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RESEARCH ARTICLE



Introduction to the study of chromosomal and reproductive patterns in Paraneoptera

Ilya A. Gavrilov-Zimin¹, Snejana M. Grozeva², Dmitrii A. Gapon¹, Andrei S. Kurochkin³, Katia G. Trencheva⁴, Valentina G. Kuznetsova¹

 Zoological Institute, Russian Academy of Sciences, Universitetskaya emb. 1, St. Petersburg, 199034, Russia
Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Blvd Tsar Osvoboditel 1, Sofia 1000, Bulgaria 3 Samara National Research University, Moskovskoe Shosse, 34, Samara 443086, Russia
University of Forestry, Blvd Kliment Ochridski 10, Sofia 1756, Bulgaria

Corresponding author: Ilya A. Gavrilov-Zimin (coccids@gmail.com)

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Abstract

This paper opens the themed issue (a monograph) "Aberrant cytogenetic and reproductive patterns in the evolution of Paraneoptera", prepared by a Russian-Bulgarian research team on the basis of long-term collaborative studies. In this first part of the issue, we provide the basic introductory information, describe the material involved and the methods applied, and give terminology and nomenclature of used taxonomic names.

Keywords

Aphids, cicadas, lice, moss-bugs, psocids, psyllids, scale insects, thrips, true bugs, whiteflies, zorapters

Introduction

The predominant reproductive strategy in eukaryotic organisms is bisexual reproduction which involves the formation and fusion of gametes, namely, sperm from the testes and eggs from the ovaries. This is also true for all major insect groups, in which, however, bisexuality is often combined with numerous aberrant modes of reproduction (White 1973; Ivanova-Kazas 1995; Simon et al. 2003; De Meeûs et al. 2007; Vershinina and Kuznetsova 2016; Leather and Hardie 2017; Gokhman and Kuznetsova 2018). These latter can characterize high-rank taxa or be found in separate genera and species within a group that mainly reproduces bisexually. The large insect supercohort Paraneoptera provides a unique opportunity to study almost the entire spectrum of aberrant reproductive strategies as well as genetic and chromosomal systems known in insects in general, such as ovoviviparity and viviparity, neoteny and paedogenesis, larval meiosis, achiasmate and inverted meiosis, parthenogenesis and polyploidization, dizygotic embryonal development, very peculiar types of mating, a huge variety of sex determination mechanisms, etc. It is important to point out that some reproductive patterns are often highly variable between or even within insect species.

In this monograph, we attempt to summarize results of our own long-term investigations in the field and available literature data in order to give an overall picture of distribution of different reproductive characteristics within and among higher taxa of Paraneoptera. The supercohort Paraneoptera comprises about 130,000 recent species in the world fauna and is traditionally subdivided into the orders Zoraptera, Copeognatha (=Psocoptera), Parasita (=Phthiraptera), Thysanoptera, and the superorder Arthroidignatha (=Hemiptera sensu stricto) with two large orders, Heteroptera (true bugs) with about 45,000 species and Homoptera with about 66,000 species (Poisson and Pesson 1951; Henry 2017; Gavrilov-Zimin 2018; Kluge 2020) (Figs 1, 2). The last group is the most taxonomically diverse and combines five recent suborders, Aphidinea (about 5,000 species), Coccinea (8,000 species), Psyllinea (3,500 species), Aleyrodinea (1,500 species) and Cicadinea (47,000 species), which are quite divergent from each other morphologically, anatomically, cytogenetically, etc. Heteroptera compete with them in diversity and even surpass them in some aspects. This taxon is usually considered in the rank from order to suborder and, together with Coleorrhyncha (about 30 species), is often included in the higher taxon Heteropteroidea. Heteroptera are divided into seven infraorders, Enicocephalomorpha, Dipsocoromorpha, Nepomorpha, Gerromorpha, Leptopodomorpha, Cimicomorpha, and Pentatomomorpha; they include aquatic, semi-aquatic, surfacedwelling, terrestrial, carnivorous, blood-sucking, herbivorous, and parasitic representatives. The current level of knowledge of the cytogenetics and reproductive biology varies significantly between different groups of Paraneoptera. For example, among Aphidinea, Coccinea, Psyllinea, Cicadinea, and Heteroptera, several thousand species from all large families have been studied in this respect. On the other hand, among Zoraptera, Copeognatha, Parasita, Thysanoptera, and Aleyrodinea only occasional species from a small number of families have been analyzed, and both cytogenetic and reproductive characteristics of these groups are currently poorly known. The reason for this is largely due to difficulties in collecting these insects which are very small and lead a hidden life style.

We hope that our review issue will be useful for specialists in entomology, cytogenetics and evolutionary biology, as well as for those in the field of plant protection, veterinary and medicine. Many scale insects and aphids as well as some thrips, psyllids and true bugs are important pests of agricultural and ornamental plants and carriers of pathogenic viruses, whereas many lice and some true bugs are ectoparasites of invertebrate and vertebrate animals, including humans. Comparative knowledge



Figure 1. a-k Species from main taxonomic groups of Paraneoptera insects **a** imago and larvae of *Clematoscenea* sp. (Copeognatha), Singapore, photo and "Creative Commons" license of "Budak" (see Acknowledgements) **b** poultry fluff louse *Goniocotes gallinae* (de Geer, 1778) (Parasita), photo and "Creative Commons" license of "Da Re" (see Acknowledgements) **c** imago of *Taeniotrips inconsequens* (Uzel, 1895), Poland, photo and "Creative Commons" license of "Budak" (see Acknowledgements) **c** imago of *Taeniotrips inconsequens* (Uzel, 1895), Poland, photo and "Creative Commons" license of "Riszard" (see Acknowledgements) **d** colony of viviparous parthenogenetic females of *Tuberolachus silignus* (Gmelin, 1790) (Aphidinea), Samara Prov. of Russia, photo of A.S. Kurochkin **f** adult female with wax ovisac and larvae of *Icerya purchasi* Maskell, 1879, Turkey, photo of A.S. Kurochkin **g** females of *Rhodococcus* sp. (Coccinea), attended by ant, Kazakhstan, photo of A.S. Kurochkin **h** imago and **i** larva of *Psylla carpinicola* Crawford, 1914 (Psyllinea), USA, photos and "Creative Commons" license of Katja Schulz (see Acknowledgements) **j** females and male of *Aleyrodes lonicerae* Walker, 1852 (Aleyrodinea), Samara Prov. of Russia, photo of A.S. Kurochkin **k** larvae of *Trialeurodes lauri* (Signoret, 1862), Turkey, photo of A.S. Kurochkin.



Figure 2. a–**p** Species from main taxonomic groups of Paraneoptera insects (continuation) **a** *Pyrops candelar*ia (Linnaeus, 1758) (Cicadinea, Fulgoridae), photo and "Creative Commons" license of "Sterling Sheehy" (see Acknowledgements) b Cicadetta montana (Scopoli, 1772) (Cicadinea, Cicadidae), photo of E.Yu. Kirtsideli, PhD c Cercopis vulnerata Rossi, 1807 (Cicadinea, Cercopidae), photo of E.Yu. Kirtsideli, PhD d Centrotus cornutus (Linnaeus, 1758) (Cicadinea, Membracidae), photo of E.Yu. Kirtsideli, PhD e Pantinia darwini China 1962 (Coleorrhyncha, Peloridiidae), photo and "Creative Commons" license of "Sterling Sheehy" (see Acknowledgements) f Glaenocorisa propinqua (Fieber, 1860) (Heteroptera, Corixidae), photo of D.A. Gapon g Gerris sphagnetorum Gaunitz, 1947 (Heteroptera, Gerridae) h Chartoscirta elegantula (Fallén, 1807) (Heteroptera, Saldidae), photo of E.Yu. Kirtsideli, PhD i Anthocoris nemorum (Linnaeus, 1761) (Heteroptera, Anthocoridae), photo of E.Yu. Kirtsideli, PhD j Cimex hemipterus (Fabricius, 1803) (Heteroptera, Cimicidae), photo of D.A. Gapon k Hesperoctenes fumarius (Westwood, 1874) (Heteroptera, Polyctenidae), photo and "Creative Commons" license of "CBG Photography Group" (see Acknowledgements) I Cremnocephalus albolineatus Reuter, 1875 (Heteroptera, Miridae), photo of D.A. Gapon m Rhynocoris annulatus (Linnaeus, 1758) (Heteroptera, Reduviidae), photo of E.Yu. Kirtsideli, PhD n Aradus laeviusculus Reuter, 1875 (Heteroptera, Aradidae), photo of D.A. Gapon **o** *Rhyparochromus phoeniceus* (Rossi, 1794) (Heteroptera, Lygaeidae), photo of D.A. Gapon p Rhaphigaster nebulosa (Poda, 1761) (Heteroptera, Pentatomidae), photo of D.A. Gapon.

of reproductive modes generated from studies across different phylogenetic lineages of Paraneoptera is essential for a better understanding of reproductive processes and underlying cytogenetic mechanisms in Insecta as a whole.

Material and methods

The issue is based on the material collected mainly by authors and sometimes by their colleagues in different regions of the world including Western and Eastern Europe, Canary Islands, Morocco, Central Russia, Russian Far East, Crimea, Caucasus, Turkey, Myanmar, Thailand, Laos, Malacca peninsula, Sumatra, Java, Borneo, Sulawesi, New Guinea, Bali, and Flores Islands, Trinidad and Tobago, New Zealand, Tasmania, Vietnam and some others. The collections available at the Zoological Institute of the Russian Academy of Sciences (**ZIN RAS**, St. Petersburg) and the Institute of Biodiversity and Ecosystem Research of the Bulgarian Academy of Sciences (**IBER BAS**, Sofia) were also used.

Taxonomic identification of insects was mainly based on traditional morphological characters, with extensive use of the structural characters of the internal reproductive system and karyotype. In most cases, the identification was carried out using either intact insects, which are mounted on pins or mounting boards, or stored in ethanol/aceto-ethanol. To study male terminalia, the pygophore (genital segment) is detached from the abdomen and boiled for several minutes in 15–20% KOH solution. Parameres and aedeagus, removed from the pygophore using finest forceps and a dissecting needle, are examined at wet preparations. To study the structure of the endosoma (the internal membranous sac of the aedeagus), the method of its hydraulic inflation by means of glass microcapillaries is used, followed by drying the endosoma in a stream of hot air in a completely inflated state (Gapon 2001). The structure of the internal ectodermal parts of the female reproductive system is studied after boiling the abdomen in alkali and mechanical removal of soft tissues. Membranous structures on wet preparations are stained with methylene blue.

In the case of aphids, scale insects, whiteflies and thrips, permanent microscopic slides are prepared from insects and mounted in Canada balsam. Material processing included a different set of steps (procedures) depending on the object or on the specific purpose of the study. For example, in Thysanoptera, Aphidinea and Coccinea, studies were carried out on (ovo)viviparous females, eggs and larval instars of both sexes, respectively, whereas in Copeognatha, Parasita, Psyllinea, Cicadinea, Coleorrhyncha, and Heteroptera they were carried out mainly on males, although in some cases, females were also involved (when reproduction is parthenogenetic or when it is necessary to identify the chromosomal mechanism of sex determination in a particular species).

Preparation of permanent microscopic slides from aphids and coccids includes the following main steps (described in Gavrilov-Zimin 2018).

Fixation

Insects, cleaned of plant tissues and/or soil particles, were fixed in 96% ethanol or (more often) in a mixture consisting of 1 part of glacial acetic acid and 3 parts of 96%

ethanol (a Carnoy's fixative). In our experience, the latter fixation is preferable due to the subsequent use of acid stains, for example, staining with acid fuchsin + pink lignin dissolved in Essig's aphid fluid (see below). The use of acetoethanol also prevents superfluous dehydration of the fixed material. The volume of fixative must significantly (20 and more times) exceed the volume of the material. Fixed material is preserved in a dark place and, if possible, in a refrigerator. The minimum fixation time is 2–3 hours.

Preliminary anatomizing

The insects were taken out of the fixative, put on the object glass in a drop of ethanol or distilled water and cut along the lateral body margin using a small blade.

Clarification

The insects were placed in 8–15% water solution of sodium hydroxide (NaOH) or potassium hydroxide (KOH) and heated in a water bath or on any hot plate (about 60 °C) until the cuticle becomes translucent. The time of heating was selected experimentally for each object. With weakly sclerotized females (most mealybugs), 10–20 minutes of heating is usually enough. Heavily sclerotized and pigmented specimens demand 1–1.5 hours of heating. On the other hand, delicate soft females of Xylococcinae (Margarodidae s.l.) can be simply clarified in cold KOH or NaOH (about 20 °C) during several hours.

Secondary anatomizing

Specimens were placed in a small amount of hot potash, and all internal organs were removed by light pressure on the cuticle using thin hooks. This is usually needed to change potash (hot or cold) several times until all the body internal content is removed. The specimens were then transferred to water for complete removing the potash.

Staining

The most common method was originally developed for aphids (Essig 1948). The stain mixture consisting of 5 ml of acid fuchsin (4% water solution) and 10 ml of pink lignin (2% water solution) are dissolved in 100 ml of Essig's fluid. The so-called "Essig's fluid" can be prepared as follows: 20 parts of lactic acid (80–90% solution), 2 parts of phenol solution (1 gram phenol in 15 ml of distilled water), 4 parts of glacial acetic acid and 1 part of distilled water. Both mixtures are preserved separately in the refrigerator until use. Just before staining the material, several ml of Essig's fluid should be poured in a small tube by adding 3–4 drops of stain mixture. Material can be stained directly in this tube for 20–30 min at 60 °C or for several hours at room temperature. Weakly sclerotized or poorly fixed specimens need longer staining. When successful staining

of the object is reached, its sclerotized parts, such as antennae, legs, different setae and wax glands become well visible through the translucent background of the cuticle.

An older, but simpler and cheaper method of staining is based on the use of only fuchsin diluted in distilled water or in 96% ethanol until saturation. You can also take 1 g of basic fuchsin in 100 ml of 96% ethanol or 0.5 g of acid fuchsin in 25 ml of 10% water solution of 30% HCl and 300 ml of distilled water. The stain mixture by Dr. Jean-François Germain (Montpellier, France) consisting of acid fuchsin diluted until saturation in the mixture of distilled water, lactic acid (80–90% solution) and glycerol (1:1:1) also gives excellent results (Gavrilov-Zimin 2018).

After staining of any type, the material should be washed several times in 96% ethanol until the excess stain is removed.

Oil impregnation

Canada balsam, which is usually used for the preparation of permanent slides, does not mix with water or ethanol. The specimens need, therefore, to be impregnated with an intermediate fluid, which can mix with both. This can be either a clove or bergamot oils, but other plant oils can also be tested if needed. The specimens should be placed in oil for 20–30 min while can be preserved in it for a longer time. If the acceptable oils are absent, it is possible (but undesirable!) to use xylene, toluene or something similar as an intermediate fluid. It is well known that a small amount of 96% ethanol can be mixed with a large amount of xylene or toluene. Therefore, the specimens can be get out of ethanol, air dried for several seconds and placed then in xylene or toluene for 20–30 min.

Mounting

Following the oil or xylene/toluene impregnation, the specimens should be placed on a clean slide and excess oil must be removed with filter paper. Dorsal and ventral sides of the specimens, which were previously cut along the entire body margin, should be placed in the same plane. Then, a small drop of Canada balsam is dripping on the specimen(s) and covered with a cover slip. The slides are now ready for study, but care must be taken during several weeks until the slide is completely dried. Either thermostat or a drying box can be used to speed up drying. Dried slides can be stored in a dust-free place for an unlimited time at temperatures not higher than 35–40 °C.

Microscopic preparations were also prepared to study the reproductive biology, genetic systems and karyotypes of scale insects and aphids. With adult insects, both laid eggs and larval instars were fixed in acetoethanol (1:3) for at least 24 hours. The specimens were then dissected under a stereomicroscope and anatomized in a drop of 45% acetic acid. The simplest method for preparation of chromosome slides is based on staining with acetoorcein or acetocarmin or (better) with lactic acid solutions of these stains. This method gives acceptable results even for not well fixed material, being

usually used for temporary slides only. For example, young embryos or gonads are stained by squashing in a drop of lactoacetorcein (50 ml 85% lactic acid : 2 g orcein : 50 ml glacial acetic acid). The cover slip can be fringed with rubber glue/cement, the slide will then be acceptable for study during a long time, especially if stored in the re-frigerator. More complicated methods are based on staining with hematoxylin (see, for example, Dikshith 1964) or with Shiff's reagent, the so-called Feulgen-Giemsa method described in Grozeva and Nokkala (1996). This method is widely used in cytogenetic studies of many insects including paraneopterans (see below); however, our experience with this method on scale insects and, in particular, on mealybugs, was negative. The main problem we encountered was an unpredictable influence of Shiff's reagent on different species or even on different developmental stages of the same organ, which was previously noted also by other researches (see Romeis 1953).

The recent studies of chromosomes and internal reproductive organs of Paraneoptera insects other than Aphidinea and Coccinea, i.e. Zoraptera, Copeognatha, Parasita, Coleorrhyncha, Heteroptera, Cicadinea, and Psyllinea, were carried out using a different and wider range of methods and procedures.

Sampling, fixation and storage of material

Male and female specimens, both newly emerged and older larvae, collected in the field, were fixed immediately in a freshly prepared Carnoy's fixative (3:1) and refrigerated then in the laboratory at 4 °C until needed. If it was possible, some insects were brought to the laboratory alive. These were given a short hypotonic treatment with 1% tri-sodium citrate solution (Na₃C₆H₅O₇) for 5 min before the specimens were fixed in a fresh Carnoy's.

Study of the anatomy of testes in males and ovaries in females

The study was carried out on both live and fixed insects. In both male and female specimens, the abdomen was separated from the body and opened on a microscope slide in a drop of 45% acetic acid. The testes and ovaries were dissected out and analyzed under a stereomicroscope. In our anatomical research, we confined ourselves mainly to studying the number of testicular follicles and ovarioles, their shape and position on the sperm duct and oviduct, respectively. In separate cases, we studied the male internal reproductive system in general, with reference to the structure of the testes, presence/absence and shape of seminal vesicle(s), accessory glands and some additional associated structures. In psyllids and zorapters, we also analyzed the arrangement of spermatocytes within the follicle and the sequential stages of sperm formation. For this purpose, follicles were put on the slide in a drop of 45% acetic acid; coverslip was put on the drop and was allowed to settle without squashing. When all streaming was ceased, the slide was squashed gently allowing the spermatocytes to remain intact and retain their original location within the follicle.

Study of chromosomes and meiosis

Slide preparation

Chromosome preparations from the male specimens (fixed in a Carnoy's fixative) were made and stained as follows: testes were removed from the abdomen in 45% acetic acid. In some cases (when specimens were not fixed), they were removed in 1% tri-sodium citrate solution (Na₂C₄H₅O₇) for 5 min, fixed in a fresh Carnoy's fixative and transferred then into a drop of 45% acetic acid on a slide. Testes were counted and cut into pieces (if large), mature sperms were largely removed, and squash preparations were made. The preparations were first examined by phase contrast to assess their quality and the presence of chromosomal divisions. After freezing off the coverslips in dry ice (a dry-ice technique by Conger and Fairchilld 1953), slides were dehydrated in fresh fixative solution for 30 min and air-dried. Chromosome preparations from female specimens were made and stained as follows: mature eggs were extracted from the abdomen and placed individually on slides in a drop of 45% acetic acid. After the chorion was removed and yolk became transparent, the eggs were squashed, and slides were made permanent by a dry-ice technique. In some cases (mainly in psyllid research), part of the material (both males and females) was both fixed and stored in 96% alcohol. In the laboratory, each of those specimens was dissected; the abdomen was immersed in the Carnoy's fixative while the head and thorax part was stored in alcohol for subsequent sequencing. This allowed both chromosomal and haplotype (DNA barcoding) analyses of the same individual, which was very important for the purposes of accurate taxonomic identification of individuals (Nokkala et al. 2015, 2017, 2019).

Chromosome staining techniques

Conventional staining

Air-dried slides were stained according to the Schiff-Giemsa protocol first developed by Puro and Nokkala (1977) and then slightly modified by Grozeva and Nokkala (1996) for the study of true bugs. In brief, slides prepared from the testes were immersed in 1N HCl at room temperature for 15 min, hydrolyzed in 1N HCl at 60 °C for 8 min and stained with Schiff's reagent for 20 min. Unreacted Schiff's reagent was rinsed of thoroughly with distilled water, the slides were immersed in Sorensen's phosphate buffer, pH 6.8, for 5 min, and stained with 2% Giemsa in Sorensen's buffer for 20–30 min. When adequate staining was achieved, the slides were rinsed briefly with distilled water, air-dried, and mounted in Entellan (a mounting medium). With slides prepared from the ovaries, a slightly modified Schiff-Giemsa method was used. Slides were subjected to hydrolysis in 1 N HCl first at room temperature for 20 min and then at 60 °C for 8 min, and stained in Schiff's reagent for 20 min. After rinsing thoroughly in distilled water, the slides were additionally stained with 4% Giemsa in Sørensen's buffer (pH 6.8) for 20 min. The slides were rinsed briefly in distilled water, air-dried and mounted in Entellan.

As it will be discussed in subsequent parts (papers) of the monograph, almost all Paraneoptera insects have holokinetic chromosomes that display a very limited number of distinctive characters (markers) making it difficult and often completely impossible to identify homologues in the karyotype and trace the behavior of chromosomes in meiosis and reproductive cycles in general. The search for chromosomal markers in "holokinetic insects" is therefore of particular importance. In these insects, including objects of this study, such techniques as C-banding, AgNOR-staining, DNA specific fluorochrome banding and fluorescence in situ hybridization (**FISH**) are widely used. Below, we will provide a brief description of these approaches and note their capabilities and goals in studies of karyotypes and gametogenesis in Paraneoptera.

Sequential staining

C-banding

Chromosomes are known to consist of euchromatin and heterochromatin, which have different staining properties. C-banding technique detects blocks of constitutive heterochromatin (C-bands) consisting of satellite DNAs, which are highly repetitive sequences of DNA with no known genes, and remain condensed all throughout the cell cycle. In monocentric chromosomes, C-bands are present mainly in the centromeric regions, although they may also occur at any other position along the chromosomes. In holokinetic chromosomes, C-bands are mainly confined to terminal portions of the chromosomes, although they are also present in nucleolar constrictions occupying one or both sides of the constriction (NOR-associated heterochromatin) and sometimes also in interstitial regions of the chromosome. In our studies, we used a slight variation of the conventional C-banding procedure which was developed by Sumner (1972) and up to the present time is widely used in various laboratories of the world. Slides were aged at 37 °C for 7–10 days, treated with 0.2 N HCl for 20 min at room temperature, immersed in a saturated solution of Ba(OH), at room temperature for 1 to 14 min (time depends on the object), rinsed three times in water, immersed in $2 \times$ SSC (sodium chloride 0.3M and 0.03M trisodium citrate, pH 7.0) at 60 °C for 1 hr, thoroughly rinsed, air-dried and stained with 4-5% Giemsa solution in Sørensen's phosphate buffer, pH 6.8. When appropriately stained, the preparations were rinsed briefly with distilled water, air dried, and mounted in Entellan.

AgNOR-staining

Nucleolus organizer regions (NORs), which give rise to the interphase nucleoli, are defined as nucleolar components containing ribosomal genes and the argyrophilic NOR-associated proteins (AgNOR proteins), which bind silver ions. AgNOR proteins are selectively stained by impregnation with silver nitrate (AgNO₃) and can be

identified by light microscopy as well-defined black dots exclusively localized on the NOR-carrying chromosomes and throughout the nucleolar area in interphase nuclei. The NORs stained by silver are called "AgNORs". In our studies, we used a "one-step" silver-staining method by Howell and Black (1980), which is the most frequently employed technique for AgNOR protein visualization in routine cytogenetic studies of different eukaryotes, including insects. Slides were incubated in standard saline citrate (SSC) solution at 65 °C or in 0.2 M HCl at room temperature for 30 min and treated in 50% AgNO₃ with gelatin as a developer (0.2 g gelatin, 10 ml distilled water, and 0.1 ml concentrated formic acid HCOOH), in the ratio 2: 1, in a moist chamber at 65 °C for 4–8 min (time is chosen empirically). The staining reaction was followed under the microscope. When the desired degree of staining was observed, the reaction was halted by rinsing with distilled water, and the preparations were dried and embedded in Entellan. The most important detail in the whole process of the experiment was to avoid the light.

Molecular cytogenetic techniques

Fluorochrome banding and fluorescence in situ hybridization (FISH) are excellent molecular cytogenetic tools which provide various possibilities in the study of chromosome structure and genome organization and contribute to a better characterization of the karyotype and meiosis.

Base-specific fluorochrome staining

Constitutive heterochromatin (C-heterochromatin; see above) can be enriched with G-C (guanine-cytosine) or A-T (adenine-thymine) base pairs of DNA. The most widely used base-specific fluorochromes, CMA₃ (chromomycin A₃) and DAPI (4',6-diamidino-2-phenylindole), are fluorescent dyes that bind strongly to GC-rich and AT-rich regions in DNA, respectively, and reveal, thus, the molecular composition of C-heterochromatin. Comparative patterns of fluorochrome banding allow the identification of homologous chromosomes in the karyotype. In our studies, we carried out DAPI/CMA, double staining following mainly Schweizer (1980). The AT-specific fluorescent dye DAPI and GC-specific dye CMA₃ were dissolved in McIlvaine's citric acid/NaHP buffer at pH 7, and in the diluted (1:1) pH 7 buffer containing 5 mM MgCl₂, respectively. Chromosomal preparations were stained for 25-45 min with CMA₂ (0.5 mg/ml), briefly rinsed with buffer, stained with AT-specific antibiotic distamycin A (DA) (0.1 mg/ml) for 5–15 min, again briefly washed, and finally stained with DAPI (0.6 mg/ml) for 20-30 min. To improve staining reaction, we added 5% methanol in the fluorescent staining solutions. After fluorochrome staining, slides were washed twice in 70% ethanol for 30 min and stained with 4% Giemsa for C-banding. The preparations were then rinsed with buffer, air-dried, mounted in a mixture of 70% glycerol and pH 7.0 McIlvaine's buffer (1:1) and sealed with rubber solution. To prevent fading of CMA₃-fluorescence, we added 1% n-propyl-gallate in the mounting medium. Prior to examination, the preparations were stored in the dark for several days, by which time both the chromomycin A₃ and DAPI fluorescence are stabilized.

Fluorescence in situ hybridization (FISH)

FISH technique, developed about 50 years ago (Gall and Pardue 1969; John et al. 1969), is powerful for the physical mapping of genes and defined DNA sequences directly on chromosomes by hybridization of complementary fluorescently labeled DNA probes on cytological preparations. This technique is very helpful in chromosome-based genome assemblies, providing information on the fine architecture of genomes and their evolution. In our studies, we mainly used two-color FISH for mapping the multigene family of rDNA and the insect-type telomeric motif (TTAGG). We aimed to study the number and the distribution of rDNA loci and use them as markers for the identification of specific chromosomes and comparative chromosome mapping as well as for tracing chromosome behavior during meiosis and gametogenesis in general. Another goal was to find out whether a particular taxon has retained the evolutionarily ancestral "insect" motive of telomeres (TTAGG), and, if not, at what stages of the evolution losses, gains or changes of this motif happened. We have developed and published detailed FISH protocols (Grozeva et al. 2015; Kuznetsova et al. 2015) specific to several model hemipteroid species, including the common bedbug *Cimex lectularius* Linnaeus, 1758 (Heteroptera, Cimicidae) and the representatives of the spittlebug genus Philaenus Stål, 1864 (Auchenorrhyncha, Aphrophoridae), which is taxonomically challenging due to outstanding color polymorphism of the species involved (e.g. Drosopoulos et al. 2010). Although these protocols were developed for C. lectularius and Philaenus spp., they have been successfully used since then for many other hemipteran insects (see e.g., Golub et al. 2018, 2019; Maryańska-Nadachowska et al. 2018).

The target chromosome preparations were prepared some time prior to hybridization to allow thorough drying and aging of the chromatin on the slide by incubation at 60 °C for at least a few hours. The 18S rDNA probe was amplified by PCR and labelled with biotin-11-dUTP (Fermentas, Vilnius, Lithuania) using genomic DNA of the true bug *Pyrrhocoris apterus* (Linnaeus, 1758): an initial denaturation period of 3 min at 94 °C was followed by 33 cycles of 30 s at 94 °C, annealing for 30 s at 50 °C and extension for 1.5 min at 72 °C, with a final extension step of 3 min at 72 °C. The telomere probe (TTAGG), was amplified by PCR and labeled with rhodamine-5-dUTP (GeneCraft, Köln, Germany): an initial denaturation period of 3 min at 94 °C was followed by 30 cycles of 45 s at 94 °C, annealing for 30 sec at 50 °C and extension for 50 sec at 72 °C, with a final extension step of 3 min at 72 °C. The chromosome preparations were treated with 100 μ g/ml RNase A and 5 mg/ml pepsin solution to remove excess RNA and proteins. Chromosomes were denatured in the hybridization mixture containing labeled 18S rDNA and (TTAGG), probes with an addition of salmon sperm DNA blocking reagent and then hybridized for 42 h at 37 °C. 18S rDNA probes were detected with NeutrAvidin-Fluorescein conjugate (Invitrogen, Carlsbad, CA, USA). The chromosomes were mounted in an antifade medium (ProLong Gold antifade reagent with DAPI, Invitrogen) and covered with a glass coverslip.

Terminology

Considering that the terminology used to describe different aspects of reproductive biology and ontogenesis is not very well known to entomologists, and in the same time the meaning of individual terms varies in the literature, below we provide an annotated list of the most important terms used in this field.

- **Arrhenotoky** parthenogenetic mode where females produce only males from unfertilized eggs). There are two forms: haplodiploidy (males are haploid due to direct development from haploid eggs) and diploid arrhenotoky (males develop from diploid eggs, similar to automictic thelytoky).
- **Contagious parthenogenesis** a process involving rare functional males produced by a parthenogenetic lineage, which mate with bisexually reproducing females resulting in fertile parthenogenetic offspring.
- **Cyclic parthenogenesis** the regular alternation of bisexual and parthenogenetic reproduction in the same species.
- **Deuterotoky** parthenogenetic mode where females and males are produced from unfertilized eggs.
- **Exuviatrium** sclerotized larval exuvium, which is used by the next larva-like instar (including neotenic female) as a shelter. *Exuviatrial* female has minute, rudimentary legs and lays eggs just inside exuviatrium. The term was introduced by Gavrilov-Zimin (2018).
- **Gynandromorphism** the phenomenon by which an individual is a sexual mosaic exhibiting characters of both sexes in various parts of the body; *bilateral gynan-dromorphs* are insects with male and female tissues distributed nearly bilaterally.
- Larva preadult instar of postembryonal development. Different instars are usually designated by numbers (I, II, III, IV, etc.) according to the number of molts which the animal underwent after the birth. Kluge (2010b) suggested using special Latin names for such instars: *primolarva*, *secundolarva*, *tertiolarva*, etc. In the situations when the total number of the instars is unknown he recommended naming instars, starting from the oldest one: *ultimolarva* (preadult instar), *penultimolarva*, etc.
- Neoteny bisexual reproduction of preimaginal instars. The term was originally introduced by Kollmann (1884) for salamanders, but now is widely used for different vertebrate and invertebrate animals.
- **Nymph** larval instar with wing buds (*protoptera*). These instars can also be named as *primo-*, *secundo-*, *tertio-*, *ultimo-*, *penultimo-*nymphs, etc.
- **Occasional eggs retention** occasional cases of starting the embryonic development inside mother's body due to unpredictable reasons, such as premature death of the mother, sudden change of environmental conditions, etc.
- **Oviparity** laying eggs before starting of embryogenesis; all embryonal development occurs outside the mother's body.
- **Ovoviviparity** laying eggs with fully or partly developed embryo inside; embryo starts to develop inside the mother's body; egg is covered with a chorion and

contains sufficient yolk to nourish the embryo until hatching without receiving aliment from the maternal organism.

- **Complete ovoviviparity** laying eggs with fully developed embryo inside; hatching of the primolarva occurs just after the oviposition.
- **Incomplete ovoviviparity** laying eggs with partly developed embryo inside; hatching of the primolarva occurs sometimes after oviposition.
- **Facultative ovoviviparity** individual and geographical variation at the stage of the embryonal development inside of laying egg, from cleavage divisions to complete embryogenesis.
- **Obligate ovoviviparity** invariable laying egg at a certain stage of embryonal development in all specimens of a taxon.
- Paedogenesis parthenogenetic reproduction of preimaginal instars. The term was introduced by Baer (1866) for larval parthenogenesis of some Cecidomyiidae (Diptera) discovered by Wagner (1862).
- **Paternal genome elimination** (PGE) a mode of reproduction where only the female genome is transmitted to offspring (sometimes also referred to as pseudo-arrhenotoky or parahaploidy). Paternal genome set is eliminated or inactivated in early embryogenesis (males are somatically haploid) or during spermatogenesis (males are somatically diploid; however, the paternal genome is eliminated, partly or to-tally inactivated by chromatin condensation, also referred to as paternal genome heterochromatinization).
- Ploidy restoration a process accompanying meiosis during automictic parthenogenetic development. There are three mechanisms known to date: *premeiotic doubling* of chromosomes with standard meiosis afterwards; *postmeiotic restoration* where haploid ootids fuse and produce a diploid nucleus (also known as 'central fusion'); and *meiotic restoration* fusion of secondary oocytes with second polar body following second meiotic division.
- **Protopteron** (plural **protoptera**) wing buds, flattened structures possessed by nymphs from which the wings will develop into imago. The term was introduced by Kluge (2005, 2010a, b).
- Pseudopuparium immovable apodal ultimolarva of whiteflies (Homoptera: Aleyrodinea); this pseudopuparium does not have protoptera (wing buds), but molts to a winged imago having well-developed legs and antennae. In contrast to the true puparium (Cyclorrhapha and Strepsiptera; Diptera), there is no pupa inside ultimolarval exuvium of whiteflies, and imaginal cuticle is forming just under larval cuticle.
- **Puparium** larval exuvium which covers a pupa, quiescent instar which molts to imago.
- **Thelytoky** parthenogenetic mode where females produce only females from unfertilized eggs. There are two forms described in animals – apomixis and automixis.
- **apomixis** a mode where a single mitotic-like division in unfertilized eggs results in genetic identity of the mother and her offspring (=ameiotic parthenogenesis).
- **automixis** a mode where egg cells are produced by meiosis, the diploid state of the offspring being restored by the fusion of meiotic products.

Viviparity – laying primolarvae, which are not covered by a chorion (Fig. 1d); whole embryogenesis occurs inside the mother's body with receiving nutriment via special maternal placenta-like structures or from other organs of embryonal and/or maternal origin. Of the different distinguishable variants of true viviparity (Hagan 1951), Paraneoptera were suggested to have the so-called (*pseudo*)*placental viviparity* in viviparous species. In this case, embryonic and/or maternal tissues form a placenta-like structure for embryo nourishment (see the second chapter of this monograph). It seems however that there are no fundamental differences between terms "placenta" and "pseudoplacenta"; these morphologically similar structures have arisen many times independently in different phylogenetic lines of invertebrates and viviparous vertebrates.

Higher classification and nomenclature

The system of higher taxa names, used in this monograph, follows hierarchical rank and typified (for superfamily and lower rank names) nomenclature. For taxa of rank above the suborder, circumscriptional names are used, based on their priority. For taxa of the suborder and infraorder ranks, circumscriptional names are used inside Copeognatha, Parasita, and Thysanoptera, whereas the typified names are used inside Arthroidignatha (=Hemiptera sensu stricto). The system and comments are adopted mainly from Gavrilov-Zimin and Danzig (2012) and Gavrilov-Zimin (2018); however, some conflicting approaches are also mentioned.

The widely known name Paraneoptera Martynov, 1923 was originally introduced with an uncertain inclusion of Zoraptera Silvestri, 1913, but subsequently Martynov (1938) explicitly placed this order in Paraneoptera.

The name Copeognatha Enderlein, 1903 is only one year older than the name Psocoptera Shipley, 1904, used for the same taxon. Another widely used name Corrodentia Burmeister, 1839 was proposed originally for a polyphyletic taxon including not only psocids, but also unrelated Polyneoptera and Neuroptera insects (Isoptera + Embioptera and Conyopterigidae, respectively).

The oldest name covered all lice is Parasita Latreille, 1796, which has priority over the frequently used names Anoplura Leach, 1815 and Phthiraptera Haeckel, 1896. Moreover, the last name was originally proposed for sucking lice only being, thus, a junior synonym of Siphunculata Latreille, 1802 (see for details Kluge 2020: 536, 545).

The name Hemiptera Linnaeus, 1758, frequently used in the literature as an order name for all "rhynchotous" insects, is nowadays a very ambiguous term since: 1) this name was used by C. Linnaeus for "rhynchotous" + thrips together; therefore it is an older synonym for Condylognatha Börner, 1904; 2) for many years up to now, this name has been used by numerous authors for true bugs (Heteroptera) only; 3) there are at least two separate orders (Heteroptera and Homoptera) within the "order Hemiptera" accepted by different authors. A similar taxonomic ambiguity concerns the well-known and widely used name Rhynchota Burmeister, 1835, which originally also included Siphunculata. Moreover, this name is preoccupied by Rhynchota Billberg, 1820 (=Aphaniptera Kirby et Spence, 1815) (Kluge 2010a). The oldest name for the taxon [aphids + scale insects + whiteflies + psyllids + cicadas + true bugs + moss bugs] is Arthroidignatha Spinola, 1850 (Kluge 2000, 2010a, b, 2020).

As for the widely known and frequently discussed order name Homoptera Latreille, 1810, there is no good reason to reject it. It originally covered all hemipteroid insects without true bugs but with thrips. However, all subsequent authors accepted this group without thrips, and Westwood (1838) seems to be the first who did it. Later, Pearce (1936) introduced the name Homopterida for the same group of taxa (i.e. without thrips). The concept of Homoptera sensu Westwood, 1838 as a paraphyletic group (for review, see for example, Dohlen and Moran 1995 or Gullan 1999), takes into account some facts and ignores others. According to cladistics, the problem comes down to considering synapomorphies of the Hemelytrata Fallén, 1829 (Cicadinea + Coleorrhyncha + Heteroptera) in contrast to synapomorphies of the Homoptera. Some authors (e.g., Gullan 1999) suppose that Homoptera are characterized by plesiomorphic characters only. Indeed, it is not easy to find reliable synapomorphies for all very diverse groups of Homoptera. However, such characters as the wing-coupling apparatus, the presence of the fields of wax glands and filter chamber of the digestive tract as well as the ability to produce honeydew can be considered as synapomorphies of Homoptera (Lambdin 2001; D'Urso 2002; Gavrilov-Zimin and Danzig 2012; Gavrilov-Zimin 2020). There is no reason to ignore these characters and consider only the probable morphological synapomorphies of Hemelytrata (see, e.g., Emeljanov 1987; Kluge 2020) or accept unconditionally untestable and controversial molecular cladograms based on a small number of occasionally selected species. Some of these cladograms (Campbell et al. 1995; Dohlen and Moran 1995; Johnson et al. 2018) support Homoptera as a paraphyletic group, whereas others (e.g. Song et al. 2012) - as a holophyletic one. A detailed historical revision of different phylogenetic reconstructions of "rhynchotous" insects was given by Brożek et al. (2003) and Forero (2008) and therefore will not be repeated here. In any case, regardless of whether further investigations will support or not the paraphyly of the Homoptera, there is no reason to reject this taxonomic name. Cladistic rejecting paraphyletic taxa is based not on scientific arguments but on voluntarism. There is no biological reason to suppose that species in paraphyletic taxa should be less related to each other than those in holophyletic taxa. This main conceptual contradiction between cladistic taxonomy (in its original W. Hennig's sense) and evolutionary taxonomy has been addressed in many publications (e.g., Simpson 1961; Mayr 1974; Mayr and Ashlock 1991; Gorochov 2001; Kerzhner and Danzig 2001; Hołyński 2005; Rasnitsyn 2010). Moreover, paraphyly of a taxon is closely connected with our subjective view of taxon boundaries. For example, if we include fossil ancestor groups of Arthroidignatha (in particular, Archescytinoidea) in Homoptera, the latter will evidently be paraphyletic; on the other hand, if we include Archescytinoidea in Hemelytrata (Cicadinea+Coleorrhyncha+Heteroptera), the latter will be paraphyletic. The factual paleontological data on the appearance of different Arthroidignatha groups are provided in the scheme of Shcherbakov and Popov (2002).

Concerning the frequently used name Sterno(r)rhynch(i)(a) (= Coccinea + Aphidinea + Aleyrodinea + Psillinea), we are not sure about the commonly discussed synapomorphies of this group. For example, according to the Shcherbakov and Popov' scheme (2002), Sternorhynchi are polyphyletic. Moreover, Sternorhynchi Amyot et Serville, 1843 is a junior synonym of Plantisuga Dumeril, 1805 (Kluge 2010a).

Summarizing all of the above, we recognize scale insects, aphids, psyllids, whiteflies and cicadas as suborders of the order Homoptera sensu Westwood, 1838, and use the ending "-nea" for all typified suborder names within Homoptera (Aphidinea, Coccinea, Aleyrodinea, Psyllinea, Cicadinea) following Pesson (1951). The International Code of Zoological Nomenclature does not regulate the taxonomic names above "family group" and we follow the principle introduced by Rohdendorf (1977) and consider the suborder names as the family-group ones.

Supercohors Paraneoptera Martynov, 1923

Ordo Zoraptera Silvestri, 1913

Cohors Acercaria Börner, 1904

Superordo Panpsocoptera Crampton, 1938

Ordo Copeognatha Enderlein, 1903 (= Psocoptera Shipley, 1904)

Ordo **Parasita** Latreille, 1796

Subcohors **Hemiptera** Linnaeus, 1758 (= Condylognatha Börner, 1904, non Hemiptera auct.)

Ordo Thysanoptera Haliday, 1836

Superordo **Arthroidignatha** Spinola, 1850 (= Hemiptera auct., non Linnaeus, 1758;

= Rhynchota auct., non Burmeister, 1835)

Ordo Coleorrhyncha Meyers et China, 1929

Ordo Heteroptera Latreille, 1810 (= Hemiptera auct., non Linnaeus, 1758)

Ordo **Homoptera** sensu Westwood, 1838, non Latreille, 1810 (= Homopterida Pearce, 1936)

Subordo Cicadinea Batsch, 1789

Subordo Psyllinea Latreille, 1807

Subordo Aleyrodinea Newman, 1834

Subordo Aphidinea Latreille, 1802

Superfamilia **Phylloxeroidea** Herrich-Schaeffer, 1854 Superfamilia **Aphidoidea** Latreille, 1802

Subordo **Coccinea Fallén, 1814** (= Coccoidea auct., Gallinsecta De Geer, 1776)

Superfamilia **Orthezioidea Amyot et Serville, 1843** (=Paleococcoidea Borchsenius, 1950; = Archeococcidea Bodenheimer, 1952)

Superfamilia **Coccoidea Fallén, 1814** (=Neococcoidea Borchsenius, 1950; = Neococcidea Bodenheimer, 1952)

Author contributions

I.G.-Z. and V.K. contributed equally to the paper; they conceived and designed the project and prepared the manuscript draft. I.G.-Z. also wrote the section "Higher classification and nomenclature". S.G. edited and commented on the draft manuscript and also prepared some paragraphs in the section Material & Methods. D.G. and A.K. collected and identified part of the material, provided some illustrations and made several additions to the text. K.T. collected and identified part of the material.

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- Fig. 1a "Budak", https://flickr.com/photos/budak/50355864921//
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- Fig. 1c "Riszard", https://flickr.com/photos/ricosz/16894219932/

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• Fig. 2a, e "Sterling Sheehy", https://www.inaturalist.org/guide_taxa/1040701

• Fig. 2k "CBG Photography Group, Centre for Biodiversity Genomics", https://v3.boldsystems.org/index.php/Taxbrowser_Taxonpage?taxid=669605.

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REVIEW ARTICLE



Egg retention, viviparity and ovoviviparity in Paraneoptera

Ilya A. Gavrilov-Zimin¹

Zoological Institute, Russian Academy of Sciences, Universitetskaya nab. 1, St. Petersburg, 199034, Russia

Corresponding author: Ilya A. Gavrilov-Zimin (coccids@gmail.com)

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Abstract

This article is a second part of the themed issue "Aberrant cytogenetic and reproductive patterns in the evolution of Paraneoptera insects", prepared by the Russian-Bulgarian research team. Here, analysis of aberrations related to the egg development is provided based on literature data and the author's own investigations. Evolutionary aspects of ovoviviparity/viviparity are also briefly discussed. Material and methods, terminology and nomenclature of taxonomic names are listed in the first paper of the issue (Gavrilov-Zimin et al. 2021).

Keywords

Embryogenesis, oviposition, neoteny, paedogenesis

In most Paraneoptera insects, embryogenesis starts only after the egg is laid outside the mother's body. Cleavage is usually meroblastic being evidenced by the presence of a large amount of yolk in the egg. The zygotic nucleus undergoes divisions and gives origin to blastomeres and vitellophages (Fig. 1). Blastomeres migrate to the egg surface and form blastoderm. The vitellophages are few in number and dispersed between yolk drops. The blastoderm differentiates into serosa and germ band. The invaginations of the germ band into the yolk (anatrepsis) together with the intensive divisions of its cells lead to the emergence of the embryo and amnion. At maximal invagination, the germ band has a characteristic S-shaped form. When the invagination is finished, the inner germ band cells give rise to mesoderm and preliminary organogenesis starts. At



Figure 1. Main stages of typical Paraneoptera meroblastic embryogenesis (on the example of scale insects).

the same time, buds of appendages appear and the embryo starts to turn backwards in comparison to its initial position (katatrepsis). The serosa degenerates whereas the amnion gives rise to the yolk epithelium. Finally, all yolk is consumed and the embryo achieves the size and form of the primolarva (Fig. 1). This general scheme of the embryonal development may have various modifications in different groups (see for reviews: Hagan 1951; Buchner 1965; Zakhvatkin 1975; Haga 1985).

The modes of egg retention are diverse and originated many times in different phylogenetic lineages of Paraneoptera (Fig. 9). So, different variants of ovoviviparity and/or placental viviparity are observed even in the most archaic Paraneoptera, in some species of Copeognatha from the families Archipsocidae, Trogiidae, and Pseudocaeciliidae (Fernando 1934; Jentsch 1936; Mockford 1957; Wong and Thornton 1968). In the most studied viviparous psocid species, *Archipsocopsis fernandi* (Pearman, 1934), the whole embryonal development occurs inside the mother's ovarium; the egg lacks a chorion and "yolk-cells", but demonstrates a meroblastic cleavage (Fig. 2b); the serosa fuses with the wall of the ovarian tubule and forms a placenta-like organ for temporary nutrition of the embryo (Fig. 2b). It appears that all 18 species of the genus *Archipsocopsis* Badonell, 1948 are viviparous and lack gonapophyses, in contrast to the closely related and normally oviparous genus *Archipsocus* Hagen, 1882, whose females have gonapophyses (New 1987: 7). At least, some Parasita, for example, Mallophaga lice of the genus *Meinertzhageniella* Eichler, 1940, as well as Siphunculata lice *Polyplax serrata* (Burmeister, 1839) and *Hoplopleura* sp. show ovoviviparous reproduction (Eichler 1946; Golub and Nokkala 2004), but the general picture of the ovoviviparity/viviparity in Copeognatha and Parasita is presently unclear because of the poorly studied reproductive patterns in most species of these groups.

In Thysanoptera, different (ovo)viviparous species were reported in the suborder Tubulifera, family Phlaeothripidae (e.g. Bagnell 1921; John 1923; Hood 1934: 71; Hathaway 1938; Bournier 1966), but in most of these reports, the authors did not provide a clear difference between viviparity and ovoviviparity. Some species of Tubulifera convincingly show facultative and incomplete ovoviviparity by laying eggs at different stages of embryogenesis (Viswanathan and Ananthakrishnan 1973; Ananthakrisnan and Dhileepan 1984; Dhileepan and Ananthakrisnan 1987; Nagrale 2012), which is similar to the same modes of oviposition in scale insects (see below).

It appears that no viviparous or ovoviviparous species have been found up to now in three suborders of Homoptera: Cicadinea, Psyllinea, and Aleyrodinea (Fig. 3), which combine together more than 50,000 recent species. Meanwhile, the phylogenetic lineage Aphidococca (suborders Aphidinea+Coccinea) shows numerous species, genera and families, which exhibit the embryonic development inside the mother's body (Fig. 9).

Among Aphidinea, only Adelgidae and Phylloxeridae (comprising together about 140 species in the world fauna) are obligately oviparous and even keep the ovipositor in adult females, whereas all other families (with about 5000 species) demonstrate obligate viviparity (rarely ovoviviparity) in parthenogenetic generations retaining oviparity in the bisexual generation only (Hille Ris Lambers 1950; Blackman 1987; Favret et al. 2016). It is interesting to note that all examples of aphid ovoviviparity were found by Hille Ris Lambers (1950) in the family Eriosomatidae (=Pemphigidae), i.e. in the most "primitive" group of "true aphids". The uniqueness of the aphid viviparity lies in the very precocious start of the embryogenesis in the parthenogenetic egg, before the birth of the mother itself. Such eggs are very small, lacking yolk and chorion; the entire embryogenesis of the follicular epithelium (Fig. 4) (Uichanco 1924; Hagan 1951; Blackman 1987).

The most diverse examples of egg retention and (ovo)viviparity are known in scale insects (see for review: Gavrilov-Zimin 2018). The most ancient and "primitive" scale



Figure 2. Embryonal development of *Archipsocopsis fernandi* (Copeognatha) (after Fernando 1934) **a** formation of endoderm (sagittal section) **b** fusion of serosa with the ovarian tubule to form a nutrient placenta-like organ (sagittal section).



Figure 3. Approximate numbers of oviparous and ovoviviparous/viviparous species in different groups of Paraneoptera.



Figure 4. Developing embryo in vitellarium of a viviparous parthenogenetic aphid female of *Uroleucon tanaceti* (Linnaeus, 1758) (after Uichanco 1924, with modifications of Hagan 1951).

insects from the tribes Matsucoccini and Steingeliini (family Margarodidae), are characterized by facultative ovoviviparity when the embryogenesis starts inside the mother's body and, at least, some eggs undergo complete embryogenesis before oviposition, whereas other eggs of the same female are laid at early stages of the development. In such cases, the external incubation period (after the moment of oviposition) varies significantly from several days to one month (e.g., Bodenheimer and Harpaz 1955; Mc-Kenzie 1943). Obviously, this reproductive mode has originated in scale insect females as a result of neoteny and loss of the imaginal structures of the reproductive system (for example, ovipositor); i.e. facultative ovoviviparity can be considered as an apomorphy of Coccinea (Fig. 5) (Gavrilov-Zimin 2018). Probably, facultative incomplete ovoviviparity is also present in other archaeococcids of the tribe Xylococcini and, at least, in some Cryptokermesini (Margarodidae) (Vayssière and Hughes-Schrader 1948; Gavrilov-Zimin 2018). The small, morphologically aberrant archaeococcid families Xenococcidae, Phenacoleachiidae, and Carayonemidae are probably characterized by obligate ovoviviparity (Silvestri 1924, 1926; Gullan and Cook 2001; Kozár and Foldi 2001). On the other hand, at least some species of Kuwaniini, Coelostomidiini, and Margarodinae s.s. show normal oviparity with the beginning of the cleavage division after oviposition. The most diverse archaeococcid group, the Monophlebinae, is unfortunately very poorly studied in terms of embryology and reproductive biology, except



Figure 5. Different modes of reproduction in scale insects (Coccinea).

only the tribe Iceryini (Hughes-Schrader 1948; Gavrilov-Zimin 2018). On the one hand, six Monophlebinae genera are characterized by the presence of a marsupium (Fig. 6) and, at least, in *Steatococcus samaraius* Morrison, 1927 eggs are laid in the marsupium just prior to katatrepsis suggesting that incomplete ovoviviparity occurs. Moreover, females of *Steatococcus hystrix* Gavrilov-Zimin et Stekolshikov, 2018 (from Mali, Africa) contain the embryos with visible appendages even before the marsupial pouch is formed. On the other hand, at least some species of *Crypticerya* Cockerell, 1895 and *Icerya* Signoret, 1876 exhibit obligate complete ovoviviparity and lay fully developed embryos beneath the body (Gavrilov-Zimin 2018). Eggs of Ortheziidae (at least in such common species as *Orthezia urticae* Linnaeus, 1758, *Newsteadia floccosa* (De Geer, 1778), and *Insignorthezia insignis* (Browne, 1887)), are full of different inclusions and it is rather difficult to understand at which moment the cleavage starts, although it most likely happens after the oviposition (Gavrilov-Zimin 2018).

The origin of the neococcid (superfamily Coccoidea) phylogenetic line was probably correlated with obligate complete ovoviviviparity (Gavrilov-Zimin and Danzig 2012; Danzig and Gavrilov-Zimin 2014; Gavrilov-Zimin 2018). This character was probably inherited by mealybugs (Pseudococcidae), the most primitive group of neococcids, from the obligate ovoviviparous ancestral family Phenacoleachiidae. It



Figure 6. Schematic illustration of marsupium in *Etropera* spp. (Coccinea: Margarodidae) in vertical and horizontal projections.

is known in numerous archaic genera of mealybugs including *Puto* Signoret, 1876, *Rastrococcus* Ferris, 1954, *Heliococcus* Šulc, 1912, *Fonscolombia* Lichtenstein, 1877, *Phenacoccus* Cockerell, 1893, *Paraputo* Laing, 1929, *Formicococcus* Takahashi, 1928 and many more divergent genera, for example all legless mealybugs (generic group *Antonina* Signoret, 1872), the species-rich genus *Mirococcopsis* Borchsenius, 1948, and numerous other small and monotypic genera. To date, more than 500 obligate ovoviviparous species of mealybugs from more than 60 genera have been reported, which is about 25% of the mealybug diversity in the global fauna. Moreover, there is no doubt that the real number of ovoviviparous mealybugs will increase due to further studies. A lot of species with complete ovoviviparity are known in other neococcid families: Eriococcidae, Micrococcidae, Coccidae, Aclerdidae, Dactylopiidae, Keriidae, Stictococcidae, Sterolecaniidae s.l., Beesoniidae, and Diaspididae (see: Gavrilov-Zimin 2018 for more detailed review). Viviparity in scale insects has been discovered till now only in three neococcid genera, including *Apiomorpha* Rübsaamen, 1894 (family Eriococcidae), *Stictococcus* Cockerell, 1903, and *Parastictococcus* Richard, 1971 (both from the

family Stictococcidae). Eggs of the studied species from these genera are very small and yolk-poor; the developing embryo receives nutrition from the mother's body through placenta-like structures (Buchner 1957, 1963, 1965) and does not have a chorion, which is always present in ovoviviparous species.

In general, the evolution of scale insects seems to show multiple cyclic conversions from oviparous reproduction to ovoviviparous/viviparous reproduction with the emergence of new peculiar adaptations for eggs' protection (Fig. 7). Thus, in archaeococcids, the initial facultative ovoviviparity with the formation of loose ovisac ("primitive" genera of Xylococcinae s.l., most of Callipappinnae s.l.) evolves into normal oviparity in their probable descendants (Margarodinae s.s., some Monophlebinae and Ortheziidae) showing different new adaptations, such as laying eggs in a special cavity under the body or in a solid wax sac behind the body. In turn, some divergent Monophlebinae and their descendants (Phenacoleachiidae, Carayonemidae, and "primitive" neococcids) demonstrate again incomplete or complete ovoviviparity putting partly developed embryos inside the marsupium or laying fully developed embryos outside the body. Among neococcids, complete ovoviviparity of "primitive" mealybugs like Puto, Rastrococcus, Paraputo, Heliococcus, etc. (see above) evolves into incomplete oviparity (or almost normal oviparity) of some divergent mealybugs (like Pseudococcus Westwood, 1840, Atrococcus Goux, 1941 and others). "Primitive" soft scales (like Pulvinariini and Eriopeltinae) form a loose ovisac as in their faraway ancestors from Monophlebinae and Xylococcinae, but in contrast to the last, they use for the ovisac construction not multilocular pores, but tubular ducts of different structure. In turn, many divergent Coccidae and Kermesidae again lay partly developed eggs in a cavity under the body, that sometimes (in Kermes Boitard, 1828) looks like the marsupium of giant scales. The most aberrant and divergent families Asterolecaniidae s.l., Diaspididae and Phoenicococcidae s.l. again restore obligate complete ovoviviparity in many genera (Gavrilov-Zimin 2018).

In the large order Heteroptera, examples of viviparity or ovoviviparity have been reported for the families Polyctenidae, Cimicidae, Anthocoridae, Plokiophilidae, Microphysidae, and also for some species of Aradidae and Lygaeidae (e.g. Hagan 1931, 1951; Carayon 1956, 1961). The most studied viviparous species of Heteroptera is the polyctenid Hesperoctenes fumarius (Westwood, 1874), an ectoparasite of bats. The oviduct of this bug does not have a spermatheca or any similar organ; during copulation the sperm pass directly into the lower part of the common oviduct, then migrate to the paired oviducts and pass through the walls of oviducts in the haemocoel. The ovulation, fertilization and at least part of the embryonal development of the egg occur in immature insects following, thus, a paedogenetic mode (Hagan 1931: 38, 1951: 396). The egg lacks chorion and yolk receiving the nutrition for embryonal development from the follicular epithelium of the mother's body. At the stage of katatrepsis, the embryo forms peculiar structures, pleuropodial extensions, which grow and surround the embryo by a pleuropodial sheath (Fig. 8). This sheath, probably, plays a role of the placenta in the nutrition of the embryo (Hagan 1951: 400). A similar embryonal organ was also found in the viviparous Physopleurella pessoni Carayon, 1956 (Anthocoridae) (Carayon 1956: 109). In many other Anthocoridae and also in Cimicidae, eggs have a chorion and only a part of the embryogenesis occurs inside the mother's ovary



Figure 7. Cyclic evolutional conversions of reproduction pattern in Coccinea from oviparous to (ovo) viviparous variants with the emergence of new modes of eggs protection.



Figure 8. Pleuropodial extension (left figure) and pleuropodial sheath (right figure) in the embryo of viviparous *Hesperoctenes fumarius* (Heteroptera) (after Hagan 1931, 1951).

(Carayon 1961, 1966: 179) and so, incomplete ovoviviparity takes place. On the other hand, the absolute majority of Heteroptera demonstrate usual oviposition of the eggs prior to embryogenesis.

The very irregular distribution of (ovo)viviparous taxa among Paraneoptera (Fig. 9) (and among animals as a whole) denotes multiple and separate origins of this mode of reproduction in different phylogenetic lines. This is confirmed by all comparative stud-



Figure 9. The phylogenetic tree of Paraneoptera based on Shcherbakov and Popov (2002), Kluge (2020), Gavrilov-Zimin (2020) with modifications. Higher rank taxa, including viviparous and ovoviviparous genera/families, are highlighted in green. Bold lines are used for paraphyletic taxa.

ies of the problem (see, for example, the last reviews of Batygina et al. 2006; Ostrovsky et al. 2016). On the other hand, until recent times there was no clear understanding of the reasons for the emergence of ovovivipity/viviparity within originally oviparous taxa. During centuries, ecological reasons of viviparity origin have been considered as most likely (see for review: Hagan 1951). Some authors tried to associate the emergence of viviparity with the life in dry climate, others – with the life in wet climate, cold or hot environment, quality of food, passive or active mode of animal life, etc. Another common approach to the problem is the hypothesis of significant evolutionary advantages of viviparity, since developing embryos are protected by the mother's body (see, for example, Hagan 1951; Meier et al. 1999; Ostrovsky et al. 2016 and large lists of references in these reviews). On the other hand, it seems that, in spite of the "adaptive advantage" hypothesis, both viviparous and ovoviviparous taxa are very

few in nature in comparison with oviparous ones. Even among vertebrate animals, this mode of reproduction characterizes only mammals, small number of reptiles and fishes, whereas most vertebrates are oviparous. As for invertebrates, the (ovo)viviparous species comprise at best only several percent of the total number of species, being known as occasional occurrences in many large phyla. Moreover, viviparous taxa (these are usually occasional genera, rarely the whole families and very rarely higher rank taxa) are characterized by depressed taxonomic and morpho-anatomical diversity. Paraneoptera insects illustrate this situation especially clearly. This huge group of insects comprises about 115,000 species in the world fauna with only 5-6% of them being ovoviviparous or viviparous (Fig. 3), and most (> 5000) of these ovoviviparous/ viviparous species of Paraneoptera are known in Aphidinea, i.e. in the group, which is characterized by very low morpho-anatomical diversity and includes species, genera and even families identified by metrical characters only. In the largest animal group, Coleoptera, comprising about 400,000 species, only occasional species from several families were found to be ovoviviparous or viviparous (Iwan 2000; B. Zilberman, personal communication). In the large insect order Diptera, comprising about 125,000 species, 61 events of independent origin of different variants of facultative/obligate ovoviviparity and viviparity were reported, including occasional species from different families as well as several small families with all species being viviparous (Meier et al. 1999). Gavrilov-Zimin (2018) hypothesized that evolutionary transformation of oviparity to ovoviviparity and, further, to true viviparity was an alternative way of phylogenesis, that occurs when usual oviposition comes into conflict with different morphological or physiological apomorphies of the ancestral species and its descendants. According to this hypothesis, most common reasons for the obligate egg retention are different variants of paedogenesis and neoteny, when the reproducing larva or nymph has lost special adult structures responsible for oviposition, with quick passage of the egg through the oviducts and fertilization of the egg in ectodermal parts of the oviduct (where a spermatheca is located). This presumption is rather clearly illustrated by Paraneoptera insects. Phylogenetic lineage Aphidococca is fully paedogenetic/neotenic; many viviparous true bugs and psocids have clear features of larvalization; all viviparous trips are known in the suborder Tubulifera, which is characterized by the loss of ovipositor, etc. A similar situation also occurs in the cases of different morphological or physiological transformations, which are related not with paedogenesis, but with changes in the imaginal reproductive system. Thus, as shown earlier by some authors (for example, Carayon 1961), parthenogenesis and change of fertilization location, from ectodermal to mesodermal parts of genitalia (up to the point of fertilization in vitellarium), are important preconditions to the origin of ovoviviparity/viviparity. The egg can start developing only after fertilization or when fertilization is not needed. If the egg is fertilized inside the vitellarium, it has enough time for the embryo to complete development before oviposition. For example, in viviparous/ovoviviparous true bugs (Heteroptera, Cimicoidea) of the families Anthocoridae, Cimicidae, Polyctenidae, and Plokiophilidae, the unique traumatic insemination and fertilization takes place; the male punctures the female body with its copulatory organ and injects sperm

outside the female reproductive system. The insemination in this case is correlated with the structures of the so-called "paragenital system" consisting of "spermalege" ("organ of Ribago" or "organ of Berlese"), "seminal conceptacles", "spermodes" and "syncitial bodies", which is used for transporting and temporarily preserving the spermatozoa before their arriving into ovarioles (Carayon 1966).

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REVIEW ARTICLE



Aberrant ontogeneses and life cycles in Paraneoptera

Ilya A. Gavrilov-Zimin¹

S.I. Vavilov Institute for the History of Science and Technology of the Russian Academy of Sciences, Universitetskaya nab. 5, St. Petersburg, 199034, Russia

Corresponding author: Ilya A. Gavrilov-Zimin (coccids@gmail.com)

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Abstract

The paper is a third part of the themed issue "Aberrant cytogenetic and reproductive patterns in the evolution of Paraneoptera", prepared by a Russian-Bulgarian research team on the basis of long-term collaborative studies. This chapter reviews different peculiar aberrations in the ontogenesis of Paraneoptera, such as the appearance of the quiescent apodal and/or arostrate instars, exuviatrial, pupillarial and pseudopupillarial development, cyclic parthenogenesis, etc. The material and methods, terminology and the nomenclature of the used taxonomic names are listed in the first chapter of the issue (Gavrilov-Zimin et al. 2021).

Keywords

Exuviatrium, imago, larvae, metamorphosis, nymphs, protoptera, pseudopuparium

The postembryonic ontogenesis of most Paraneoptera exhibits simple direct development from primolarva to imago and includes 5–6 immature instars in both sexes (see, for example, Poisson and Pesson 1951; Zakhvatkin 1975; Štys and Davidova-Vilimova 1989; Gavrilov-Zimin 2018; Kluge 2020) with the presence of protoptera (wing buds) in instars 3–5(6), which are named "nymphs" in contrast to first larval instars lacking protoptera (Fig. 1). All postembryonic instars of such ontogenesis are actively mobile and feeding. This type of development is undoubtedly a plesiomorphic, archaic condition, inherited by Copeognatha (Psocoptera) (Fig. 2) from the common ancestor of all Paraneoptera and shared with the most other "hemimetabolous" insects.



Figure 1. Ontogenesis in different groups of Paraneoptera.



Figure 2. Larval instars of *Ectopsocus meridionalis* Ribaga, 1904 (Copeognatha) (from Weber 1936). Larva V has a similar habitus with larva IV and is not figured.

The normal number of larval instars in Copeognatha is six, but in some rare cases this number can decrease to five, four or even three, this being associated with neoteny and alary polymorphism in the corresponding species (New and Lienhard 2007: 20–21, 113). One further interesting aberration of Copeognatha development is known in the European species *Prionoglaris stygia* Enderlein, 1909 (Prionoglarididae), which demonstrates a change of the initial type of the buccal apparatus to another type in course of the preimaginal ontogenesis (Ball 1936).

The small group Parasita (Mallophaga+Siphunculata+Rhyncophthirina), which originated from Copeognatha, is characterized by simplified ontogenesis with only 3 immature instars and a total lack of the protoptera and wings (Séguy 1951; Kluge 2020).

Psyllinea, Cicadinea, Heteroptera, and Coleorrhyncha generally retain the archaic "hemimetabolous" mode of the development and life cycle (Figs 1, 3), which may be monovoltine or polyvoltine, depending on species and climatic conditions,



Figure 3. Ontogenesis of *Blissus leucopterus* (Say, 1832) (Heteroptera) (from Packard and Benton 1937) A eggs **B-F** larval instars **G** imago.

as in many other insects. Minute aberrations are connected with an unusual prolongaltion of the larval stage of the development (as in the family Cicadidae) or with a rear reduction of the number of the larval instars (as in Coleorrhyncha and in some species of Heteroptera). Thus, the periodical cicadas of the genus Magicicada Davis, 1925 show 13- or 17-year life cycles in different species with the duration of the imaginal instar 4-6 weeks only (Williams and Simon 1995). The decrease of the number of larval instars (from usual five to four) is known in Cicadinea for some brachypterous species of the tribe Almanini, family Dictyopharidae (Emeljanov 1980). The reduction of the number of the larval instars to four was noted in sporadic species of Heteroptera from the families Veliidae, Mesoveliidae, Nepidae, Nabidae, Anthocoridae, Cimicidae, Microphysidae, Miridae, Tingidae, Reduviidae, Tessaratomidae, while the vast majority of true bugs have five larval instars (see for review: Štys and Davidova-Vilimova 1989). The parasitic true bug family Polyctenidae, which is characterized by viviparity and paedogenesis, shows only three larval instars (Hagan 1951: 396; Maa 1959; Štys and Davidova-Vilimova 1989). All these instars have protoptera, which probably testifies the loss of two first larval instars in such ontogenesis (Štys and Davidova-Vilimova 1989). On the other hand, 6 larval instars are known only in several species of Miridae and Piesmatidae true bugs, which demonstrate variation in number of immature instars from 4 to 6 (Štys and Davidova-Vilimova 1989).

Species of the small relict order Coleorrhyncha have only 4 larval instars (China 1962; Evans 1981).

Comparatively small groups of Paraneoptera, such as thrips (Thysanoptera), lice (Parasita), whiteflies (Aleyrodinea), scale insects (Coccinea) and aphids (Aphidinea) show various curious aberrations in the postembryonic development (Fig. 1). In contrast to other Paraneoptera, thrips (Thysanoptera), whiteflies (Aleyrodinea) and scale insects (Coccinea) have ontogenesis with one or several immobile instars. Thus, ontogenesis of thrips (Thysanoptera) shows various patterns in different families, but their most primitive ontogenesis includes two first mobile larval instars in both sexes, two quiescent starving nymphs with partly reduced mouthparts and a mobile imago with normally developed legs, antennae, wings and mouthparts (Figs 1, 4) (Pesson 1951; Kluge 2020).

In whiteflies, the larva in all known species loses mobility after the first molt, and next three larval instars have only vestigial legs and are absolutely immobile (Fig. 1); moreover, all immature stages do not have protoptera; the ultimolarva (pseudopuparium) additionally is able to survive a long period of starvation. The pseudopuparium molts into the imago of both sexes which have well developed legs, antennae and wings. This ontogenesis is in fact similar to the metamorphosis of the holometabolous insects and is the most aberrant not only amongst Paraneoptera, but of Insecta as a whole.

In scale insects (Coccinea) two preadult instars of male are quiescent (arostrate and with non-segmented appendages). Such instars are in fact analogous to pupal instars of Holometabola (Gabritschesky 1923; Zakhvatkin 1975; Gavrilov-Zimin 2018). When such instars have protopter they can be named as quiescent nymphs. Adult males of



Figure 4. Ontogenesis of *Taeniothrips inconsequens* (Uzel, 1895) (Thysanoptera) (from Cameron and Trenerne 1918).

all scale insects are arostrate, but usually have normally developed legs and wings. In the female life cycle of all scale insects the normal imaginal stage is absent and larva of third of forth stage (neotenic female) is able to copulate with adult male and reproduce progeny. In some archaeococcids of the family Margarodidae s.l. (subfamilies Margarodinae s.s., Xylococcinae, Callipappinae) the second and third (if present) female instars are apodous, but actively suck sap from its host plant, whereas the neotenous female is mobile, has legs, but is arostrate (Fig. 5). On the other hand, most other archaeococcids



Figure 5. The archaic cycle of Matsucoccini (Coccinea) pattern. L - larva; N - nymph.

(Margarodidae: Monophlebinae, Ortheziidae, Phenacoleachiidae, Carayonemidae) and many neococcids (superfamily Coccoidea) have simple direct ontogenesis of females, with all stages mobile (Fig. 6). Such neococcids as Aclerdidae, Asterolecaniidae s.l., Keriidae, Beesoniidae, Phoenicococcidae, Diaspididae, along with some species and genera of Pseudococcidae, Eriococcidae and Coccidae lose their legs during the first or last molt of females without alternation of movable/immovable instars (Fig. 7).

Borchsenius (1956) presumed that the original ontogenesis of Coccinea was similar to that of whiteflies (Aleyrodinea), i.e. apodal stages were present in both female



Figure 6. Life cycle of Monophlebinae (Coccinea) pattern.

and male ontogenesis. However, this presumption was not supported by any detail argumentation or comparative analysis of the life cycles of different scale insects and other Paraneoptera. The opposite hypothesis was provided and comprehensively argued by Danzig (1980). She supposed that the ancestor of all scale insects had a simple direct ontogenesis similar to that of Psyllinea, Cicadinea and Heteroptera. Then in course of the evolution of Coccinea the ontogenesis became more complete in males only, whereas females retained the direct cycle, but lost the winged imago (neoteny). In the frame of such approach the alteration of mobile/immobile stages and



Figure 7. Life cycle of apodal neococcids (Coccoidea) pattern.

the aphagia of adult females in some Margarodidae s.l. was considered as a collateral evolutionary occurrence. Recently, two modern investigations provided important new data for understanding the evolution of scale insect ontogenesis and as a result Borchsenius' (1956) idea starts to seem more reliable. Firstly, Kluge (2010) studied several species of scale insects from different families (*Orthezia urticae* Linnaeus, 1758, *Icerya* sp. and *Coccus hesperidum* Linnaeus, 1758) and discovered paradoxical transformation of legs and antennae in the course of molts of these species from one larval instar to another (see below). Secondly, Gavrilov-Zimin (2018) comprehensively analyzed the data on life cycles of all studied archeococcids in combination with



Figure 8. Simplified scheme of paradoxical and usual molts in scale insects. 1, 2, 3, 4 – molts from one instar to another.

comparative morphological analysis of all families, subfamilies and tribes of Orthezioidea. Both studies evidenced that the complicated ontogenesis with the alternation of mobile/immobile stages and with the arostrate imago of both sexes was initial in scale insect evolution and such ontogenesis may be considered as an apomorphy of suborder Coccinea.

Three scale insect species (from the families Ortheziidae, Margarodidae and Coccidae), studied by Kluge (2010), do not have any apodous stages in the female life cycle, but are characterized with a unique transformation of legs and antennae in course of the molt of one larval instar to another. Most of the internal soft tissues of every appendage, including the majority of muscles, degenerate before the molt and then emerge anew (Fig. 8). Moreover, the proximal segment of each appendage (coxa and scapus) newly grows in an unusual inverted position and everts only during ecdysis. As a result, the larva cannot move during the molt. This phenomenon occurs during all



Figure 9. The evolution of ontogenesis in different groups of scale insects.

molts in female life cycle and during two first molts in male life cycle, whereas subsequent male molts (nymph I to nymph II and nymph II to adult male) are implemented without degeneration as in most other insects.

As it was noted above, the ontogenesis with arostrate imago and apodal larvae of both sexes is considered now as a most primitive in scale insect evolution (Gavrilov-Zimin 2018). The appearance of such ontogenesis as well as all other variants of complicated metamorphosis, including holometabolism, was probably connected with the development of the larval instars in narrow shelters under the high pressure of unspecialized predators which decreases the number of openly lived insects in the late Paleozoic and Mesozoic biotopes (Rasnitsyn 1980). An ancestral group for both aphids and scale insect, as well as for all other Homoptera, was extinct Archescytinoidea (Popov 1980; Shcherbakov and Popov 2002). The Archescytinoidea lived in Permian geologic period (late Paleozoic Era) and were trophically connected with Gymnospermae trees. Females of Archescytinoidea laid eggs in unripe strobili of Gymnosperms and larvae dwelt there until ripe strobilus would dehisce (Popov 1980; Shcherbakov and Popov 2002). Such mode of life exactly permitted to protect immature stages from the predators. The sedentary life in strobili and then in cracks of tree bark was probably led to more and more significant difference between the larval instars and imago. As a result larvae of scale insect reduced and lost legs, and such apodous instars started to occupy most of the time of the life cycle. Additionally, in the condition of immobility the apodal body was probably more protected from entomopathogenic fungi, being evenly covered with wax. In contrast, unprotected imago started to be a short-lived instar with reduced mouthparts. Significant morphological contrast between the immobile apodous larva and highly mobile imago led to the appearance of the quiescent nymphal stages and so to the complicated metamorphosis (Fig. 9) - see for more details Gavrilov-Zimin (2018: 54-56).

In some groups of scale insects, the female remains to live and reproduce inside the cuticle of the ultimolarva (Fig. 10). Such remarkable ontogenesis is known in some archeococcids of the subfamilies Callipappinae and Margarodinae (Morrison 1928; Vayssiére and Hughes-Schrader 1948; Morales 1991; Foldi 2005; Foldi and Gullan 2014; Gavrilov-Zimin 2018) and in some neococcids: in several genera of Phoenicococcidae s.l. (Stickney 1934), in about 60 genera of Diaspididae (Howell and Tippins 1990; Danzig 1993), in occasional species of Beesoniidae (Takagi 1992) and Eriococcidae (Gullan and Williams 2016). Thus, in the following genera of the tribe Cryptokermesini (Callipappinae): Cryptokermes Hempel, 1900, Paracoelostoma Morrison, 1927 and Ultracoelostoma Cockerell, 1902 the secundolarvae of both sexes secrete a resinous protective test that enlarges during subsequent development of the insect. The tertiolarva and neotenic female remain inside this test and moreover, inside the exuviae of the previous instar. Such instars are often considered as "pupillarial" (see, for example, Danzig 1993; Foldi and Gullan 2014; Gullan and Williams 2016) but this is incorrect, because the true puparium is the cover of the pupa, whereas scale insect females never have pupal instars in their ontogenesis. Gavrilov-Zimin (2018: 20, 59) introduced the new term "exuviatrium" for the larval exuvium which is used by the next larva-like instar (including neotenic female) as a shelter. Correspondingly, the species with such a peculiarity may be named "exuviatrial". In the genus *Mimosicerya* Cockerell, 1902 (also Cryptokermesini) female instars do not secrete any protective test, but the adult female is also exuviatrial, because it lives and lays eggs inside the



Figure 10. Exuviatrial and pupillarial instars in ontogenesis of different scale insects.

strongly sclerotized ultimolarval exuvium. It seems rather obvious that such a mode of ontogenesis originated several times in the evolution of scale insects. Ontogenesis of Cryptokermesini probably originated from the archaic ontogenesis of Coelostomidiini ancestors, which is proved by the absence of mouthparts in the neotenic female and/or by the presence immobile stages of the ontogenesis. On the other hand, the ontogenesis of different exuviatrial neococcids (some of Eriococcidae, Phoenicococcidae s.l., Diaspididae and Beesoniidae) clearly originated from advanced pattern of ontogenesis of Monophlebinae-Pseudococcidae, since in all mentioned families the adult females are sap sucking (Gavrilov-Zimin 2018).

The true pupillarial development is now known in some scale insects of the family Phoenicococcidae s.l. only, in which quiescent male instars molt inside the exuvium of the secundolarva (Stickney 1934) (Fig. 10). On the other hand, the other species of this family, for example, *Colobopyga coperniciae* Ferris, 1952, are characterized by dwarfish apterous exuviatrial neotenic males, having only 2 immature instars (Köhler 1987).

Mating of scale insect winged males with apterous larva-like females and parthenogenetic reproduction of lava-like females are usually considered as examples of neoteny and paedogenesis, starting probably from the papers of Börner (1910) and Gabritschesky (1923). This approach is based on the comparison of female and male ontogenesis and the presence of more numerous male instars in contrast to female ones in the life cycle: female has only 3–4 instars, all of which are always larva-like, whereas male has 5 instars, one or two of which are quiescent nymphs (with protoptera) and one is the alate male imago (Fig. 11). Moreover some species from different scale insect families (as in archaeococcids as well as in neococcids) show obligate or facultative presence of larva-like males (Fig. 11) (see for review Gavrilov-Zimin 2018). In case of facultative appearance of larva-like males they are present in the population together with the normal alate males which undergo complicated individual development, including 2–3 larval and 1–2 quiescent nymphal instars. It was clearly demonstrated in some species that the apterous males have fewer instars than alate males – three or even two immature instars instead of four (Hadzybeyli 1958, 1969; Hafez and Salama 1967; Köhler 1987).

On the other hand, the apterous males of Phoenicococcidae s.l. (according to Stickney 1934), Xenococcidae (according to Williams 1986, 1998 and Kishimoto-Yamada et al. 2005), *Acropygorthezia* (according to LaPolla et al. 2008), and *Puto superbus* (Leonardi, 1907) (according to Gavrilov-Zimin 2018) have the same number of quiescent arostarte instar(s) before molting into apterous males.

In Stictococcidae according to Richard (1971) both apterous and alate males have four instars, which is less than the usual number (five) in alate males of other studied scale insects. Moreover, the loss of mouthparts in Stictococcidae males occurs during the first molt. This fact may be considered as an evolutionary loss of the second feeding larva in the ontogenesis (Fig. 11).

Borchsenius (1956) disputed the neoteny in scale insects and explained the evolution of coccid ontogenesis in the frame of "larvalization" of both females and apterous males. He supposed that the evolutionary reduction of the general number of instars was connected with the loss of quiescent instars, but not with the loss of imaginal instar itself. This idea is contradicted by the following facts: 1) All cytogenetically studied scale insect males have spermatogonial meiosis in third instar, whereas fourth instar and adult male have fully developed sperm bundles in their testes; the oogenesis also occurs in third



Figure 11. Neoteny and larvalization of males in different families of scale insects (Coccinea).

instar of female and so, this instar may be clearly considered as a reproduced neotenic tertiolarva. 2) The real imaginal larvalization with the absence of nymphs may be observed in aphids, sister group to scale insects. Apterous larva-like females and males of aphids (excluding *Stomaphis* Walker, 1870 discussed below) usually have the same number of instars (five) in their ontogenesis as alate females of the same population (Fig. 15). So, the true larvalization is not connected with the reduction of the number of instars, but with their modification only. In this meaning, the term "larvalization" may be used at least for apterous males of *Acropygorthezia* (Ortheziidae), Xenococcidae, Phoenicococcidae, and Stictococcidae, which save quiescent preadult instars in their ontogenesis (Fig. 11).

In many scale insects, for example, in such archeococcids as *Gueriniella* Fernald, 1903 or different species of *Icerya* Signoret, 1876, and in numerous species of neococcids from different families, males are unknown and probably completely absent. In these cases, the female tertiolarva reproduces in a parthenogenetic or hermaphroditic way and so can be considered as a paedogenetic female.

The ontogenesis of aphids (Aphidinea), usually consisting of 6 instars (egg, 4 larval instars and imago) (Figs 15, 16), is complicated in most cases by cyclic parthenogenesis with a regular alternation of bisexual and parthenogenetic generations and with or without a regular alternation of the host plants (Mordvilko 1914, 1934, 1935; Pesson 1951; Popova 1967; Blackman 1987; Moran 1992). In the archaic aphid superfamily Phylloxeroidea, both parthenogenetic and bisexual generations lay eggs (Fig. 12), whereas in the "advanced" superfamily Aphidoidea parthenogenetic females produce offspring by placental viviparity or ovoviviparity (see also the second paper (chapter) in the present Issue). In so-called "holocyclic" aphids the life cycle includes: 1) a bisexual generation (wingless larva-like or alate females and males in different families of aphids), which copulates and produces eggs; 2) a generation of wingless (rarely alate) females "fundatrices", which hatch from the eggs and then produce next generation by parthenogenesis; 3) several or many wingless parthenogenetic generations of females ("virginoparae"); 4) a generation of alate females which may or may not migrate to another host plant, another part of the same plant (monoecious cycle) or another species of host plant (dioecious cycle) and then give rise a new generation by parthenogenesis; 5) several or many wingless or alate parthenogenetic generations of females on the same or on the secondary host plant; 6) a generation of alate females, so-called "sexuparae" which migrate back to primary host plant (in dioecious cycle) and parthenogenetically produce the bisexual generation or "gynoparae", which produce only females or "androparae", which produce only males (Fig. 13). In the whole cycle (Fig. 16) the wintering egg gives rise to fundatrix female, which produce virginoparae and/or migrant females by thelytokous parthenogenesis during the summer time; in the autumn the generation of sexuparae females appears and produce females and males by deuterotokous parthenogenesis; these sexual instars copulate and the females lay overwintering eggs. In course of the development of the parthenogenetic egg only one maturation division of meiosis takes place without reduction of the diploid number of chromosomes and with a throwing out one polar body; however, the crossing over of the homologous chromosomes probably occurs



Figure 12. Biennial life cycle of *Adelges nordmannianae* (Eckstein, 1890) (Aphidinea), from Pesson, 1951, with changes. Stages **1–13** occur during first year on *Picea orientalis* (Linnaeus, 1763): **1** female "sexupara", migrated from fir (June) **2–3** larval instars on spruce (July) **4–5** female and male (July) **6** oviposition (July) 7 wintering larva (August-March) 7^{bis}–**8** female "fundatrix" (April) **9** oviposition (April) **10–11** larva, producing a gall on twig of spruce (Mai) **12** nymph (June) **13** migrating female (July). Stages **14–24** occur during second year on *Abies nordmanniana* (Steven, 1838): **14–15** females, migrating from spruce lay eggs (July) **16** overwintering larva (August-April) **17–18** parthenogenetic female and its oviposition (Mai) **19–23** new parthenogenetic generations (Mai-June) **24** alate female, migrating to spruce (June).

in early prophase (Blackman 1987: 177). In sexuparae females the oocytes destined to produce male, the X chromosomes form a bivalent with two homologues joined end-to-end (Fig. 16), and then one of X-chromosome degenerates, whereas the other one divides equationally with the autosomes (Orlando 1974; Blackman 1987: 172). During male meiosis one of the secondary spermatocytes gets an X-chromosome and more cytoplasm than the other spermatocyte, which degenerates, which is a unique feature of the Aphidoid genetic system (Blackman 1987; Gavrilov-Zimin et al. 2015).



Figure 13. Generalized scheme of the annual cycle of generations in holocyclic aphids with wingless "virginoparae" and wingless larva-like "sexuales".

Some species of aphids, especially in tropical climates, demonstrate a simplified ("anholocyclic") life cycle with only parthenogenetic generations and without regular changing of the host plants. Often the number of parthenogenetic generations may be 15–20 per year and sometimes up to 40 per year (Mordvilko 1934: 37; Gullan and Martin 2009). On the other hand, in some aphid species from the families Greenideidae and Aphididae the annual cycle may be reduced (in some parts of the species range) to only two generations: bisexual generation give rise a generation of fundatrices, which parthenogenetically produces new bisexual generation (Fig. 14) (Takahashi 1959; Strathdee and Bale 1995; Stekolshchikov and Khruleva 2014).

The arostrate instars in aphids are known as a whole bisexual arostrate generation in all species of Phylloxeridae and Pemphigidae (Mordvilko 1914; Popova 1967). All four larval instars and imago (of both sexes) in this generation do not have mouthparts and do not increase the body size during molts (Fig. 1). In the aphid genus *Stomaphis* Walker, 1870 (Lachnidae) only neotenic males are arostrate, whereas all female instars have well developed mouthparts (Mamontova 2008, 2012; Depa et al. 2015). The species of this genus save only two or three (instead of four) immature instars in ontogenesis (Fig. 5), that is considered as a clear example of male neoteny (Mamontova 2008, 2012; Depa et al. 2015).



Figure 14. Generalized scheme of the simplified annual cycle of some species of Greenideidae (Aphidinea).



Figure 15. Ontogenesis and larvalization of aphids (Aphidinea).



Figure 16. Cyclic parthenogenesis and its cytogenetic mechanisms in holocyclic aphids with diploid chromosome number 4.

There are no doubts that the ancestral ontogenesis and life cycle of aphids was based on obligatory bisexual reproduction as in most other insects and Paraneoptera in particular. The appearance of intricate aphid cycles with an obligate alternation of bisexuality and parthenogenesis was connected with the original adaptation of the group to the temperate climate of the Holarctic (Mordvilko 1934: 47, 1935: 34), where an absolute majority of aphid species, including all archaic groups, are still found. On the contrary, aphids in the tropical zone of the world and in the Southern Hemisphere are comparatively rare and represented by some "advanced" families only. The evolutionary appearance of the aphid cyclic parthenogenesis, based on the unique "Aphidoid" genetic system, is considered as an apomorphic character of the suborder Aphidinea (Moran 1992; Gavrilov-Zimin et al. 2015). This genetic system excludes the reduction of the modern aphid life-cycle to only one bisexual generation (see also the next papers(chapters) of this Issue).



Figure 17. The phylogenetic tree of Paraneoptera based on Shcherbakov and Popov (2002), Kluge (2020), Gavrilov-Zimin (2020) with modifications. The phylogenetic lines with the quiescent larval instars in ontogenesis are indicated by black solid circles (•); the lines with general reduction of the number of larval instars are indicated by black solid squares (**n**). Bold lines are used for paraphyletic taxa.

The overall picture of ontogenesis in Paraneoptera shows peculiar and even enigmatic evolutionary parallelisms – the independent appearance of the similar aberrations in related, but not sister phylogenetic lineages. Such parallelisms are also known in other fields of Paraneoptera biology – in morphology, anatomy, cytogenetics, reproductive biology, etc. (see for details: Gavrilov-Zimin et al. 2015; Gavrilov-Zimin 2020). Thus, the quiescent instars are present in ontogenesis of Thysanoptera and in two of five suborders of Homoptera: Coccinea and Aleyrodinea, which are not sister to each other according to the current interpretation of the phylogeny (see Fig. 17). The general reduction of the number of larval instars from 5–6 to 2–4 occurs in Parasita, Thysanoptera, Coleorrhyncha, Aphidinea, Aleyrodinea, Coccinea (especially in females) and also in occasional genera and families of Copeognatha and Heteroptera, whereas Psyllinea and Cicadinea show a rather high (5) and stable number of the larval instars. It seems that the reduction of the number of instars was associated with different causes in various groups of Paraneoptera. In some cases, the true imaginal instar disappears and the previous larval instars start to reproduce in course of neoteny or paedogenesis (as in aphids, scale insects and probably in some booklice and some true bugs). In lice the ontogenetic reduction probably connects with the loss of as true imaginal as well as of true first larval instars in view of the so-called embryonal molt. In whiteflies and thrips some intermediate larval instars were probably "merged" in one or two quiescent larval instars. However, the questions, connected with the clear interpretation of homology/non-homology of the instars in the ontogenesis of most Paraneoptera are presently rather controversial and very poorly studied.

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REVIEW ARTICLE



Comparative analysis of chromosome numbers and sex chromosome systems in Paraneoptera (Insecta)

Valentina G. Kuznetsova¹, Ilya A. Gavrilov-Zimin¹, Snejana M. Grozeva², Natalia V. Golub¹

 I Zoological Institute, Russian Academy of Sciences, Universitetskaya emb. 1, St. Petersburg, 199034, Russia
Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Blvd Tsar Osvoboditel 1, Sofia 1000, Bulgaria

Corresponding author: Valentina G. Kuznetsova (valentina_kuznetsova@yahoo.com)

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Abstract

This article is part (the 4th article) of the themed issue (a monograph) "Aberrant cytogenetic and reproductive patterns in the evolution of Paraneoptera". The purpose of this article is to consider chromosome structure and evolution, chromosome numbers and sex chromosome systems, which all together constitute the chromosomal basis of reproduction and are essential for reproductive success. We are based on our own observations and literature data available for all major lineages of Paraneoptera including Zoraptera (angel insects), Copeognatha (=Psocoptera; bark lice), Parasita (=Phthiraptera s. str; true lice), Thysanoptera (thrips), Homoptera (scale insects, aphids, jumping plant-lice, whiteflies, and true hoppers), Heteroptera (true bugs), and Coleorrhyncha (moss bugs). Terminology, nomenclature, classification, and the study methods are given in the first paper of the issue (Gavrilov-Zimin et al. 2021).

Keywords

Chromosome number variability, holokinetic chromosomes, monocentric chromosomes, rates of chromosome number evolution, sex chromosomes

Introduction

The structure of chromosomes and mechanisms of their evolution, the number of chromosomes and the chromosomal sex determination constitute the foundation of reproduction and are crucially important for reproductive success.

Chromosome structure and mechanisms of chromosome evolution in Paraneoptera

Nearly all Paraneoptera insects are characterized by holokinetic chromosomes. The only intriguing exception to this rule is the order Thysanoptera (thrips) in which all so far studied representatives of both suborders, Terebrantia and Tubulifera, were shown to have monocentric chromosomes (Brito et al. 2010). Holokinetic chromosomes are, thus, an additional synapomorphy of Paraneoptera, but without Thysanoptera. Monocentric and holokinetic chromosomes make up two main types of chromosomes among eukaryotes. Chromosome segregation during cell division is known to depend on specific chromosomal regions called centromeres and observed with the light microscope. Accurate chromosome segregation requires each chromosome's centromere to build a kinetochore, a large and complex protein assemblage that connects chromosomes somes to microtubules of the mitotic and meiotic spindles to distribute the replicated genome from a mother cell to its daughter cells (Westhorpe and Straight 2013).

Most animal and plant species have monocentric chromosomes with centromeres restricted to a defined chromosomal region, the so-called primary constriction, first described by a German biologist and a founder of cytogenetics, Walther Flemming (Flemming 1882). At mitotic anaphase, spindle microtubules attach to the centromeres (actually kinetochores) and bring chromosomes to the poles with the centromere leading. Holokinetic chromosomes, unlike monocentric chromosomes, do not have a localized centromere, and centromeric determinants are dispersed along their whole or almost whole length. Microtubules become attached along the entire length of holokinetic chromosomes, which, therefore, move as linear bars toward the poles at anaphase (Drinnenberg et al. 2014). Holokinetic chromosomes are often referred to as holocentric chromosomes; accordingly, the phenomenon itself became known as holocentricity. However, holokinetic chromosomes do not have a proper centromere in the sense of a primary constriction connecting sister chromatids, how is the case in monocentric chromosomes. At metaphase, holokinetic chromosomes show sister chromatids separated from each other by a regular distance. The term "holocentric" does not, thus, reflect adequately the morphology and behavior of these unusual chromosomes (Mola and Papeschi 2006; Guerra et al. 2010), a conclusion we agree with.

Holokinetic chromosomes, as distinct from monocentric chromosomes, were recognized as late as the mid-1930s (Schrader 1935), and one of the first groups of insects in which these chromosomes were discovered and studied were paraneopteran insects, namely mealybugs (Pseudococcidae, Homoptera) (Hughes-Schrader and Ris 1941; Hughes-Schrader 1942, 1948; Hughes-Schrader and Schrader 1961). Since

then, many original articles, reviews and discussion papers about holokinetic chromosomes have been published and an impressive series of conceptual advances in the topic have accumulated (Nokkala et al. 2004, 2006; Guerra et al. 2010; Melters et al. 2012; Drinnenberg et al. 2014; Zedek and Bureš 2018; Mandrioli and Manicardi 2020; Ruckman et al. 2020; Senaratne et al. 2021). The ancestral insects are believed to be monocentric (Melters et al. 2012; Drinnenberg et al. 2014). Holokinetic chromosomes appear to have evolved independently in multiple eukaryotic lineages by convergent evolution (Melters et al. 2012). Among insects, they are currently known in Odonata (Palaeoptera); Dermaptera (Polyneoptera); Copeognatha [=Psocoptera], Parasita [=Phthiraptera], Homoptera, and Heteroptera (Paraneoptera); Lepidoptera and Trichoptera (Oligoneoptera) (White 1973). More recently, it has been shown that Coleorrhyncha and enigmatic Zoraptera (both from Paraneoptera) also have holokinetic chromosomes (Kuznetsova et al. 2002, 2015; Grozeva et al. 2014). Thus, holokinetic chromosomes occur in every major phylogenetic lineage of Pterygota suggesting that they are likely to have evolved at least four times independently in insect evolution (Drinnenberg et al. 2014; Kuznetsova and Aguin-Pombo 2015).

The transition from monocentric to holokinetic state occurred, apparently, by accident; however, holokinetic state itself is speculated (Zedek and Bureš 2018) to represent a potential advantage in exploiting new habitats and adapting to stressing conditions. On the other hand, number of chiasmata (crossingovers) in holokinetic bivalents is limited to one or two only (Nokkala et al. 2004), what can be an evolutionary disadvantage, once the organism adapts to its new habitat (Zedek and Bureš 2018).

One of the most significant advances is the recent discovery that independent transitions to a holokinetic state in insects were associated with the recurrent loss of a centromeric histone H3 variant (CenH3), which is an essential chromatin component of centromeres in most eukaryotes (Drinnenberg et al. 2014; Senaratne et al. 2021). The question is what happened first: the acquisition of the derived holocentric state or the loss of CenH3. Taking into account that CenH3 loss is only identified in "holokinetic" insect lineages (Drinnenberg et al. 2014), the emergence of the "holokinetic" state is likely to have proceeded and subsequently allowed the loss of CenH3 (Senaratne et al. 2021). This sequence of evolutionary events, i.e. the loss of CenH3 after the establishment of "holokinetic" chromosome structure, is also supported by the fact that some "holokinetic" insects do have CenH3 homologs. These insects could therefore represent an intermediate form, leading to the establishment of the CenH3-deficient state (Senaratne et al. 2021).

Chromosomal mechanisms of sex determination in Paraneoptera

The vast majority of paraneopteran insects have male heterogametic sex determination system. These insects carry either an XX/XY or an XX/X(0) system. Some species have complex systems with multiple X or (much rarer) multiple Y-chromosomes, which evolve mainly by fissions of the ancestral sex chromosomes. Others possess so-called neo-XY systems that have derived from the X(0) system by the fusion of the X chromosome with

an autosome, resulting in a neo-X chromosome, and the homologue is transformed into a neo-Y chromosome (White 1973; Blackman 1995). A number of more complicated sex determination systems are also known in paraneopteran insects. Some insects display haplodiploidy (arrhenotoky) where males are haploid and develop from unfertilized eggs, while females are diploid and develop from fertilized eggs (e.g. thrips, whiteflies and some genera of scale insects). Some insects have paternal genome elimination (PGE) where both sexes develop as diploids, but maleness is determined by inactivation or loss of paternal chromosomes, making males functionally haploid (e.g. true lice, scale insects and whiteflies). Finally, true parthenogenesis exists in some paraneopteran insects occurring either in separate species or on higher taxa level, when female embryos develop from unfertilized diploid eggs (see Vershinina and Kuznetsova 2016).

Chromosome number evolution in Paraneoptera

One of the basic features of holokinetic chromosomes is that their fragments retain centromere activity and the ability to segregate to the poles, which has been demonstrated in experiments with X-ray irradiation (Hughes-Schrader and Ris 1941; Brown and Nelson-Rees 1961; Hughes-Schrader and Schrader 1961).

Moreover, it has been shown in experiments on the Mediterranean flour moth *Ephestia kuehniella* Zeller, 1879 (Lepidoptera, Pyralidae) that radiation-induced chromosome fragments are regularly inherited by both somatic and germ cells and can be transmitted through more than 50 generations suggesting that they persist as long as their active kinetochore elements are preserved (Marec et al. 2001). Due to this feature, chromosomal rearrangements (tandem fusions, fissions, etc.) can arise and be transmitted to daughter cells at successive cell divisions in holokinetic organisms. In contrast, in organisms with localized centromeres, any cells with changes that result in acentric or dicentric chromosomes will be eliminated as such chromosomes will not segregate normally at mitosis, which will result in a loss of genetic material and probably inviable gametes (Blackman et al. 2000; Escudero et al. 2013). Thus, the presence of centromere is a limiting factor in chromosome evolution within monocentric groups.

Chromosome number is highly variable across insects as a whole (Blackmon et al. 2017). Changes in chromosome number can happen due to different mechanisms (Sylvester et al. 2020). Decrease in number can be a result of chromosome fusions, whereas increase in number can occur due to simple chromosome fissions, the addition or loss of a whole chromosome (aneuploidy as trisomy and monosomy, respectively), or the addition of a whole chromosome set (polyploidy). Theoretically, holokinetic chromosome structure facilitates the successful inheritance of novel fusion chromosomes or fission fragments, and species with holokinetic chromosomes should therefore tolerate structural rearrangements of chromosomes better than species with monocentric chromosomes. Because of this, "holokinetic" taxa should have higher rates of chromosome number evolution compared to "monocentric" taxa (Hill et al. 2019). Indeed, there are a number of great examples of rapid chromosome number evolution in holokinetic organisms, both plants and invertebrate animals. So, a large holokinetic angiosperm

genus Carex (Linnaeus, 1753) (Cyperaceae) demonstrates an exceptional chromosome number series with nearly continuous range from 2n=12 to 2n=124 and substantial variation of the 2n within many species (see for references Escudero et al. 2013). The holokinetic insect order Lepidoptera provides the most impressive examples of this kind showing the highest variance in chromosome number within a species and between species within a genus, the highest single count and polymorphisms in counts that do not affect fertility in crosses (Hill et al. 2019). So, within a widespread Eurasian butterfly Leptidea sinapis (Linnaeus, 1758) (Pieridae), the 2n gradually decreases from 106 in Spain to 56 in eastern Kazakhstan (Lukhtanov et al. 2011). The large blue butterfly subgenus Agrodiaetus Hübner, 1822 belonging to the genus Polyommatus Latreille, 1804 (Lycaenidae) exhibits unusual interspecific diversity in chromosome number varying from n=10 to n=134 (Kandul et al. 2007). Another blue butterfly species, Polyommatus (Plebicula) atlanticus (Elwes, 1906), displays 2n=ca. 448-452, the highest chromosome number among all the non-polyploid eukaryotic organisms (Bureš and Zedek 2014; Lukhtanov 2015). There are equally outstanding examples among Paraneoptera. The standard karyotype of the human bed bug Cimex lectularius Linnaeus, 1758 (Heteroptera, Cimicidae) includes 2n=29(26+X,X,Y) in males (Ueshima 1979). However, in a number of European populations males have very varied chromosome numbers, 2n=29-37, 40, 42, 47; this variability is explained by an increase of the number of X-chromosomes as a result of intense processes of fragmentation of the original X-chromosomes (Sadílek et al. 2013). An endemic Australian scale insect genus Apiomorpha Rübsaamen, 1894 (Coccinea, Eriococcidae) exhibits an extraordinary 48-fold

Although variations in chromosome number of related species are probably due to both fissions and fusions of holokinetic chromosomes, fusions are suggested to be more common. The point is that a chromosome, be it holokinetic or monocentric, has to display two functional telomeres in order to survive a mitotic cycle. A fusion chromosome always displays functional telomeres originated from the ancestral chromosomes, whereas a fission chromosome has to be able to develop a functional telomere *de novo* (Nokkala et al. 2007). However, the ability of holokinetic fragments to restore telomeres *de novo* by telomerase has been repeatedly confirmed in various insects (e.g. Mohan et al. 2011; Mandrioli et al. 2014; for other examples, see Kuznetsova et al. 2020).

variation in chromosome number (2n=4-192) and extensive chromosomal variation

within numerous morphologically defined species (Cook 2000).

To test whether insects with monocentric and holokinetic chromosomes differ in the amount and rate of chromosomal rearrangements, Ruckman et al. (2020) undertook an extensive analysis of chromosome counts across 22 insect orders. The authors focused, on the one hand, on "monocentric" orders Blattodea, Coleoptera, Diptera, Hymenoptera, Isoptera, Neuroptera, and Phasmatodea and, on the other hand, on "holokinetic" orders Odonata, Hemiptera, and Lepidoptera. To exclude polyploidy as a source of "aberrant" (derived) chromosome numbers, the authors explored two models for the evolution of chromosome number, one of which included both fusion/ fission and polyploidy, and the other only fusion/fission events. It is of interest that the analysis covering a total of 4,393 species and 599 genera, and using various approaches detected no significant difference between taxa with different chromosome types suggesting that characteristics other than "holocentricity" and "monocentricity" (e.g. meiotic drive, polyploidy events and population size) can be key to determine rates of chromosome number changes (Ruckman et al. 2020). Although this suggestion was based on extensive analysed material (a large number of species, genera, and orders), it requires more solid confirmation. For example, regarding Paraneoptera, representing one of the largest and most diverse insect lineages, the authors addressed only 1,695 species, while the number of paraneopteran species with known chromosome numbers is several time higher. To expand the possibilities of future analyses, we give below a brief overview of chromosome number variations found in each of the major phylogenetic lineages of Paraneoptera, including Zoraptera, Copeognatha (=Psocoptera), Parasita (=Phthiraptera), Thysanoptera, Homoptera, Heteroptera, and Coleorrhyncha.

Material and methods

The material for this review study was the representatives of all major phylogenetic lineages of Paraneoptera, including Zoraptera (angel insects), Copeognatha (=Psocoptera; bark lice), Parazita (=Phthiraptera; true lice), Thysanoptera (thrips), Homoptera (scale insects, aphids, jumping plant-lice, whiteflies, and true hoppers), Heteroptera (true bugs), and Coleorrhyncha (moss bugs). In most cases, chromosome analysis was performed using both conventional cytogenetic techniques (Giemsa, C-banding, Ag-NOR-staining, base-specific fluorochrome banding) and FISH mainly with rDNA and telomeric (TTAGG)_n probes. Methods, terminology, nomenclature, and classification are given in the first paper of this issue (Gavrilov-Zimin et al. 2021).

Review and discussion

Here, we review and discuss basic data on chromosome numbers and sex chromosome systems separately for each Paraneoptera order or, in some cases (e.g. Homoptera), for suborders within the order.

Zoraptera

Zoraptera (known as angel insects) are minute, less than 3 mm long, insects of cryptic habits living under bark, in humus, termite nests, etc. This is one of the lesser-known insect lineages in the world in terms of distribution, diversity, mode of life, reproductive biology, genetics, etc. The order currently contains a single genus *Zorotypus* Silvestri, 1913 constituting the family Zorotypidae, with 44 extant and 13 fossil species (Mashimo et al. 2014; Villamizar and González-Montana 2018). The majority of zorapteran species are tropical, with just a few exceptions, e.g. *Zorotypus hubbardi* Caudell, 1918 widespread in the United States. *Z. hubbardi* is the first and still the only zorapteran

species studied in terms of karyotype (Kuznetsova et al. 2002). Males of this species were shown to have 2n=38(36A+XY) and holokinetic chromosomes. Autosomes can be classified into two size groups, with three larger pairs and six pairs showing an even gradation in size, respectively. X- and Y-chromosomes form a chiasmatic bivalent in meiosis, suggesting that sex chromosome system is of a neo-XY type and could result from an X-autosome fusion in the initial karyotype of 2n=40(38A+X). This is consistent with the fact that in insects the only known chiasmatic sex chromosome system is neo-XY (Blackman 1995). The autosomally derived Y chromosome of *Z. hubbardi* is still homologous with the autosomal part of the neo-X that is evidenced by their synapsis in meiosis. This suggests a relatively recent origin of this sex determining system in *Z. hubbardi* (Kuznetsova et al. 2002).

Copeognatha

Copeognatha (=Psocoptera; bark lice) are mostly small insects inhabiting terrestrial ecosystems; representatives of certain families are closely related to human dwellings and even considered as pests in storage facilities. Psocoptera are a basal taxon of Paraneoptera (Yoshizawa and Saigusa 2001) comprising 5,941 species in 485 genera and 41 families unequally distributed between the three suborders, Trogiomorpha, Troctomorpha, and Psocomorpha. Trogiomorpha comprise 418 recent species (58 genera, 7 families) and retain most archaic features. Troctomorpha comprise 536 species (86 genera, 9 families) and include highly specialized forms. The largest suborder Psocomorpha, considered as the most evolutionarily advanced group of Copeognatha, comprises 5,028 species (330 genera, 27 families) (Mockford 2018).

To date, 90 bark lice species (about 1.5% of the described ones) from 51 genera and 21 families have been studied cytogenetically. Of these, 80 species (43 genera, 16 families) belong to Psocomorpha (reviewed in Golub and Nokkala 2009; see also Golub et al. 2019), 6 species (5 genera, 3 families) to Trogiomorpha, and 4 species (2 genera, 2 families) to Troctomorpha. Diploid chromosome numbers range from 13/14 (male/female) found in two species of Psocomorpha to 29/30 reported for two species of Trogiomorpha; a sex chromosome system X(0) is characteristic of all bark lice species, with the exception of two species of the family Amphipsocidae (Psocomorpha). Amphipsocus japonicus Enderlein, 1906 and Kolbia quisquiliarum Bertkau, 1882 have a neo-XY system, which evolved due to chromosomal rearrangements involving autosomes and sex chromosomes in an ancestral karyotype with an X(0) system (Golub and Nokkala 2001, 2009). Psocoptera demonstrate a clear mode at 2n=16A+XX/X(0). This chromosome complement is found in nearly 90% of the studied species (71 species, 36 genera, 15 families) and in each of the 3 suborders. It is assumed that the modal karyotype (type or basic) is the ancestral one in the evolution of Copeognatha (Wong and Thornton 1966; Golub 1999; Golub and Nokkala 2009). This assumption is confirmed by the following facts. First, both species with a derived sex chromosome system have 2n=16(14A+neo-XY) in males, indicating that translocation of the X to an autosome has most likely occurred in a male ancestor with 2n=17(16A+X). Second, all

known triploid parthenogenetic species, *Valenzuela labinae* Lienhard, 2006, *V. flafidus* (Stephens, 1836) (Caeciliusidae), *Ectopsocus meridionalis* Ribaga, 1904 (Ectopsocidae), and *Peripsocus subfasciatus* (Rambur, 1842) (Peripsocidae), have 2n=3x=27(24A+XXX). Their karyotype arose from the putative ancestral one, 2n=18(16A+XX), by adding one more haploid set, n=9(8A+X) (Nokkala and Golub 2002, 2006). The remaining derivative karyotypes most likely evolved through chromosome fusion/fission events leading to an increase or decrease in the number of autosomes in the karyotype. In Trogiomorpha, chromosome fissions seem to have prevailed, whereas in Troctomorpha and Psocomorpha, on the contrary, the fusion processes predominated. However, there is still very little data to confirm this hypothesis.

Chromosomes of Copeognatha are comparatively small and of similar size being, therefore, hard to distinguish from each other in the karyotype when the standard techniques of chromosome staining are used. The applying of banding techniques is scarce in this group. Only three species, all from the suborder Psocomorpha, Psococerastis gibbosa (Sulzer, 1766), Blaste conspurcata (Rambur, 1842), and Amphipsocus *japonicus*, were studied using C-banding, silver impregnation and sequence-specific fluorochromes CMA, and DAPI (Golub and Nokkala 2001; Golub et al. 2004). It was shown that nucleolus organizer regions (NORs) are located differently in these species: on an autosomal bivalent, on the X-chromosome, and on the neo-XY bivalent, respectively. It was also shown that these species have a small amount of C-heterochromatin, which localizes as a tiny blocks in terminal regions of chromosomes and consists of AT-rich DNA, except for the NOR regions, which are both AT- and GC-rich, just like the X-chromosomes. Some minor differences in the above characters were observed between species and between different chromosomes of a particular species. It was repeatedly shown, that fluorescence in situ hybridization (FISH) helps identifying individual chromosomes and tracing, thus, their behavior in meiosis of "holokinetic" insects (Panzera et al. 2012, 2015; Maryańska-Nadachowska et al. 2013, 2018; Mandrioli et al. 2014; Kuznetsova et al. 2015; Anjos et al. 2016; Golub et al. 2017; Salanitro et al. 2017; Grozeva et al. 2019). Some higher insect taxa were shown to differ in the presence/absence of the insect-type telomere motif (TTAGG), (reviewed in Kuznetsova et al. 2020). Within Copeognatha, several Psocomorpha species were studied using FISH (Frydrychová et al. 2004; Golub et al. 2019). In each of these species, 18S rDNA-FISH revealed two large clusters of the rRNA genes located on a medium-sized bivalent, and (TTAGG),-FISH revealed signals at the telomeres of their chromosomes. These data suggest that Copeognatha, at least in the suborder Psocomorpha, display the telomere motif (TTAGG), considered ancestral for the class Insecta in general (Frydrychová et al. 2004; Kuznetsova et al. 2020).

Parasita

Parasita (=Phthiraptera; true lice), the closest relatives of Copeognatha, include obligate ectoparasites of birds and mammals. The order is divided into Mallophaga (Amblycera + Ischnocera) and Rhynchophthirina known as chewing or biting lice, respectively,

and Siphunculata (=Anoplura) known as sucking lice (Smith et al. 2011; Kluge 2020). There are about 5,000 known species, of which 550 species (50 genera, 15 families) belong to Siphunculata; 1,341 species (76 genera, 7 families) to Amblycera; 3,120 species (130 genera, 3 families) to Ischnocera; and only 3 species (genus Haematomyzus Piaget, 1869, Haematomyzidae) to Rhynchophthirina (Price et al. 2003; Durden 2019). Chromosomal data, although extremely scarce and fragmentary, are presently known for each of the suborders, with the exception of Rhynchophthirina. Of these, 2 species (2 genera, 2 families) belong to Amblycera, 4 species (3 genera, 2 families) to Ischnocera, and 7 species (5 genera, 5 families) to Siphunculata (Tombesi et al. 1999; Golub and Nokkala 2004; Bressa et al. 2015; for other reference see Tombesi and Papeschi 1993). Surprisingly, among as few as 13 studied species, as many as 7 different chromosome counts were found. These latter vary from 2n=4 to 2n=24, and sex chromosomes were not distinguished in any of the studied species. It is impossible now to establish modal numbers (type or basic numbers) either for Parasita as a whole or for separate taxa within the order. The lowest count is found in Amblycera (Gyropus ovalis Burmeister, 1838), and the highest count is found in Ischnocera (Chelopistes meleagridis (Linnaeus, 1758) as Gonioides stylifer Nitzsch, 1818).

C-banding experiments failed to reveal constitutive heterochromatin in karyotypes of human head- and-body lice, *Pediculus humanus capitis* De Geer, 1778 and *Pediculus humanus humanus* Linnaeus, 1758 (Pediculidae, Siphunculata) (Bressa et al. 2015). The genomic study of *P. h. humanus* showed its telomeric DNA to consist of the TTAGG repeats (Kirkness et al. 2010). Using a genotyping approach, Filia et al. (2017) have established that both *P. h. capitis* and *P. h. humanus* reproduce through paternal genome elimination (PGE), an unusual genetic system when males transmit only their maternally derived chromosomes.

Thysanoptera

Thysanoptera (thrips, also known as thunder flies) encompass minute insects, which are usually only a few mm in length. Most thrips feed on fungi, flowers and leaves of green plants, less often on mosses and detritus. Thrips are distributed worldwide being more abundant in tropical, subtropical, and temperate regions. Thysanoptera are a monophyletic group subdividing into the two suborders, Terebrantia with about 2,400 described species in 8 families, and Tubulifera with about 3,500 species in a single family, the Phlaeothripidae. The majority of known thrips species are placed in the two largest families, the Phlaeothripidae (Tubulifera) and Thripidae (Terebrantia) (Mound and Minaei 2007). Most studied Thysanoptera species possess a haplo-diploid reproductive mode and reproduce via arrhenotoky (Nault et al. 2006) where unfertilized eggs develop parthenogenetically into males, which are always haploid, and fertilized eggs develop into females, which are always diploid; so males only transmit their maternal genome to the offspring (see Vershinina and Kuznetsova 2016). A number of thrips are known to be obligatory thelytokous (Nguyen et al. 2015). Moreover, three reproductive modes, thelytoky, arrhenotoky, and even deuterotoky, were documented

in *Thrips tabaci* Lindeman, 1889 populations collected from onion fields in New York (Nault et al. 2006).

The degree of cytogenetic knowledge of thrips is negligible. Risler and Kempter (1961) compiled all chromosome numbers known for Thysanoptera (10 species in total) studied by the beginning of 60s of the last century. Another 7 species originating from Northeast Brazil were karyotyped by Brito et al. (2010). Most of what is known concerning the chromosomes of thrips is due this latest publication. According to Brito et al. (2010), karyotypes are currently known for 17 species (about 0.3% of the described ones) of which 6 species in 4 genera are from the Phlaeothripidae and 11 species in 9 genera are from the Thripidae. It was unexpected to find out that thrips, unlike all other Paraneoptera insects, have monocentric chromosomes. The lowest and the highest haploid numbers of chromosomes in male thrips are 10 (i.e., 2n=20) and approximately 50-53 (i.e., 2n=100-106), respectively. The lowest count was found in both Tubulifera (Haplothrips tritici (Kurdjumov, 1912)) and Terebrantia (Taeniothrips simplex Morison, 1930), and the highest count is found in Terebrantia, in Aptinothrips rutua (Prussard-Radulesco, 1930). Chromosome numbers represent a nearly continuous range in each of the suborders, varying in Tubulifera from n=10 to n=15, with intermediate values 12, 13, 15 and in Terebrantia from n=10 to n=50-53, with intermediate values 14, 15, 16, 17, 18, 20, 21. In Heliothrips haemorrhoidalis (Bouche, 1833) and Taeniothrips inconsequens (Uzel, 1895), both from the Thripidae, different chromosome numbers were reported by different authors (n=16, 21 and 26/28, and n=16 and 18/20, respectively); however, no information other than chromosome counts is given in the original publications (see for references Risler and Kempter 1961 and also Brito et al. 2010). Besides, two "cytotypes" were found by Brito et al. (2010) in Gynaikothrips uzeli (Zimmermann, 1900) (Phlaeothripidae) originating from two isolated localities, one with n=13 and karyotype formula of n=4M+8SM+1A, and the other with n=15 and karyotype formula of n=4M+10SM+1A. Brito et al. (2010) also constructed karyotype formulas for another four species and for each of the G. uzeli "cytotypes". A great variation in both number and morphology of chromosomes was revealed at family, genus and species levels, with usually no clear correlations between changes in chromosome numbers and karyotype structure. The exception seems to be the two studied representatives of the genus Gynaikothrips Zimmermann, 1900, G. uzeli (with its two cytotypes) and G. ficorum Marchal, 1908. The species status of G. ficorum remains uncertain for many years. Mound et al. (2016) have suggested that G. ficorum is probably a form of G. uzeli, and morphological differences between different populations of these thrips can be due to different hosts and latitude. According to Brito et al. (2010), differences in chromosome number between G. uzeli (the cytotype A with n=13) and G. ficorum (n=15) as well as differences in chromosome morphology between G. uzeli (the cytotype B with n=4M+10SM+1A) and G. ficorum (n=4M+7SM+6T) indicate that both G. uzeli and G. ficorum are distinct species and that pericentric inversions led to the differentiation of their karyotypes. It should be noted, however, that both hypotheses, especially the last one, need further research and more convincing chromosomal and also molecular confirmations.
Unfortunately, based on the available data, it is currently impossible to speculate on mechanisms underlying chromosome number diversity in Thysanoptera. It can be assumed that some species of thrips are polyploids. As mentioned above, thelytokous reproduction occurs in some species, including the greenhouse thrips, *Heliothrips haemorrhoidalisis*. This species was suggested to reproduce by automixis through fusion between the second polar body nucleus and the egg nucleus, so called terminal fusion (Bournier 1956). This conclusion was made on the basis that chromosome number was reduced during oogenesis from 2n=42 to 2n=21, followed by the re-establishment of 42 chromosomes. More recently, the analysis of genetic diversity at two nuclear loci has indicated that *H. haemorrhoidalis* may be polyploid (and potentially hexaploid), which does warrant future cytogenetic investigation (Nguyen et al. 2015).

Sex chromosomes were not detected in any species of thrips (Brito et al. 2010), and the cytogenetic mechanisms involved in sex determination in these insects are not yet fully understood. We can assume that they have a chromosomal single-locus sex determination, as is the case with haplodiploid Hymenoptera where sex determination involves no heteromorphic sex chromosomes and sex is determined by a single locus (heterozygotes at the sex locus develop into females and hemizygotes develop into males) (Wilgenburg et al. 2006). The presence of the (TTAGG)_n telomere motif was detected in chromosomes of *Parthenothrips dracaenae* (Heeger, 1854) (Frydrychová et al. 2004).

Coccinea

Coccinea (scale insects) are sap-sucking phytophagous insects, among which there are many pests of agricultural and ornamental plants, as well as producers of natural dyes, lacquers and waxes. Scale insects represent a moderate-sized group of sternorrhynchous Homoptera comprising more than 8,000 species in the world fauna; they are subdivided into 19-36 recent families, depending on the taxonomic and phylogenetic views of different authors. Here, we follow the traditional system of 19 "large" families, without dividing Margarodidae, Pseudococcidae, Eriococcidae, and Asterolecaniidae into minute "families" with overlapping characters (see for details Danzig 1980; Gavrilov-Zimin 2018a). Scale insects have a wide range of chromosome numbers and also unique variety of sex determination mechanisms and mode of reproduction, including hermaphroditism, paternal genome heterochromatinization (differential inactivation) and elimination (PGH and PGE, respectively), different types of haplodiploidy (both usual haplodiploidy and the so-called Lecanoid and Diaspidoid systems with some variations), various modes of parthenogenesis, etc. (Hughes-Schrader 1948; Nur 1980; Gavrilov 2007; Gavrilov-Zimin et al. 2015). As mentioned at the beginning of this paper, mealybugs (Pseudococcidae) were one of the first groups in which holokinetic chromosomes were discovered as a phenomen and studied (Hughes-Schrader and Ris 1941; Hughes-Schrader 1942, 1948; Hughes-Schrader and Schrader 1961). Scale insects, especially some mealybugs (*Planococcus* spp.), have long been used as a model system to study the mechanisms underlying resistance of holokinetic chromosomes

to ionizing radiation as well as the phenomenon of genome stability in holokinetic organisms (Hughes-Schrader and Ris 1941; Brown and Nelson-Rees 1961; Hughes-Schrader and Schrader 1961; Mohan et al. 2012).

Chromosome numbers have been reported for 506 species of scale insects belonging to 15 families (reviewed by Gavrilov 2007 and Gavrilov-Zimin et al. 2015; see also Gavrilov-Zimin 2016, 2017, 2018b, 2020), thus comprising about 6% of the total number of coccid species described to date. The lowest chromosome number, 2n=4, is found in 21 species of the tribe Icervini (Margarodidae) and in several species of the endemic Australian gall-inducing felt-scale genus Apiomorpha (Eriococcidae), while the highest number, 2n≈192, is found in Apiomorpha macqueeni Froggatt, 1929 (Hughes-Schrader 1948; Cook 2000, 2001; see for review Gavrilov 2007). Three values, 2n=8, 10, and 18, can be suggested today as modal ones for Coccinea as a whole. They prevail in large and comparatively better studied families Pseudococcidae s.l. (138 species in 50 genera), Eriococcidae s.l. (98 species in 19 genera), Diaspididae (141 species in 68 genera), Coccidae (56 species in 31 genera), and Margarodidae s.l. (39 species in 21 genera). Moreover, most studied species of the small families Dactylopiidae and Phoenicococcidae s.l. have 2n=10, and the only species of the ancient monotypic family Phenacoleachiidae, Phenacoleachia zealandica (Maskell, 1891), has 2n=8. All other families are very poorly studied (Ortheziidae, Xenococcidae, Kermesidae, Aclerdidae, Conchaspididae, Asterolecaniidae s.l., and Kerriidae) or not studied at all (small families Carayonemidae, Stictococcidae, Micrococcidae, and Beesoniidae) (see Gavrilov 2007; Gavrilov-Zimin et al. 2015).

In some cases, an increase in the number of chromosomes is a result of polyploidy or the presence of B-chromosomes. Both triploid and tetraploid forms were described by Nur (1979) in the soft scale (Coccidae) species *Physokermes hemicryphus* (Dalman, 1826) and *Saissetia coffeae* (Walker, 1852). We can assume that polyploidy actually occurs in scale insects more often what is indirectly indicated by that some congeneric species of soft scales, felt scales, and mealybugs differ from each other in chromosome number three or four times (Gavrilov 2007). The presence of some chromosomes, additional to the standard chromosome number and suggested to be B-chromosomes, has been confirmed in many publications (Gavrilov 2007). However, only in some species, e.g. *Pseudococcus viburni* (Signoret, 1875) (Pseudoccocidae), B-chromosomes were studied in more details (Nechaeva et al. 2004). In this species, B-chromosomes were found to be large (these chromosomes are usually small although exceptions are known, e.g. Maryańska-Nadachowska 1999) and completely heterochromatic (after C-banding); they varied in number from zero to 2 within an individual, with a single B- chromosome being most frequent.

The number of chromosomes is quite often stable or slightly variable within genera and within many higher rank taxa of scale insects (see Gavrilov 2007; Gavrilov-Zimin et al. 2015). Here are examples coming from some comparatively better studied groups. The mealybug genera *Pseudococcus* Westwood, 1840 and *Phenacoccus* Cockerell 1893 (Pseudococcidae) are characterized by a karyotype with 2n=10, which was found in 13 species (of the 15 studied ones) and in 19 species (of the 20 studied ones), respectively. The felt scale genus *Eriococcus* Targioni Tozzetti, 1868 (Eriococcidae) was found to have 2n=18 in 13 of the 18 studied species. In the family Diaspididae, which is most species-rich (2,650 species in 418 genera) and the best cytogenetically studied (141 species in 68 genera), the 2n varies from 6 to 18 but the overwhelming majority of species have 2n=8. However, there are exceptions. Some scale insect genera demonstrate a significant or even extraordinary variation of chromosome number; moreover, different diploid numbers can be found in the same nominal species. Most impressive example of such variation is the aforementioned feltscale genus *Apiomorpha*, where 42 diploid counts, ranging from 2n=4 to $2n\approx192$, have been found in 47 studied species, including undescribed ones. Moreover, extensive chromosomal variation was observed within many morphologically defined species of *Apiomorpha*, suggesting the involvement of chromosomal changes in the divergence of this lineage and in the generation of cryptic species (Cook 2000, 2001; Mills and Cook 2010).

Scale insects are characterized by a huge variety of mechanisms of sex determination (see for references Nur 1980; Gavrilov-Zimin and Kuznetsova 2007; Gavrilov 2007; Gavrilov-Zimin et al. 2015). Sex chromosomes have been identified in only a small number of species. The XX/X(0) system was reported for some primitive taxa, including genera of the most ancient families Margarodidae and Ortheziidae (archaeococcids) (see for references Gavrilov-Zimin 2018a) and was assumed to be ancestral for Coccinea in general (Nur 1980; Gavrilov-Zimin and Danzig 2012). The same system was also identified in several studied species of the genus Puto Signoret, 1875 from the family Pseudococcidae (neococcids). There are known species with multiple sex chromosomes that have arisen presumably because of fissions (fragmentations) of the original X-chromosomes. Matsucoccus gallicolus Morrison, 1939 (Margarodidae) is a unique species with 12Xs in females and, thus, 6Xs in males (Hughes-Schrader 1948), the number being probably the highest among both Paraneoptera and Insecta in general. Although even higher numbers of X-chromosomes (up to 21) were reported for a bed bug species Cimex lectularius Linnaeus, 1758 (Heteroptera) (Sadílek et al. 2013), they all exist in the form of polymorphism.

Currently, there are no confirmed cases of the XY system in Coccinea. Two species, *Praelongorthezia praelonga* (Douglas, 1891) with 2n=16 (Ortheziidae) and the Australian felt scale *Lachnodius eucalypti* (Maskell, 1892) with 2n=18 (Eriococcidae), were shown to have the same number of chromosomes in both males and females. It has been reported (see for references Gavrilov 2007) that these species do not have the PGH/PGE systems, what could explain the observed phenomenon, and that their males have no morphologically distinguishable (heteromorphic) pair of chromosomes, which would unambiguously assume an XY system. Such an uncertain situation is usually called 2n-2n in scale insects. However, it is known that sex chromosomes can be homomorphic, with little or no divergence in size, and such examples are often found in insects, including those of Paraneoptera (e.g. Rebagliati and Mola 2010; Golub et al. 2015). It should also be added that the degree of divergence is not necessarily associated with sex chromosome age, with examples of young heteromorphic systems and, on the contrary, old homomorphic systems (see Furman et al. 2020 for references). To get more information on what kind of sex determination system these species actually have, we need in chromosomal and molecular markers making it possible to identify sex chromosomes in the genome. Molecular methods based on genome coverage from next generation sequencing data, which are commonly used to distinguish sex chromosomes from autosomes and X and Y chromosomes from each other, have never been used in Coccinea. This approach exploits the difference in sex chromosome ploidy between males and females with XX/XY systems considering that X-linked genes show half the number of genomic reads in males compared to females, and that Y-linked reads are absent in females (Palmer et al. 2019). New cytogenetic methods are still very rarely used in the cytogenetics of scale insects. Nechaeva et al. (2004) used several chromosome banding techniques to study Pseudococcus viburni. This species is known to have a Lecanoid sex determination system, which is characterized by heterochromatinization and genetic inactivation of one haploid set of chromosomes in male embryos; however, genes for *rRNA* were shown to remain active in the heterochromatic haploid set of male embryos. Mohan et al. (2011) detected the (TTAGG), telomere motif in chromosomes of Planococcus lilacinus (Cockerell, 1905). Moreover, these authors also detected telomerase activity at the sites of chromosome breaks initiated by radiation in this species, which implies the possibility of *de novo* telomere formation at the ends of the fragmented chromosomes.

Aphidinea

Aphidinea (aphids) are a moderate-sized group of sternorrhynchous Homoptera, with approximately 5,000 described species distributed mainly throughout the temperate regions of the globe (Favret and Eades 2009). Aphidinea are considered as a sister taxon to Coccinea (e.g. Shcherbakov 2007) and are sometimes united with it in the higher taxon Aphidococca (Kluge 2010; Gavrilov-Zimin et al. 2015). The close relationship of these groups is well supported by numerous morphological, anatomical, embryological, cytogenetic, physiological and other characters. Aphids are small plant sucking insects, very important as agricultural pests and active vectors of crop viruses. They typically exhibit cyclical parthenogenesis, alternating a single annual (sometimes biennial) bisexual generation with several (or numerous) unisexual (all-female) generations reproducing by apomictic parthenogenesis. The bisexual generation may be lost secondarily, so that reproduction is then exclusively by thelytoky (Blackman et al. 2000).

Opinions differ as to the higher classification within Aphidinea, in particular regarding the number of accepted families and their relationships. In the well-known taxonomic catalogue of aphids (Remaudière and Remaudière 1997), all recent "true aphids" are placed in the only family, Aphididae. However, different authors accept 6 to 13 true aphid families, in addition to the families of "not true aphids", Adelgidae and Phylloxeridae (Börner 1952; Shaposhnikov 1964; Heie 1987; Heie and Wegierek 2009a, 2009b). Along with two latter families, we accept 12 more recent families of Aphidinea; by now, chromosome numbers have been reported for 1,113 species belonging to all but one (Tamaliidae) families, which is about 22% of the total number of described aphid species (reviewed in Gavrilov-Zimin et al. 2015). The smallest 2n currently known in aphids is 4 being reported for three species of the family Aphididae, *Gypsoaphis oestlundi* Hottes, 1930, *Myzaphis rosarum* (Kaltenbach, 1843), and *Amphorophora tuberculata* Brown et Blackman, 1985. This chromosome number was also reported for a population of *Chaitophorus eucomelas* Koch, 1854 (Chaitophoridae) from Peru; however, several other populations of this species sampled from Israel, Great Britain, and South Africa were found to have high chromosome numbers, 36 or 40 (see for references Gavrilov-Zimin et al. 2015). The highest count, 2n=72, is found in *Amphorophora sensoriata* Mason, 1923 (Blackman 1980).

Like the aforementioned scale insect genus Apiomorpha showing the entire chromosome number range known in Coccinea, the aphid genus Amphorophora Buckton, 1876 is unique among Aphidinea demonstrating the entire range of chromosome numbers, from 4 to 72, known in aphids (Blackman 1980, 1985). This genus was shown to have 10 different chromosome counts (4, 10, 12, 14, 18, 20, 30, 40, 48, and 72) in 19 studied species and subspecies (see Gavrilov-Zimin et al. 2015). Chromosome numbers vary, although within narrower limits, in some other genera of Aphidinea, e.g. Phylloxera Boyer de Fonscolombe, 1834 (6-22), Glyphina Koch, 1856 (8-55), Forda von Heyden, 1837 (18-30), Tetraneura Hartig, 1841 (10-26), Cinara Curtis, 1835 (10-22), Lachnus Burmeister, 1835 (8-38), Euceraphis Walker, 1870 (8-22), Chaitophorus Koch, 1854 (4-40), and Trama von Heyden, 1837 (8-23) (see Gavrilov-Zimin et al. 2015). Almost permanently parthenogenetic genus Trama shows great interspecific and intraspecific diversity of structurally heterozygous karyotypes (Blackman et al. 2000). Only in a few collecting sites of southern England, chromosome number was found to vary from 14 to 23 in T. troglodytes von Heyden, 1837, from 9 to 12 in T. caudata del Guercio, 1909, and from 10 to 14 in T. maritima (Eastop, 1953). It suggests a high rate of karyotype evolution in the above species. The observed karyotype variability appeared to have no association with host plant. This is so even with the most polyphagous species, T. troglodytes, which evidences that no host race or biotype formation is occurring or even may occur in the absence of bisexual reproduction (Blackman et al. 2000). This hypothesis is consistent with DNA sequence data evidencing that specimens of *T. troglodytes* sampled from different plants have the same (or very similar) mitochondrial DNA haplotype (Normark 1999). An association between chromosome number and host plant has been described in the corn leaf aphid Rhopalosiphum maidis (Fitch, 1856), which has 2n=10 on barley in the northern hemisphere but 2n=8 on maize, sorghum, and Johnson grass (Sorghum halepense (Linnaeus, 1753) in all parts of the world (Brown and Blackman 1988), and in species of the genus Sitobion Mordvilko, 1914, which show 2n=12 on ferns while 2n=18 on grasses (Hales et al. 1997).

The karyotypes of some *Trama* species were found to include a variable number of small mainly heterochromatic chromosomal elements. It is of interest that intraspecific changes in 2n mostly involved these heterochromatic elements but not the euchromatic chromosomes, which remain relatively stable in both number and size (Blackman et al. 2000). In bisexual aphid species that display an XX/X(0) sex chromosome

composition, X-chromosomes are known to have much heterochromatin and also carry *rRNA* genes (Blackman 1985; Mandrioli et al. 1999; Criniti et al. 2009). The rDNA-FISH experiments carried out on different *Trama* species, showed one to six hybridization signals on heterochromatic elements suggesting that they could have originated from the redundant X chromatin and most likely represent B-chromosomes (Blackman et al. 2020). In species of the genus *Euceraphis* and some related genera, one or more B-chromosomes, also presumably of X chromosomal origin, have also been identified or at least suggested (Blackman 1988). Despite the above cases of chromosome number variability, most aphid genera have a remarkably stable number of chromosomes. For example, in the large genus *Dysaphis* Börner, 1931, all of the 31 studied species were shown to have 2n=12; in the species-rich genus *Aphis* Linnaeus, 1758, the absolute majority of nearly 100 studied species have 2n=8 (for other examples, see Gavrilov-Zimin et al. 2015).

Karyotypes including 2n=8, 10 and 12 can be considered today as modal karyotypes for Aphidinea as a whole. These numbers clearly prevail in the largest (3,035 species in 273 genera) and the best cytogenetically studied (601 species in 119 genera) family Aphididae. These numbers or, at least, some of them are found and prevail in other relatively better studied families, including Drepanosiphidae (141 studied species in 48 genera; 2n=6–48 with the numbers 8, 4, and 18 being, accordingly, most common), Eriosomatidae (86 species in 28 genera; 2n=6–38 with 10, 12, 20 being most common), and Lachnidae (72 species in 11 genera; 2n=6–60 with 10, 12, 14 being most common). Finally, in the family Hormaphididae (with 25 studied species in 9 genera; 2n=8–50), 2n=12 was more common than others. All other families are poorly studied without giving the opportunity to identify modal values.

Like some primitive scale insects, aphids have an XX/X(0) sex determination. The transition between parthenogenetic and bisexual reproduction in the complicated aphid life cycle involves a number of peculiar cytogenetic processes still not studied and understood in necessary details. For example, in order for cyclical parthenogenesis to occur, all the progeny developing from fertilized eggs has to be XX female, whereas all sperm must have only one X-chromosome. This is brought about by the elimination of one of the two X-chromosomes during the single maturation division of the parthenogenetic egg what happens once a year (Orlando 1974; Blackman and Hales 1986; Blackman 1987; Jaquiéry et al. 2012). On the other hand, the formation of the female-only parthenogenetic progeny from the bisexual population involves the elimination of male gametes that do not carry an X. Thus, the X-chromosome of aphids is transmitted half of the time by males and half of the time by females (see the whole annual cycle of Acyrthosiphon pisum (M. Harris, 1776) on Fig. 1 in Jaquiéry et al. 2012). In aphids, sex determination, instead of being achieved by stochastic combination of male and female chromosome sets during fertilization (as it happens with other X(0) organisms), is mediated by endocrine factors in response to environmental stimuli (Manicardi et al. 2015). Such a complicated and unique (possibly within Insecta in general) system highlights a special "Aphidoid type" of sex determination in parallel with such unusual systems as Lecanoid and Diaspidoid known in Coccinea (Gavrilov-Zimin et al. 2015).

In addition to the aforementioned *Trama* spp, a number of other aphid species have multiple sex chromosomes originated most likely via X-chromosome fissions, although other mechanisms can also be suggested. Some species of the families Adelgidae and Greenideidae have up to four pairs of X-chromosomes, and some species of the families Phylloxeridae, Eriosomatidae, Lachnidae, and Drepanosiphidae have two pairs of sex chromosomes (see Gavrilov-Zimin et al. 2015). In all above species, despite the presence of numerous sex chromosomes, the sex determination system still remains the same, $X_n X_n (0)$ (male/female). Hales (1989) described a peculiar Xchromosome constitution in the obligately holocyclic species *Schoutedenia lutea* (van der Goot, 1917) (Greenideidae). The female karyotype of this species consists of 4 long chromosomes and 12 short chromosomes. Some embryonic cells, assumed to be male cells, showed 12 short and only 2 long chromosomes suggesting that *S. lutea* has

male cells, showed 12 short and only 2 long chromosomes suggesting that *S. tutea* has $2n=12A+X_1X_1X_2X_2/X_1X_2$ (female/male). This sex chromosome constitution, which is complex in itself, is further complicated by the fusion between sex chromosomes and a pair of autosomes leading to that the sex chromosome constitution of this species turns into an even more complex structure (X_1+A_1 ; X_2+A_2 ; X_1 ; X_2). The latter might be expected to persist only if it conferred some powerful adaptive advantage to the species; however, speculations on possible explanations were argued to be premature in the absence of information on male meiosis in *S. lutea* (Hales 1989).

Despite the large number of karyotyped species, there is not much information yet about the chromatin structure and organization of aphid chromosomes. However, the information that is available is very interesting (e.g. Blackman 1985; Mandrioli et al. 1999, 2014; Blackman et al. 2000; Criniti et al. 2009; Monti et al. 2011; Manicardi et al. 2015). Genes for rRNA (18S, 5.8S, 28S), known to be located on both autosomes and sex chromosomes in other holokinetic groups (see elsewhere), are located (except for a few cases) in aphids exclusively on X chromosomes and mostly correspond to the C-positive heterochromatic areas at one of their ends (see Manicardi et al. 2015). Such a conserved position of C-blocks and *rRNA* genes is probably due to the fact that they are involved in the association of X chromosomes to each other and in the delay in their separation from each other during the complicated processes of transition between parthenogenetic and sexual reproduction in aphids (Orlando 1974; Blackman and Hales 1986). At least four aphid species were shown to have the insect-type motif of telomere (TTAGG), (see for references Vershinina and Kuznetsova 2016); however, in the Russian wheat aphid, Diuraphis noxia (Kurdjumov, 1913), this telomeric sequence was not identified (Novotna et al. 2011). An interesting finding made on the TTAGG-positive Myzus persicae (Sulzer, 1776) is that its telomerase is capable of producing telomeres de novo to stabilize chromosome fragments, if they arise (Mandrioli et al. 2014 and references therein).

Aleyrodinea

Aleyrodinea (whiteflies) represent a small suborder of sternorrhynchous Homoptera with a single family Aleyrodidae that includes about 1,600 described species in the world fauna (Ouvrard and Martin 2018). Whiteflies are small (1–3 mm in body length)

sap-sucking phytophagous insects; some species are serious pests of various crops and ornamentals while others are capable of vectoring viruses in agricultural crops. White-flies are of cytogenetic interest because of their haploid males (White 1973), although the presumption of haplodiploidy for the group in general is based on scant information coming from very few species (Blackman 1995; Blackman and Cahill 1998).

To date, only four whitefly species have been studied cytologically. Male haploid chromosome numbers are known for Trialeurodes vaporariorum (Westwood, 1856), n=11, Aleurotulus nephrolepidis (Quaintance, 1900), n=13, and for Aleyrodes proletella (Linnaeus, 1758), n=13 and/or 14 (Thomsen 1927). Both male (n=10) and female (2n=20) numbers are known for Bemisia tabaci (Gennadius, 1889) complex sampled from 4 populations representing either sibling species or host races and/or biotypes (Blackman and Cahill 1998). To test the aforementioned haplodiploidy hypothesis, the last authors made chromosomal preparations from eggs extracted from both unmated and mated females of *B. tabaci*. They observed that in the first case, the laid eggs had 10 chromosomes, whereas in the second case they had either 10 or 20 chromosomes suggesting, thus, that fertilized females of B. tabaci are capable to give bisexual offspring including diploid females and haploid males. These observations show that sex determination in whiteflies is more complex than true haplodiploidy. We can assume that B. tabaci exhibits haplodiploidy in the form of paternal genome elimination (PGE) as is the case in some Hymenoptera species (Crozier 1975; Bull 1983), some Coccinea (see above) and in a number of other insects (see Vershinina and Kuznetsova 2016; Gokhman and Kuznetsova 2018). More special research is needed to understand sex determination system of whiteflies. Chromosomes of B. tabaci lack detectable centromeres being most likely holokinetic as in their relatives within the Homoptera; finally, no detectable differences in chromosome morphology were found between studied populations of *B. tabaci* (Blackman and Cahill 1998). There are data in the literature suggesting that the telomeres of whiteflies, at least in B. tabaci and Trialeurodes vaporariorum, consist of the canonical insect telomere motif (TTAGG), (Frydrychová et al. 2004; Luan et al. 2018). However, the evidence is clearly insufficient. In the first species, the conclusion about the presence of TTAGG repeats was made according to the positive results of Southern hybridization. This technique is however much less reliable than FISH, since it detects repetitive sequences located not only at telomeric sites (Frydrychová et al. 2004). In the second species, the TTAGG repeats were detected in the bacteriocyte genome (insect cells harboring symbiotic bacteria).

Psyllinea

Psyllinea (jumping plant-lice) form a moderate-sized group of sternorrhynchous Homoptera comprising nearly 4,000 species (in more than 200 genera) described from across every biogeographic region of the world, most of which from tropical and subtropical regions (Burckhardt and Ouvrard 2012; Ouvrard 2020). As with related aphids (Aphidinea), scale insects (Coccinea), and whiteflies (Aleyrodinea), psyllids feed on the phloem of vascular plants but, unlike them, they are generally oligophages

restricted to one or a few closely related host plants, particularly at the larval stage. Several species are harmful to their host plants acting as vectors for various plant diseases, as economically important pests in agriculture and forestry, and as potential biocontrol agents for some invasive plants (Burckhardt et al. 2014; Percy et al. 2018). In a recent classification of Psyllinea (Burckhardt and Ouvrard 2012), as many as 8 families have been recognized incuding Aphalaridae, Carsidaridae, Calophyidae, Homotomidae, Liviidae, Phacopteronidae, Psyllidae, and Triozidae. Cytogenetically, approximately 220 species from 55 genera (about 5.3% and 27% of their total number, respectively) have been investigated to date (Maryańska-Nadachowska and Głowacka 2005; Labina et al. 2007; Kuznetsova et al. 2012; Nokkala et al. 2019; for other references, see review of Maryańska-Nadachowska 2002). Karyotypes, mainly in terms of chromosome number and sex chromosome system, are currently known for all families with the exception of the tropical most basal family Phacopteronidae (Cho et al. 2019). Most data concern representatives of the Psyllidae (Spondiliaspidinae, Psyllinae), Aphalaridae (Aphalarinae, Rhinocolinae), and Triozidae, whereas other families have been relatively poorly studied.

With rare exceptions (Labina et al. 2009; Nokkala et al. 2013, 2015, 2019), psyllids reproduce bisexually. The bisexual species have diploid chromosome numbers varying between 7 and 27 in males with a distinct mode at 25, suggesting that this is the ancestral count of the Psyllinea. The most frequent karyotype, 2n=24A+XX/X(0), is found in approximately 80% and 52% of the studied species and genera, respectively, and in each of the studied families being, therefore, considered as evolutionarily ancestral for Psyllinea as a whole (Kuznetsova et al. 1997; Maryańska-Nadachowska 2002). All other karyotypes could have evolved independently as derived characters in different families. The number of autosomes in derived karyotypes is usually lower than the mode. An exception is the karyotype of *Pauropsylla tricheata* Pettey, 1924 (Triozidae) comprising 2n=26A+X(0) and attributing to the fission of one pair of autosomes (Maryańska-Nadachowska and Głowacka 2005). Other few cases of an increase of chromosome number are associated with B-chromosomes and polyploidy (see below).

Based on available data, it can be suggested that the ancestral Psyllinea lineage experienced a series of chromosomal rearrangements, among which chromosome fusions most likely dominated. Rearrangements, other than fusions/fissions, do not alter chromosome number and size and remain unfortunately undistinguishable in chromosome preparations because of the absence of reliable chromosomal markers. In the majority of known cases only a few chromosome fusions have occurred, resulting in insignificant differences in chromosome numbers between related species. There are two impressive exceptions. Within the family Psyllidae, which displays predominantly 2n=25, the Australian subfamily Spondiliaspidinae is characterized by very low chromosome numbers, 2n=7, 9 or 11, found in males of all so far studied species (16 species, 10 genera, tribes Ctenarytainini and Spondyliaspidini) (Maryańska-Nadachowska et al. 2001). These low-numbered karyotypes suggest their common origin, with 2n=10A+XX/X(0) being supposedly an ancestral trait in this group. Data on chromosome numbers suggest an independent reduction of 2n at least once in Ctenarytainini and at least once in

Spondiliaspidini. Another group with low chromosome numbers, 2n=11 and 2n=13 (males), is the subfamily Rhinocolinae (Aphalaridae); however, in this group, data are still available only for 4 species (see Maryańska-Nadachowska 2002).

Undoubtedly, the X(0) sex chromosome system is the ancestral one in Psyllinea. Several species have a neo-XY system or a neo-X1X,Y system. Two species, Cacopsylla sorbi (Linnaeus, 1767) and C. mali (Schmidberger, 1836), were reported to have derived sex chromosome systems originating from one or several autosome-autosome and X-autosome fusions (Grozeva and Maryańska-Nadachowska 1995; Maryańska-Nadachowska et al. 2018). In C. sorbi, different male karyotypes, 2n=24A+X and 2n=20A+neo-XY, were found in various European populations (Suomalainen and Halkka 1963; Grozeva and Maryańska-Nadachowska 1995; Maryańska-Nadachowska and Grozeva 2001; Maryańska-Nadachowska et al. 2018). The neo-XY cytotype was suggested to have originated from 2n=24A+X through at least two fusions, one between two pairs of ancestral autosomes (resulting in 22A+X), and another between one of the fusion chromosomes and the X. In C. mali, a fusion supposedly occurred first between an autosomal pair and the X in the progenitor karyotype 2n=24A+X, resulting in 2n=22A+neo-XY; a further fusion occurred between the neo-XY and another autosomal pair resulting in 2n=20A+neo-X,X,Y (neo-X,X,Y cytotype) (Grozeva and Maryańska-Nadachowska 1995). Both forms (the cytotypes) coexist within geographically distant populations and are morphologically indistinguishable from each other (Grozeva and Maryańska-Nadachowska 1995; Maryańska-Nadachowska and Grozeva 2001; Nokkala et al. 2004; Maryańska-Nadachowska et al. 2018). These forms are most likely conspecific. A reason for the neo-X,X,Y system to be present in a polymorphic state (together with the neo-XY system) has been attributed to the high frequency of unbalanced gametes produced during meiosis in X1X,Y individuals, thus, resulting in the neo-X1X,Y system being unable to become fixed in a population (Nokkala et al. 2004).

The intraspecific variation in the chromosome number of Psyllinea is sometimes related to the occurrence of B-chromosome (Maryańska-Nadachowska 2002). Bchromosomes are generally considered as extra chromosomes that are often selfish in their transmission and lack the ability to meiotic pairing unlike A chromosomes. The number of B-chromosomes can vary among populations of the same species, among individuals in a population and among cells in an individual (Camacho et al. 2000; Ahmad and Martins 2019). Two psyllid species, Rhinocola aceris Linnaeus, 1758 and Cacopsylla peregrina (Foerster, 1848), have been studied in detail in terms of B chromosome distribution, C-banding pattern and behavior in meiosis. In R. aceris, B chromosomes varied in number (from one to three) in geographically distant populations and differed from each other in size, C-banding pattern and alleged origin (Maryańska-Nadachowska 1999). In male meiosis of both R. aceris and C. peregrina, a B-chromosome and the X chromosome were observed to appear as univalents during prophase I stages, displayed a "touch and go" pairing at metaphase I, and underwent quite regular segregation at anaphase I (Nokkala et al. 2000, 2003). To account for this peculiar behavior, the authors of the works suggested that a B-chromosome was integrated into an achiasmatic segregation mechanism with the X chromosome in a place

normally occupied by a Y chromosome in species with achiasmatic XY systems. They hypothesized that Y chromosome may arise from a mitotically stable B-chromosome that was first integrated into an achiasmatic segregation mechanism with the X and, then, became fixed in the karyotype as a Y chromosome. In *R. aceris*, consecutive stages of the conversion of a B-chromosome into a Y chromosome were detected in different populations (Nokkala et al. 2000; Nokkala and Nokkala 2004). It was conjectured that a Y chromosome formed this way was a morphological Y chromosome only and carried no male determining genes (Nokkala et al. 2003). It was suggested that the same mechanism underlined the origin of the achiasmatic Y chromosome in some species of Cicadinea (see Kuznetsova and Aguin-Pombo 2015) and in *Drosophila* (Diptera) (Carvalho et al. 2009), as well as the origin of a W chromosome in Lepidoptera (Fraïsse et al. 2017).

In psyllids, all three known cases of polyploidy (three species) have been detected in the same genus Cacopsylla Ossiannilsson, 1970 (family Psyllidae), which is the most diverse genus of Psyllinea, with over 500 known species distributed throughout the Holarctic Region, and spreading into the Oriental Region (Ouvrard et al. 2015). The first of these species, Cacopsylla myrtilli W. Wagner, 1947, is widely distributed throughout the Palaearctic, while its distribution also shows a shift towards the north and/or high altitudes. Females of this species are usually triploid (2n=3x=36A+XXX) and reproduce through apomictic parthenogenesis, while rare diploids also exist. Infrequent, mainly nonfunctional, but also functional males with 2n=24A+X can be found in some populations (Nokkala et al. 2013, 2015). Another species, Cacopsylla borealis Nokkala et Nokkala, 2019, is common and widespread throughout the Palaearctic too. Its distribution range reaches from northern Fennoscandia in the West to Magadan in the East. C. borealis is a pentaploid species (2n=5x=60A+XXXXX) with apomictic parthenogenetic reproduction. No males have been recorded in C. borealis so far (Nokkala et al. 2019). The third species, Cacopsylla ledi (Flor, 1861), is widely distributed throughout Fennoscandia, Central Europe, and Russia; it occasionally forms sympatric populations with C. borealis. Its habitats are restricted to the temperate and alpine zones. The species is triploid (2n=3x=36A+XXX) and reproduces through apomictic parthenogenesis, while infrequent functional males (2n=24A+X) can be found in some populations. In such populations with rare males, infrequent diploid females (2n=24A+XX) also exist among the triploids (Nokkala et al. 2015). The above examples demonstrate the unique possibilities of cytogenetic methods for studying the nature of reproduction and the structure of populations of insects.

Cicadinea

Cicadinea (sometimes referred to as "true hoppers") are a large group of auchenorrhynchous Homoptera comprising more than 47,000 species distributed worldwide. The true hoppers are generally monophagous or narrowly oligophagous; many species are of economic significance acting as pests of agricultural crops and vectors of plant pathogens, including phytoplasmas, viruses, spiroplasmas, and bacteria. The two major lineages are recognized within the suborder: the infraorder Cicadomorpha comprising four superfamilies, Cicadoidea (cicadas), Cercopoidea (froghoppers and spittlebugs), Membracoidea (leafhoppers and treehoppers), and Myerslopoidea (ground-dwelling leafhoppers), and the infraorder Fulgoromorpha comprising the only superfamily Fulgoroidea (planthoppers) (Bartlett et al. 2018). Cicadomorpha are subdivided into 13 families with approximately 30,000 described species; the Fulgoromorpha is subdivided into about 20 families (depending on the classification followed) with more than 12,000 described species (Dietrich 2002; Cryan 2005; Deitz 2008; Bartlett et al. 2018).

Karyotypes of approximately 850 species (nearly 2% of the total number of species described) belonging to 500 genera of 31 families representing all currently recognized superfamilies of the suborder Cicadinea were studied up to now (reviewed in Kirillova 1986, 1987; Emeljanov and Kirillova 1990, 1992; Kuznetsova and Aguin-Pombo 2015; see also Maryańska-Nadachowska et al. 2016; Anjos et al. 2016, 2018; Anjos 2017; Karagyan et al. 2020). Since the late 1990s, chromosome studies of Cicadinea have been carried out using C- and AgNOR- banding, base-specific fluorochrome-banding, and FISH with a number of DNA probes. The use of these methods and approaches allowed to identify specific regions in chromosomes of Cicadinea and enhanced understanding their chromosome structure (Anjos et al. 2016, 2018; Maryańska-Nadachowska et al. 2016; Anjos 2017; Karagyan et al. 2020; for earlier publications, see Kuznetsova and Aguin-Pombo 2015). In particular, it has been shown that the chromosomes of true hoppers have, like in the majority of other Paraneoptera, the insect (TTAGG)_n motif of telomeres (Karagyan et al. 2020; for other references see Kuznetsova et al. 2020).

According to Kuznetsova and Aguin-Pombo (2015), chromosome numbers in Cicadinea range between 8 and 38 (2n, female); the lowest and the highest numbers are found in Cicadomorpha (Cicadellidae) and Fulgoromorpha (Delphacidae and Dictyopharidae), respectively. Despite the fact that since 2015 new data have appeared, the above series has remained unchanged. Chromosome numbers exceeding 38, when reported, are all related to parthenogenesis (true parthenogenesis referred to as thelytoky or gynogenesis, sometimes to as pseudogamy) and accompanying polyploidy. For example, up to 45 chromosomes have been found in pseudogamous triploid females of Muellerianella fairmarei Perris (Booij 1981, 1982) and other planthopper species belonging to the genera Ribautodelphax Wagner, 1963 (den Bieman 1988) and Delphacodes Fieber, 1866 (den Bieman and de Vrijer 1987). Cicadomorpha and Fulgoromorpha differ both in the limits of variation in chromosome number and in the modal numbers. Within each infraorder, some taxa have more than one modal number and these numbers are characteristically lower in Cicadomorpha than in Fulgoromorpha. In the latter, 2n varies from 20 (Pentastiridius hodgarti Distant, 1911 in Cixiidae) to 38 (Scolops spp. in Dictyopharidae and Paraliburnia clypealis J. Sahlberg, 1871 in Delphacidae), with strongly marked mode at 28 and the second and the third modes at 30 and 26, respectively. In Cicadomorpha, 2n varies from 8 (Orosius sp., Cicadellidae) to 32 (Peuceptyelus coriaceus Fallén, 1826, Aphrophoridae). Most species have 2n between 20 and 28, other counts being rare. Specifically, in Cercopoidea 2n varies between 14 and 32, with the mode at 26-28; in Cicadoidea between 12 and 20, with the mode at 20; in Membracoidea between 8 and 28, with the mode at 22 (Emeljanov and Kirillova 1992; Kuznetsova and Aguin-Pombo 2015; Anjos et al. 2018). In Myerslopoidea, the only studied species, *Mapuchea chilensis* (Nielson, 1996), has 2n=16+XY. The XY system in this species is most likely of a neo-XY type and indicates the derivative nature of the karyotype, which could be a result of a fusion between the original X and an autosome in the original karyotype of 2n=18+X(0) (Golub et al. 2014).

Some higher taxa of Cicadinea show stable or only slightly variable karyotypes. Quite often, the chromosome number is constant within the genus and even within the family suggesting that fusion/fission events were rare in their evolution. Supporting examples can be found in the review of Kuznetsova and Aguin-Pombo (2015) and in some more recent original publications. Some impressive examples of this sort come from the groups, which have been more fully explored, e.g. the families Dictyopharidae and Issidae (Fulgoroidea), and the cicada genus Magicicada Davis, 1925 (Cicadidae, Cicadoidea). The family Dictyopharidae is one of the largest families of planthoppers worldwide, with 720 valid species and 156 valid genera at present (Bourgoin 2016). The family is classified into the two subfamilies, Dictyopharinae and Orgeriinae. The karyotypes are known in 18 species (7 genera) of the first subfamily and in 29 species (17 genera) of the second subfamily. In Dictyopharinae, the tribe Dictyopharini (with about 14% of species and genera studied) is characterized by 2n=28A+X(0) in males. Within Orgeriinae, the most primitive tribe Ranissini (about 20% and 70%, respectively) and one of the most advanced tribes, Orgeriini (about 7% and 20%), show 2n=26A+X(0) in all the species studied. The tribe Almanini (about 20% and 60%) is characterized by 2n=24A+neo-XY. Available data suggest that the ancestral karyotype of the Dictyopharidae included 2n=28A+X(0) and karyotypic transformations in the evolution of the family occurred mainly by fusion of chromosomes (Kuznetsova 1986; Kuznetsova et al. 2009). For the world-wide family Issidae, a lot of new data have been received recently (Maryańska-Nadachowska et al. 2016). The family comprises approximately 1,000 species with around 170 genera classified within the only subfamily Issinae with the three tribes, Issini, Hemisphaeriini and Parahiraciini (Gnezdilov 2013). In general, karyotypes have been studied in 44 (5%) species from 27 (15%) genera covering all the three recognized tribes (Maryańska-Nadachowska et al. 2016 and references therein). Available data suggest that Issidae are a group characterized by a high karyotypic conservatism, which manifests itself primarily in the same karyotype, 2n=26A+XX/X(0), found in all but three studied species. The basic issid karyotype appears also conservative in structure. Every species was shown to have a very large pair of autosomes that also carry rDNA clusters what has been confirmed by both different chromosomal staining techniques (AgNOR banding and DNA-binding fluorochrome CMA₂) and rDNA-FISH. The newly obtained data support, thus, the hypothesis that the karyotype of 2n=26A+XX/X(0) has the monophyletic origin and represents an ancestral character state for the family Issidae in general (Maryańska-Nadachowska et al. 2006; Kuznetsova et al. 2010). The chromosome number decreased independently at least three times in the evolution of Issidae, and all three reduction events happened in the same tribe Issini. Males of Latilica maculipes (Melichar, 1906) and Brahmaloka

sp. were shown to have 2n=24A+X, whereas males of *Falcidius limbatus* (A. Costa, 1864) were found to have 2n=24A+neo-XY. Both derived karyotypes could have arisen by a single tandem fusion in the ancestral karyotype of 2n=26A+X, the first between two pairs of autosomes resulting in 2n=24A+X, and the second between an autosome and the X resulting in 2n=24A+neo-XY. The genus *Magicicada* (Cicadidae) inhabiting eastern United States and comprising the periodical cicadas remarkable for their 17- or 13-year synchronized life cycles and periodical mass emergence of adults, has 2n=18A+XX/X(0) in all the seven recognized species (Karagyan et al. 2020). Moreover, the same chromosome number seems to be characteristic of the family Cicadidae in general (Kuznetsova and Aguin-Pombo 2015).

The XX/X(0) sex determination is of common occurrence and seems to be an ancestral trait in both Cicadinea (Halkka 1959; Emeljanov and Kirillova 1990, 1992) and their allies (Blackman 1995). Despite evolutionary stability, in some cases the X(0) system has been replaced by an XY system in species within the same genus or even within the same family that are otherwise exclusively X(0), as is the case in the aforementioned family Dicrtyopharidae. Chromosome systems of sex determination evolved via autosome/sex chromosome fusion have been frequently reported in Cicadinea (Kuznetsova and Aguin-Pombo 2015). In a recently formed neo-XY system, the autosomally derived neo-Y chromosome and the autosomal part of the neo-X chromosome remain still homologous, and therefore synapse at prophase I of meiosis. Once a neo-XY system has arisen, it can undergo a further transformation into a multiple X,X,Y system as a result of the translocation involving the Y chromosome and another pair of autosomes. In representatives of the spittlebug genus Philaenus Stål, 1864 (Cercopoidea, Aphrophoridae), which were thoroughly studied using different methods and approaches (AgNOR- and C-banding; fluorochromes CMA, and DAPI; FISH with 18S rDNA and (TTAGG), telomeric probes), it was possible to trace almost all successive evolutionary stages of sex chromosome transformations (Maryańska-Nadachowska et al. 2012, 2013). A different, achiasmatic XY system, with a very small Y chromosome, was found in the planthopper species Limois emelianovi Oshanin, 1908 and L. kikuchii Kato, 1932 (Fulgoridae) (Kuznetsova 1986, Tian et al. 2004). It seems likely that the Y in such cases has originated from a mitotically stable B-chromosome through a mechanism suggested by Nokkala et al. (2004) and discussed in more detail above, in the psyllid part of this paper.

Theoretically, as mentioned above, fission and fusion of holokinetic chromosomes do not result in unbalanced meiotic products, and so these rearrangements may be preserved through generations and establish variations in chromosome number within populations. Yet, descriptions of chromosomal polymorphisms are quite rare in Cicadinea. We can anticipate that it is due to very few studies at the population level in this group (like in other Paraneoptera). However, some examples of polymorphism for B-chromosomes and for fission/fusion events have been described in natural populations of both leafhoppers and planthoppers (Kuznetsova and Aguin-Pombo 2015). There are also some groups of Cicadinea, in which a wide variety of chromosome numbers occurs suggesting that both fusions and fissions have established themselves during their evolution. For example, in the genus *Eurhadina* Haupt, 1929 (Cicadellidae), a full range from 2n=12 to 2n=20 was reported for only 19 studied species. The cosmopolitan genus *Empoasca* Walsh, 1862 with more than 800 nominal species (Southern and Dietrich 2010) is another group, which seems to show a striking range in chromosome number. In this genus, 12 species examined so far display 2n ranging from 16 to 22. Finally, the aforementioned spittlebug genus *Philaenus* displays 2n=20, 23, and 24 in only 12 studied species. More examples can be found in Kuznetsova and Aguin-Pombo (2015).

Heteroptera

Heteroptera (true bugs) are a very diverse group in terms of habitats (aquatic, terrestrial and parasitic on vertebrates, including human and birds) and feeding habits (phytophagous, predators, and hematophagous) (Weirauch and Schuh 2011). Several species have received intense focus for economic, medical or scientific reasons (Wang et al. 2019). Heteroptera are the largest order of Paraneoptera with more than 42,000 described species in about 90 families and seven infraorders including Leptopodomorpha, Gerromorpha, Nepomorpha, Pentatomomorpha, Cimicomorpha, Dipsocoromorpha, and Enicocephalomorpha (Štys and Kerzhner 1975; Henry 2017; Weirauch et al. 2019).

The very beginning of cytogenetic studies in Heteroptera dates back to the end of the 19th century when German biologist Hermann Henking (1891) discovered a "peculiar chromatin element" in sperm nuclei of Pyrrhocoris apterus Linnaeus, 1758 (Pyrrhocoridae), which he designated in his drawings by "x" (actually a sex chromosome). Since this great discovery, the following key developments have occurred in true bugs cytogenetics. In the period from 1905 to 1912, T.H. Montgomery and E.B. Wilson published a series of papers that actually marked the beginning of true bug cytology (see for references, Ueshima 1979). Hughes-Schrader and Schrader (1961) were the first who established that the chromosomes of true bugs lack centromeres, i.e. they are holokinetic. Ueshima (1979) published a monograph/survey devoted to cytogenetic characteristics of Heteroptera. Some of these characteristics, e.g. presence of a pair of m-chromosomes (see below) and the so-called sex chromosome "post-reduction" in male meiosis when sex chromosomes undergo equational division at anaphase I and reductional division at anaphase II, make true bugs unique among Paraneoptera and even among Insecta in general. The above monograph also contains a comprehensive check-list of chromosome numbers and sex chromosome systems known at that time for all heteropteran infraorders with the exception of Enicocephalomorpha, for which information is lacking to this day. Since Ueshima's (1979) excellent review, which until now remains the only source of information about chromosome numbers and sex chromosome mechanisms of Heteroptera, and a review by Manna (1984), a large amount of new cytogenetic data on the Heteroptera has been obtained. Papeschi and Bressa (2006) summarized and discussed data accumulated by that time on the basic aspects of true bugs cytogenetics and speculated about mechanisms of karyotype evolution in Heteroptera as a whole. According to this comprehensive review, heavily based on Ueshima's list, data were available for approximately 1,600 species belonging to 46 true bugs' families. Although the data set seemed to be impressive, the number of studied species by 2006 was no more than 4.2% of described true bug species. The authors have argued that chromosome numbers in true bugs vary from 2n=4 to 2n=80 but about 70% of the species have 12 to 34 chromosomes, with male diploid number of 14 being the most represented; sex chromosome mechanism is predominantly of the XX/ XY type (found in 71.4% of studied species), but other variants such as an XX/X(0) system (14.7%), multiple sex chromosome systems such as X₂X₂/X₂Y, X₂X₂/X₂(0), and XX/XY₂ (13.5%), as well as neo-sex chromosomes (0.5%) also occur. The main mechanisms of karyotype evolution in true bugs were argued to be fusions between autosomes, fusions between the X and an autosome, and fissions involved both autosomes and sex chromosomes. It is generally accepted that multiple sex chromosome systems in Heteroptera are the result of sex chromosome fissions (fragmentations) (Ueshima 1979). This is well supported by an example of *Cimex lectularius* that shows from 2 to 20 X-chromosomes in males of different European populations (Sadílek et al. 2013, 2019a, b). It has been hypothesized that tandem duplications in AT-rich regions on the X-chromosome increase the fragility of these regions, which induces fragmentations of the X-chromosome of C. lectularius (Sadílek et al. 2019a). In some cases, multiple systems can result from a non-disjunction (Ueshima 1979; Grozeva et al. 2011) or even a duplication of the X-chromosome (Sadílek et al. 2019b).

For a long time, the question of what mechanism, XY or X(0), was the evolutionarily initial in the Heteroptera has been actively debated. Two alternative hypotheses supported by different sources of evidence have been proposed. One of these holds that the XY system has evolved from an X(0) system (Ueshima 1979) while the other assumes that the XY mechanism is plesiomorphic, the existence of the X(0) species being a result of the repeated loss of the Y chromosome during the evolution (Nokkala and Nokkala 1983, 1984; Grozeva and Nokkala 1996; Pal and Vicoso 2015). Last listed authors argue that many true bugs have XY sex chromosomes, with the Y showing a typical reduction in size relative to the X, suggesting extensive loss of gene content on this chromosome. However, the choice between the above hypotheses still remains difficult, at least until data for the basal groups become available. At present, they are completely absent for Enicocephalomorpha and scarce for Dipsocoromorpha, in which 6 species have been studied and both systems have been found, including within the same genus *Pachycoleus* Fieber, 1860 (Grozeva and Nokkala 1996; Kuznetsova et al. 2011).

In karyotypes of many true bug species (within the infraorders Dipsocoromorpha, Nepomorpha, Leptopodomorpha, and Pentatomomorpha), a pair of so-called "m-chromosomes", has been described (Ueshima 1979; Grozeva and Nokkala 1996; Papeschi and Bressa 2006; Kuznetsova et al. 2011). The origin and significance of these peculiar chromosomes are still obscure. They behave differently from both autosomes and sex chromosomes during male meiosis. As a rule, m-chromosomes are extremely small while in some species they might be of approximately the same size as the autosomes (Grozeva et al. 2009). They are usually asynaptic and achiasmatic throughout early meiotic prophase (Ueshima 1979); however in male *Coreus marginatus* Linnaeus, 1758 (Pentatomomorpha, Coreidae), m-chromosomes were shown to undergo normal synapsis at pachytene assuming the chiasma formation (Nokkala 1986). The presence or absence of m-chromosomes is a stable character at higher taxonomic levels in

the Heteroptera (Ueshima 1979); however, there are exceptions to this rule (Grozeva and Simov 2008). The discovery of m-chromosomes in the families Dipsocoridae and Schizopteridae of the basal infraorder Dipsocoromorpha allows to suggest that m-chromosomes were present in the plesiomorphic karyotype of the Heteroptera in general (Grozeva and Nokkala 1996).

In a fair number of true bug species, the presence of some extra chromosomes in addition to the standard chromosome number has been confirmed (see for references Ueshima 1979; Kuznetsova et al. 2011). Available data point to a significant variability of these supernumeraries in terms of their size, C-heterochromatin amount and distribution, meiotic behavior and impact on segregation of A-chromosomes in the species. In most cases, these extra chromosomes are interpreted as B-chromosomes without any evidence. Poggio et al. (2013) conducted a thorough study of a population of the assassin bug *Zelurus femoralis longispinis* Lent et Wygodzinsky, 1954 (Reduviidae) polymorphic for the presence/absence of an extra chromosome. In the studied population, males with 2n=22(20A+XY) coexisted with males displaying 2n=23(20A+XY) + extra chromosome. The meiotic behavior of the extra chromosome was highly regular and similar to that of sex chromosomes. Using various cytogenetic approaches combined with a morphometric analysis of chromosome students, the authors concluded that this extra chromosome was an additional X chromosome rather than a B-chromosome (Poggio et al. 2013).

Over the past 15 years, several review papers devoted to individual higher taxa of true bugs have been published, namely, Cimicomorpha (Kuznetsova et al. 2011), Gerromorpha (Fairbairn et al. 2016), and Pentatomomorpha (Souza-Firmino et al. 2020). Besides, a great number of research papers have been published too (e.g. Panzera et al. 2010, 2012; Kuznetsova et al. 2012; Bardella et al. 2012, 2013, 2014; Chirino et al. 2013, 2017; Sadílek et al. 2013, 2019a, 2019b, 2020; Chirino and Bressa 2014; Pita et al. 2014, 2016; Kaur and Gaba 2015; Stoianova et al. 2015; Golub et al. 2016, 2017, 2018; Angus et al. 2017; Gallo et al. 2017; Grozeva et al. 2019; for other references see aforementioned reviews). As a result, the number of karyotyped species increased significantly and knowledge about karyotypes and their evolution in true bugs was expanded.

According to our rough estimates, since the last review (Papeschi and Bressa 2006) the number of cytologically studied true bug species has increased by almost 300 and, thus, reached approximately 1,900 (5% of described species). However, despite the expanded data-set, the overall picture of karyotype variability has changed significantly neither within the individual higher lineages nor within the Heteroptera in general. The lowest and the highest chromosome numbers remained the same, 2n=4 (*Lethocerus* sp., Belostomatidae, Nepomorpha) to 2n=80 (4 species of genus *Lopidea* Uhler 1872, Miridae, Cimicomorpha) as reported by Chickering (1927) and Akingbohungbe (1974), respectively; other species with these extreme counts were not found. The infraorder of semiaquatic bugs, Gerromorpha, in which the number of studied species has more than doubled (51 against 21), although enriched with new interesting data (Fairbairn 2016), can still be seen as a group only slightly varying in chromosome number, with predominantly XX/X(0) sex determination and the absence of m-chromosomes (as stated by Andersen 1982).

In recent years, knowledge of true bug cytogenetics has advanced significantly due to the use of modern techniques and approaches (chromosomal bandings, FISH, DNA content, etc.). For example, within the largest evolutionarily advanced and highly diversified infraorder Cimicomorpha, this applies to the families Cimicidae (e.g. Sadílek et al. 2019a, 2019a), Tingidae (Golub et al. 2015, 2016, 2017, 2018); Nabidae (Grozeva et al. 2004; Sadílek et al. 2020), and Reduviidae (e.g. Pita et al. 2014, 2016; Bardella et al. 2014; Grozeva et al. 2019). Specifically, Sadílek et al. (2020) using genome size data (together with rDNA-FISH) decided between two alternative hypotheses about the direction and mechanisms of karyotype evolution in the family Nabidae (Kuznetsova and Maryańska-Nadachowska 2000; Nokkala et al. 2007). The data obtained confirmed the hypothesis that the ancestral karyotype of Nabidae included 2n=16A+XY, and the karyotype 2n=32A+XY of Himacerus Wolff, 1811 spp. originated via polyploidization of autosomes (Kuznetsova et al. 2000). Then, Pita et al. (2016) and after them Grozeva et al. (2019) found out that the family Reduviidae, at least, the kissing bug subfamily Triatominae and the largest reduviid subfamily Harpactorinae, have the insect-type (TTAGG), motif of telomeres. It should be noted that in true bugs this motif was first identified in Lethocerus patruelis (Stål, 1854) from the family Belostomatidae, Nepomorpha (Kuznetsova et al. 2012), and all other true bug groups studied in this respect turned out to have lost the (TTAGG), motif (Grozeva et al. 2011; Kuznetsova et al. 2012). In a series of publications on the family Tingidae (Golub et al. 2015, 2016, 2017, 2018) it was shown, firstly, that lace bugs also lost this telomere motif and, secondly, that closely related species of lace bugs share the same or similar karyotypes (at least, the same number of autosomes) but differ in the rDNA site location. Significant advances have been made recently in cytogenetics of the true water bug infraorder Nepomorpha, the families Nepidae, Aphelocheiridae and especially Belostomatidae, which is the best-studied family within this group in terms of karyotypes, meiosis and chromosome evolution (e.g., Kuznetsova et al. 2012; Chirino et al. 2013, 2017; Grozeva et al. 2013; Wisoram et al. 2013; Gallo et al. 2017). Species of the genus Belostoma Latreille 1807 (Belostomatidae) were shown to differ from one another in chromosome number and sex chromosome systems; besides, interstitial telomere sequences (ITS) found in some species were interpreted as signs of telomere-telomere fusions that took place in the evolution of the genus (Chirino et al. 2017). In the same family Belostomatidae, the species Lethocerus patruelis (Stål, 1855) was found to have a conventional prereductional division of sex chromosomes in male meiosis, what distinguishes it from all other studied species of this family (Grozeva et al. 2013 and references therein). Although pre-reduction of sex chromosomes is not usual in Heteroptera, it does occur in some groups, and even closely related species occasionally differ in this pattern (Ueshima 1979; Grozeva et al. 2006, 2007). It shoud be noted in this regard that lace bugs (Tingidae, Cimicomorpha) are the only true bug family showing this meiotic pattern in all hitherto studied species (Ueshima 1979, Grozeva and Nokkala 2001; Golub et al. 2015, 2016, 2017, 2018). Recently, the first data on C-banding and FISH were published for the family Nepidae (Angus et al. 2017). At the same time, the first chromosomal data were obtained for the benthic true bug family Aphelocheiridae, in which

all the three studied species, *Aphelocheirus aestivalis* (Fabricius, 1794), *A. murcius* Nieser et Millán, 1989, and *Aphelocheirus* sp. (from northern Spain), were shown to have the same karyotype, 2n=22+XX/X(0) (Stoianova et al. 2017).

Coleorrhyncha

Coleorrhyncha (moss bugs or peloridiids) are little-known insects believed to be relict members of an ancient lineage of Hemiptera (Evans 1982). This taxonomically small group comprises 17 genera and 36 species of small insects (up to 5 mm long) with a cryptic lifestyle (Burckhardt 2009; Burckhardt et al. 2011). These "living fossils" inhabit temperate and sub-Antarctic rainforests of the southern Hemisphere, where



Figure 1. The mapping of diploid autosome numbers, male sex chromosome systems, and both modal and putative ancestral states of these characters onto phylogenetic tree of Paraneoptera. The phylogenetic tree is based on Shcherbakov and Popov (2002), Kluge (2020), and Gavrilov-Zimin (2020a), with modifications. Putative ancestral autosome numbers (2n) are indicated by black solid circles (\bullet); putative ancestral sex chromosome systems are indicated by black solid squares (\blacksquare).*Total 2n (sex chromosomes not identified) in female.

they live in and feed on bryophytes and hepatics without moving much (Burckhardt 2009; Shcherbakov 2014). The phylogenetic relationships of moss bugs have been a matter of debates for a long time. In the past, Coleorrhyncha have been variously assigned to the Heteroptera or to the Homoptera since they possess a mixture of cicadomorphan and bug-like characters (Bechly and Szwedo 2007). Today, they are usually considered to be the sole family (the Peloridiidae) of the separate suborder Coleorrhyncha, which is treated as the sister group to the Heteroptera (Larivière et al. 2011), though there are data supporting divergent opinions as well (e.g. Cui et al. 2013). Recently, the first cytogenetic data on Coleorrhyncha were published. The species Xenophyes cascus Bergroth, 1924 from New Zealand and Peloridium pomponorum Shcherbakov, 2014 from Chile were reported to have 27(26A+X) and 33(2n=32A+X) holokinetic chromosomes, respectively (Grozeva et al. 2014; Kuznetsova et al. 2015). Besides, both species appeared to display the inverted sequence of sex chromosome divisions during spermatocyte meiosis, the so-called sex chromosome post-reduction previously known only in the Heteroptera (Ueshima 1979; Papeschi and Bressa 2006; Kuznetsova et al. 2011). This unique feature can be considered as an additional synapomorphy of Heteroptera + Coleorrhyncha (Kuznetsova et al. 2015; Wang et al. 2019).

Conclusions

The overview presented here, shows that the supercohort Paraneoptera is a very diverse group, interesting for comparative cytogenetic studies, with different evolutionary scenarios from the maintenance of a preserved karyotype condition to greatly derived karyotype characteristics that can be traced within each of the higher-level taxa.

With only the intriguing exception of Thysanoptera, all Paraenoptera insects have holokinetic chromosomes. Paraneoptera have a great variety of sex chromosome systems, among which simple systems XX/XY and XX/X(0) clearly prevail. One or both systems are present in every major lineage, with rare exceptions where sex chromosomes either not identified or really absent (Thysanoptera, Parasita, and Aleyrodinea). The X(0) system has been recognized by different authors as the ancestral one for a number of groups including the most basal Copeognatha, and it appears to be an attractive candidate for the ancestral sex chromosome system for Paraneoptera clade in general (Fig. 1). Paraneoptera exhibit a large range of chromosome numbers varying from 2n=4 to 2n≈192. The lowest count is found in several groups including Parasita (Gyropus ovalis, Amblicera, Mallophaga), Homoptera Coccinea (at least 5 species of the genus Apiomorpha from Eriococcidae, and at least 16 species in 6 genera of the tribe Icervini, Margarodidae), Homoptera Aphidinea (Amphorophora tuberculata, Aphididae), and Heteroptera (Lethocerus sp., Belostomatidae). On the other hand, the highest count is found in the only scale insect species, Apiomorpha macqueeni. The genera Apiomorpha (Coccinea) and Amphorophora (Aphidinea) are unique showing the most extensive chromosome number variability known among Coccinea and Aphidinea, respectively: in the first, 2n ranges from 4 to 192 (48-fold variation) and in the second, 2n ranges from 4 to 72 (13-fold variation). These examples suggest a high rate of chromosome evolution in *Apiomorpha* and *Amphorophora* and the potential for holokinetic chromosomes to break and fuse. It's amazing that within *Apiomorpha*, as many as 42 chromosome counts have been reported for 47 species studied to date. It is important to note that both scale insects and aphids display very diverse and often very peculiar reproductive modes, including different types of parthenogenesis that may enable rearranged karyotypes to persist and potentially contribute to speciation events. However, all other Aphidococca as well as all other holokenetic groups of Paraneoptera display comparatively low chromosome numbers and rather little chromosome number variation at different taxonomic levels. The only other exception to this rule known today in Paraneoptera is the plant bug genus *Lopidea* (Miridae, Heteroptera) showing 2n=80 in each of the four studied species.

The currently available data suggest that the chromosome number variability in holokinetic groups of Paraneoptera is not very pronounced; it does not differ significantly from the variability observed in monocentric insects, including monocentric Thysanoptera (also classified within Paraneoptera) where 2n ranges from 20 to 100–106 (5-fold variation) in the only 17 studied species. It is worth mentioning in this regard that some other non-polyploid monocentric animals can also have high chromosome numbers as well as between-and within-species chromosome number variation (e.g. Contreras et al. 1990; Fetzner and Crandall 2001; Searle et al. 2019) including insects (e.g. Dutrillaux et al. 2007).

The significance of chromosomal rearrangements and mechanisms underlying differences in chromosome number have been debated for many years (e.g. White 1973, 1978; Grant 1981; King 1995; Schubert 2007). However, the question of how and why chromosome numbers evolve and why some groups have a wide variety of chromosome numbers, while others do not remains unanswered. As to holokinetic organisms, already in early classical publications, it was speculated that there is a certain mechanism preventing an increase in the number of chromosomes, and it happens quite rarely that spontaneous chromosome fragments are transmitted to subsequent generations and play a role in the evolution and speciation (Brown 1960; Nur et al. 1987).

As noted in the Introduction, Ruckman et al. (2020) conducted a purposeful study leading to the rather unexpected conclusion that rates of chromosome number evolution in holokinetic groups are similar to those in monocentric groups. We find that our analysis based on a large amount of data across the entire insect supercohort Paraneoptera supports the trends that have been seen by Ruckman et al. (2020). In our opinion, the hypothesis that rates of chromosome number evolution in holokinetic organisms are higher for the reason that they tolerate structural rearrangements of chromosomes better than monocentric organisms needs to be revised. We conclude that holokinetic chromosomes do have a well proven unique ability to fissions and fusions; however, these rearrangements only accidently, being probably influenced by certain environmental conditions, become drivers of evolutionary changes and speciation events, at least among Paraneoptera insects.

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ORCID

Valentina G. Kuznetsova https://orcid.org/0000-0001-8386-5453 Ilya A. Gavrilov-Zimin https://orcid.org/0000-0003-1993-5984 Snejana Grozeva https://orcid.org/0000-0003-3196-8068 Natalia V. Golub https://orcid.org/0000-0002-6048-9253