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Article

The karyotype of *Rhagades* (*Wiegelia*) *predotae* (Naufock, 1930) (Lepidoptera: Zygaenidae, Procridinae, Procridini)

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Abstract
The subfamily Procridinae of the family Zygaenidae includes five tribes: Thyrassiini, Pollanisini, Artonini, Cleleini, and Procridini. The karyotypes are known for three of them, viz. Pollanisini, Artonini and Procridini. This paper gives information about the haploid chromosome number (n = 12) of *Rhagades predotae* (Naufock, 1930) (Procridini). The evolution of the karyotypes of the genus *Rhagades* is discussed.

Key words haploid chromosome number; Zygaenidae; Procridinae; Procridini; *Rhagades; Rhagades predotae*.

Introduction

The Zygaenidae and especially its subfamily Procridinae is a very interesting and promising group of Lepidoptera for studying its genetics, morphology and biology (Efetov 1996a, b, 1997a, b, 1998a, 1999, 2001a, b, 2006, 2010; Efetov *et al.* 2006, 2011, 2014, 2019a, c, 2024b; Efetov & Gorbunov 2024; Efetov & Hayashi 2008; Efetov & Knyazev 2014; Efetov & Savchuk 2009; Efetov & Tarmann 1999b, 2013a, b, 2014, 2016a, b, 2017a, b, 2024a; Knyazev *et al.* 2015; Marianelli *et al.* 2020; Mirić *et al.* 2024) as well as the karyotypes because the haploid chromosome numbers in different species can vary from 12 to 47 (Efetov 1998b, 2001b, c, 2004; Efetov & Tarmann 1999a; Efetov *et al.* 2003, 2004, 2015, 2025; Efetov & Parshkova 2003, 2004, 2005). The genus *Rhagades* from the tribe Procridini is known now from five species inhabiting a huge territory from the Iberian Peninsula and Asia Minor to the Japanese Islands. Mainly, this distribution is represented by one species, viz. *Rhagades* (*Rhagades*) *pruni* ([Denis & Schiffermüller], 1775), which is known as polyploid with 94 chromosomes. The other four species are local endemics of the Iberian Peninsula, viz. *Rh*. (*Wiegelia*) *predotae* (Naufock, 1930), the eastern Mediterranean region, viz. *Rh*. (*Wiegelia*) *amasina* (Herrich-Schäffer, 1852), and Iran, viz. *Rh*. (*Wiegelia*) *tarmanni* Keil, 1999, and *Rh*. (*Naufockia*) *brandti* (Alberti, 1938). Hitherto, the karyotypes

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were not known for *Rh. predotae* and *Rh. tarmanni*. To fill this gap K. A. Efetov and G. M. Tarmann undertook a field trip to Spain in 2002 but this was not successful. Only the synthesis and the use of attractants from the series EFETOV-2 (Can *et al.* 2019; Efetov *et al.* 2018, 2019b, 2022, 2023, 2024a; Vrenozi *et al.* 2019; Yardim *et al.* 2024) allowed us to discover a large population of this zygaenid moth in Spain in 2018.

Materials and methods

Eggs of *Rhagades predotae* were collected on 13 July 2018 at Spain, Cuenca, 1225 m, 3 km NE of Huelamo (Efetov &Tarmann 2020). The first instar larvae hatched on 22 July 2018 and were reared up to the last instar (Figure 1) on *Prunus*. For karyological analysis only larvae of the males were used. When the larvae began to spin cocoons on 28 May 2019 the testes were removed from the dissected abdomens and then were immediately fixed in a solution of 3 parts of ethanol to 1 part of glacial acetic acid. On 16 January 2020, the gonads were stained in 2 % lacto-acetic orcein. On 20 January 2020 temporary slides were prepared in a drop of 40 % lactic acid by using the squash method. Investigation of the karyotype and calculation of the number of chromosomes was carried out in the metaphase stage with the help of light microscopy using a LOMO MICMED-6 (Russia), x 100 oil immersion objective. The images were obtained by a digital camera SONY NEX-6 (Japan).

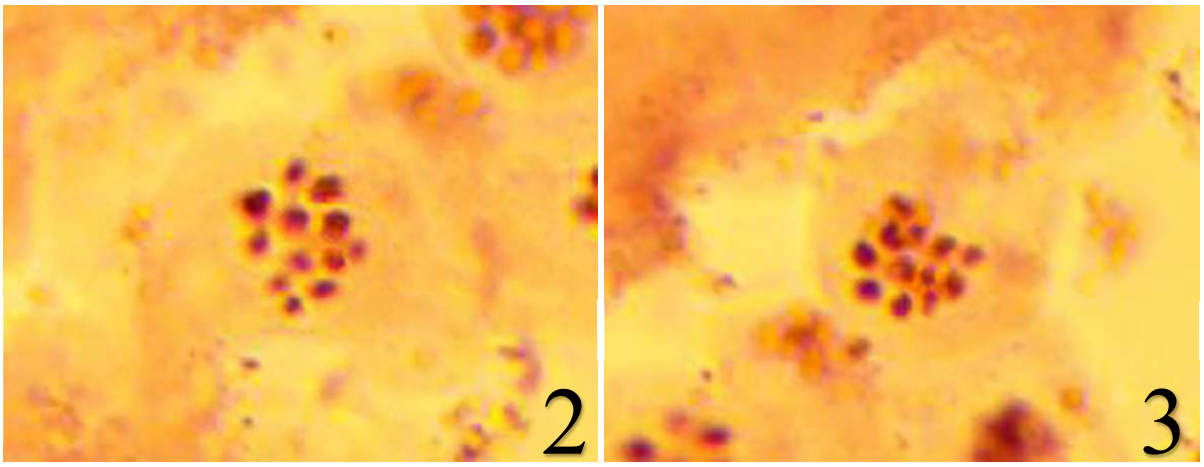


Figure 1. Adult larva of *Rhagades* (*Wiegelia*) *predotae* (Naufock, 1930). Spain, Cuenca, 3 km NE of Huelamo, ex ovo July 2018, photo 19.V.2019. Photo: K.A. Efetov.

Results and discussion

The colour of the testes of *Rhagades predotae* is dark cherry.

During the experiments we obtained three metaphase plates of good quality with a haploid chromosome number: n = 12. The chromosomes represent roundish structures of different size, at least four of them larger than others (Figures 2, 3).



Figures 2-3. *Rhagades* (*Wiegelia*) *predotae* (Naufock, 1930). Meiotic metaphase of spermatogenesis. Haploid chromosome number: n = **12**. Male specimen No. 9, preparation 509.9b. Photo: K.A. Efetov.

As is known, the modal haploid chromosome number for most Lepidoptera is 30–31 (Robinson 1971, 1990; Lukhtanov & Kuznetsova 1988), as well as for the subfamilies Zygaeninae and Chalcosiinae in Zygaenidae (Lukhtanov & Kuznetsova 1988; Tremewan 2002, 2006; Efetov & Parshkova 2003, Efetov *et al.* 2003).

However, in many species of the tribe Procridini the haploid number of chromosomes is different, e.g. in *Jordanita* (*Solaniterna*) *subsolana* (Staudinger, 1862) it is 27, in *Adscita* (*Procriterna*) *subtristis* (Staudinger, 1887) it is 17, in *Illiberis* (*Primilliberis*) *rotundata* Jordan, 1907, it is 25, in *Theresimima ampellophaga* (Bayle-Barelle, 1809) it is 28 (Efetov 2004).

It is remarkably interesting that the largest changes of chromosome numbers we can see in close relatives of the genus *Rhagades* Wallengren, 1863. In the primitive species, *Rh.* (*Naufockia*) *brandti* (Alberti, 1938), it is modal (31), but in *Rh.* (*Wiegelia*) *amasina* (Herrich-Schäffer, 1851) it is only 12, and in *Rh.* (*Rhagades*) *pruni* ([Denis & Schiffermüller], 1775) it is 47. The number of chromosomes in the endemic Iberian species *Rh.* (*Wiegelia*) *predotae* (Naufock, 1930) was hitherto unknown. The purpose of our work was to fill this knowledge gap.

Now we know that the haploid chromosome number of this species is 12 which is the same as in another closely related species of the subgenus *Wiegelia* Efetov & Tarmann, 1995, viz. *Rhagades* (*Wiegelia*) *amasina* that occurs in Bulgaria, Greece, Turkey, Syria and Lebanon.

We consider the increase in the number of chromosomes in *Rh. pruni* to be a result of polyploidy (Efetov 2001a, b). A hypothesis for the polyploid origin of high chromosome numbers in bisexual Lepidoptera was proposed by Lukhtanov & Puplesene (1999). The high chromosome number in *Rh. pruni* may have led to the high adaptability of this species, thus culminating in a very wide distribution (from the Pyrenees to Japan) and polyphagy of the larva (feeding on Rosaceae, Ericaceae, Cistaceae, Fagaceae, Rhamnaceae, Salicaceae) (Efetov & Tarmann 2024b). Species of the other subgenera of *Rhagades* have local areas of distribution and feed only on Rosaceae (Efetov 2001c).

If this hypothesis is true, the common ancestor of contemporary *Rhagades* species had the haploid chromosome number n = 31. The reduction of the chromosome number caused a new branch of evolution with a common ancestor of *Rh. predotae*, *Rh. amasina* and *Rh. pruni* having only 24 chromosomes. Later, polyploidy took place which caused the appearance of *Rhagades pruni* (Figure 4) with the haploid chromosome number 47, one of them of double size (this could be a result of chromosome junction). The chromosome number (n = 12) of *Rh. predotae* and *Rh. amasina* could appear after the secondary junction of 24 chromosomes.

Not long ago we published a large paper (Mirić *et al.* 2024) in which on the basis of DNA studies the dendrogram of the genus *Rhagades* looks slightly different, with *Rh. brandti* and *Rh. pruni* coming from one branch (Figure 5). However, this dendrogram is a result of the differences in DNA sequences and not of the evolution of the number of chromosomes. It means that both dendrograms have the right to exist.



Figure 4. Dendrogram demonstrating possible change of haploid chromosome number during evolution of *Rhagades* species. Numbers show haploid chromosome numbers.



Figure 5. Dendrogram demonstrating DNA differences between four *Rhagades* species (ex Mirić *et al.* 2024).

Conclusion

The haploid chromosome number (n = 12) was determined for *Rhagades* (*Wiegelia*) *predotae*. The number is the same as in the closely related species *Rh*. (*W*.) *amasina*.

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