*) to  $n = 51$  (*E. iranica*) (Lesse, 1960); in *Polyommatus* - from  $n = 10-11$  [*P. (Agrodiæfus) posthumus*] to  $n = 217-223$  [*P. (Lysandra) atlantica*] (Lesse, 1960, 1970).

## CONCLUSION

The following conclusions have been drawn from the results of karyotype analysis of 17 species belonging to a superfamilies of higher Lepidoptera, and from literature: 1. A stable chromosome number is characteristic in Lepidoptera. At the same time, the widest range, among insects, of chromosome number is observed. The unusual variation of this characteristic is found in various low taxa. 2. A change in chromosome number may occur in the process of species evolution. This characteristic sometimes differentiates closely related species and forms groups within a species. Chromosome breakages and unions are the mechanisms responsible for the change in the number of chromosomes. 3. Numerous rearrangements in the karyotype occur during the evolution of species without change in the chromosome number. The majority of forms studied differ in absolute and relative size. 4. Two karyotype

forms are widespread throughout the Lepidoptera. In the first, the conservative type, chromosomes diminish in size. The second type is different in that a distinctively large pair of chromosomes is found. 5. Structural rearrangements in karyotype played a more important role in Lepidoptera evolution than change in chromosome number. 6. The characteristics of karyotype structure may be used in explaining taxonomy levels of the subspecies, species, and groups of closely related species. The frequently observed lack of correlation between the level of divergence of higher taxa and the differences in relative chromosome area limits the possibility of applying this characteristic in macrotaxonomy. 7. Three basic trends may be seen in the karyotype evolution in Lepidoptera: (1) change in chromosome numbers; (2) preservation of the conservative karyotype structure; and (3) formation of a pair of large chromosomes accompanied with no change in the conservative structure of the remaining part of the genome. All 3 trends are differently marked in different taxa. The first is most pronounced in Lycaenidae and Satyridae, the second is common in all groups and karyotypes with various chromosome numbers, the third is observed sporadically in many families, but most of all in Tortricidae.

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