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## Chromosome evolution in conifer sawflies (Hymenoptera Diprionidae): role of ribosomal DNA in karyotype evolution

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The Diprionidae constitute a small hymenopteran family of about 125 species in 11 genera belonging to the infra-order Symphyta. Larvae of these defoliating insects feed on conifers.

Twenty-nine species from six genera are cytogenetically known. The modal chromosome number is  $n=7$  and only a few deviants are known. [ref.1-7 & present paper]

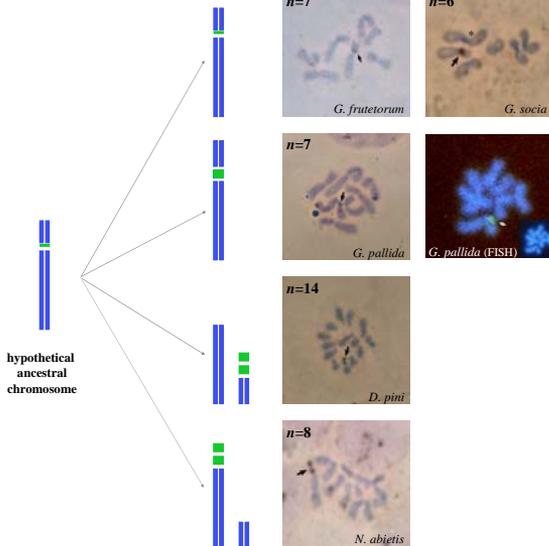


Femoral and male of *Diprion pini*. Cephalon larvae of *D. pini*. Solitary larva of *D. similis*. Cephalon larvae of *Neodiprion sertifer*.

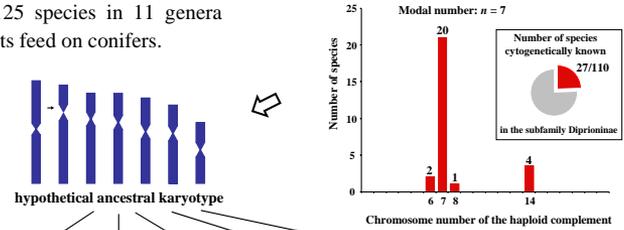
The ancestral karyotype probably consists of seven metacentric chromosomes at the broad sense ( $\sigma: n=7$ ;  $\text{♀}: 2n=14$ ). Two species of the Palearctic genus *Gilpinia* show chromosome number reduction ( $n=6$ ) while the Palearctic genus *Diprion* and one Nearctic species of the genus *Neodiprion* show a chromosome number increase ( $n=14$  and  $n=8$  respectively). [see the figure opposite & ref.1,6-8]

In the genus *Diprion*, the chromosome number doubling resulted from centric fissions. These latter involved breaking points in all the centromeric regions, including a breaking point close to an rRNA gene cluster [ref.6 & figure below]. This could be illustrative of the role of blocks of satellite or ribosomal DNA sequences in karyotype evolution and poses the question of interactions between karyotype evolution and genome organization in this family.

The aim of this study was to determine whether the only known karyotype differentiation by a single centric fission (*Neodiprion abietis*) involved a chromosome bearing rRNA genes.



*In situ* hybridization with rDNA probe to chromosomes of five diprionid species. The rDNA probe used contains the 18S, 5.8S and 28S genes plus intergenic spacers of *Drosophila melanogaster*.



hypothetical ancestral karyotype

$n=6$

$n=7$

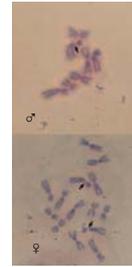
$n=7$   
with  
2<sup>nd</sup> constriction (arrow)

$n=14$   
with  
satellite (arrow)

$n=8$   
with  
satellite (arrow)



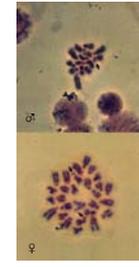
*Gilpinia socia*  
+ [ref.1]



*Gilpinia frutetorum*  
*Gilpinia virens*  
*Gilpinia hercyniae*  
*Microdiprion pallipes*  
*Macrodiprion nemoralis*  
*Neodiprion sertifer*  
+ [ref.1,2,3,4,5,7]



*Gilpinia pallida*



*Diprion pini*  
*Diprion similis*  
*Diprion jingyuanensis*  
*Diprion nipponicus*



*Neodiprion abietis*

The *in situ* hybridization experiments showed that only one chromosome bears an rRNA gene cluster in each studied species, including the non-deviant ones ( $n=7$ ).

In *N. abietis*, rDNA sequences are located on one of the fission products. Their location corresponds to the small short arm of the large pseudoacrocentric chromosome. This implies that the rDNA was located at or close to the breaking point. Indeed, repeated DNA sequences are often assumed to constitute preferential breaking points for chromosomal rearrangements.

Moreover, ISH showed that the fission process was associated with growth of rDNA. Even if the chromosome arm maintaining rDNA was different in *D. pini*, the same amplification process occurred and could have "healed" and stabilized the chromosome end [see ref.9,10].

Genome organization that results in the repeated occurrence or better survival of chromosome mutation increases the probability that this mutation become fixed. Chromosome breakage involving rDNA should have an increased occurrence and a greater chance of viability. Thus, the chromosomally conservative family Diprionidae could provide such an example: the single centric fission event involved a breaking point close to the rRNA genes cluster.