CYTOTAXONOMIC AND EVOLUTIONARY STUDIES IN *THYMUS (LABIATAE)*; RELATIONSHIPS OF THE MEMBERS OF SECTION *THYMUS* JALAS (1)

por

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Abstract

ELENA-ROSSELLÓ, J. A. (1981). Cytotaxonomic and evolutionary studies in Thymus (Labiatae); relationships of the members of section Thymus Jalas. *Anales Jard. Bot. Madrid* 38(1):51-60.

With the object of establishing the interspecific relationships between *Thymus vulgaris, Th. aestivus, Th. zigis* and *Th. hyemalis,* all belonging to the section *Thymus* Jalas, a comparative study of morphological and geographical characteristics, flowering seasons and karyology of these taxa has been undertaken along with various interspecific crosses which allow us to observe the behaviour of the hybrids.

The results of this study have lead us to propose a hypothesis concerning the origin of the said taxa as well as to reach conclussions concerning the establishement of the taxonomic units which correspond to each one of them. The arguments upon which we base our hypothesis are discussed in the present work.

Resumen

ELENA-ROSSELLÓ, J. A. (1981). Estudios citotaxonómicos y evolutivos sobre Thymus (Labiatae); relaciones entre los miembros de la sección Thymus Jalas. *Anales Jard. Bot. Madrid* 38(1):51-60 (En inglés).

Con el fin de establecer las relaciones interespecíficas entre los táxones *Thymus vulgaris, Th. aestivus, Th. zygis* y *Th. hyemalis,* hemos observado el comportamiento de los híbridos interespecíficos obtenidos artificialmente, efectuando además un estudio comparativo de las características morfológicas, geográficas, época de floración y cariología de aquéllos.

Los resultados obtenidos nos conducen a la proposición de una hipótesis acerca del origen de dichos táxones, así como al establecimiento de las unidades taxonómicas correspondientes a cada uno de ellos. Los argumentos en los que se basa dicha hipótesis son discutidos en el presente trabajo.

INTRODUCTION

The genus *Thymus* which Linnaeus established in 1735 includes a wide group of perennial aromatic plants with a generally woody stalk; the great

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diversity which the species of woody stalk present in Spain has made their taxonomy specially complicated and lacking in precision.

The species *Th. vulgaris*, the only representative of the woody stalk group in Europe, has never presented taxonomical problems to European botanists since it constitutes for them a well-characterized and stable systematic unit at all levels including the karyological. The distribution of this species in Europe is well-localized, not going beyond the limits of the Mediterranean zone and immediate neighbouring regions.

As we make our way into the Iberian Peninsula and specially when we near the subdesertic zones of southeast Spain, the situation changes radically; plants whose vegetative system reminds us of the thyme of the french «garrigues» are found occupying great extensions which receive the name of «tomillares» (thyme-beds). It is when we observe these same plants in the period of flowering that we notice a marked diversity in the Iberian Peninsula, which contrasts with the uniformity of the French thymes. Thus, while the period of flowering in France is limited to the spring, in Spain it may be prolonged during the summer months in *Th. aestivus* or during winter in *Th. hyemalis*. On the other hand there also arise differences in the corolla which may be much bigger and of a distinctly red color in *Th. longiflorus* or yellow in *Th. membranaceus*.

Within the wide group of plants similar to *Th. vulgaris*, we will deal in this work with *Th. zygis*, *Th. aestivus* and *Th. hyemalis* wich are specially difficult to differentiate from *Th. vulgaris*. Because of this and with the object of establishing the interspecific relationships amongst the said taxa, we have performed (1) a comparative study of morphological characteristics, geographical distribution, periods of flowering and karyology, and (2) various interspecific hybridizations with the aim of observing the behaviour of the hybrids: any difficulty in the interchange of genes among the taxa will be interpreted as an effect of speciation.

Materials and methods

The material used comes from native Spanish populations belonging to the taxa under study:

Th. vulgaris	-	Station	E-23	Fortuneta, Murcia
Th. zygis	-	»	E-ZA-1	Cubo del Vino, Zamora
		»	E-ZA-2	Zamora
		»	E-SA-1	Ledesma, Salamanca
		»	E-SA-3	Doñinos, Salamanca
		»	E-SA-4	Fuente S. Teresa (Sal.)
		»	E-47	Motril, Málaga
Th. aestivus	-	»	E-18	Pto. Albaida, Alicante
Th. hyemalis	-	»	E-41	Aeropuerto, Almería
		»	E-91	San José, Almería

Chromosome numbers were determined in aceto-orcein squash preparations of root tips (somatic metaphasic plaques) or stamens of flower buds (meiosis metaphasic plaques). The counts were effected on patterns observed in the clear OPL chamber. It has been necessary to observe a minimum of 50 individuals per population and per taxon (ELENA-ROSSELLÓ, 1980).

The artificial hybridizations have been performed on plants gathered in the wild and grown for one year in the experimental field of the C.E.P.E. (1) (in cold winter-quarters during the winter).

The pollinations were performed placing the pollen grains of the hermaphrodite flowers (male-fertile plants) over the female flowers (male-sterile plants). The gathering of seeds was done in the month following pollination. Table I summarizes the crosses that were executed.

RESULTS

Morphological studies

It has been observed after this comparative study of morphological characters (shape and dimensions of leaves and stalk, size and color of flowers) that the forms *Th. aestivus* and *Th. hyemalis* both present great similitude to *Th. vulgaris*. However, we must mention that certain perceptible differences do exist: 1) The plants of *Th. aestivus* are comparatively much bigger than those of *Th. vulgaris,* either as a whole or when their organs are observed separately, 2) in *Th. hyemalis* the characteristic of hairiness in the base of the leaves serves to differentiate it from *Th. vulgaris,* whose leaves lack hair, 3) *Th. zygis,* on the other hand, has flowers smaller than those of *Th. vulgaris* and of a white color and the leaves present hairiness in the base like those of *Th. hyemalis*.

Geographical studies.

We have verified that the area of distribution of the various *Thymus* is variable and depends on the plasticity of the members which comprise the group.

For the taxa used in this study we find (fig. 1): *Th. zygis* widely distributed in the Iberian Peninsula from north to south reaching even North Africa and from east to west but not reaching beyond the zones near the Mediterranean region; *Th. vulgaris,* a especies spread over the Mediterranean zone of

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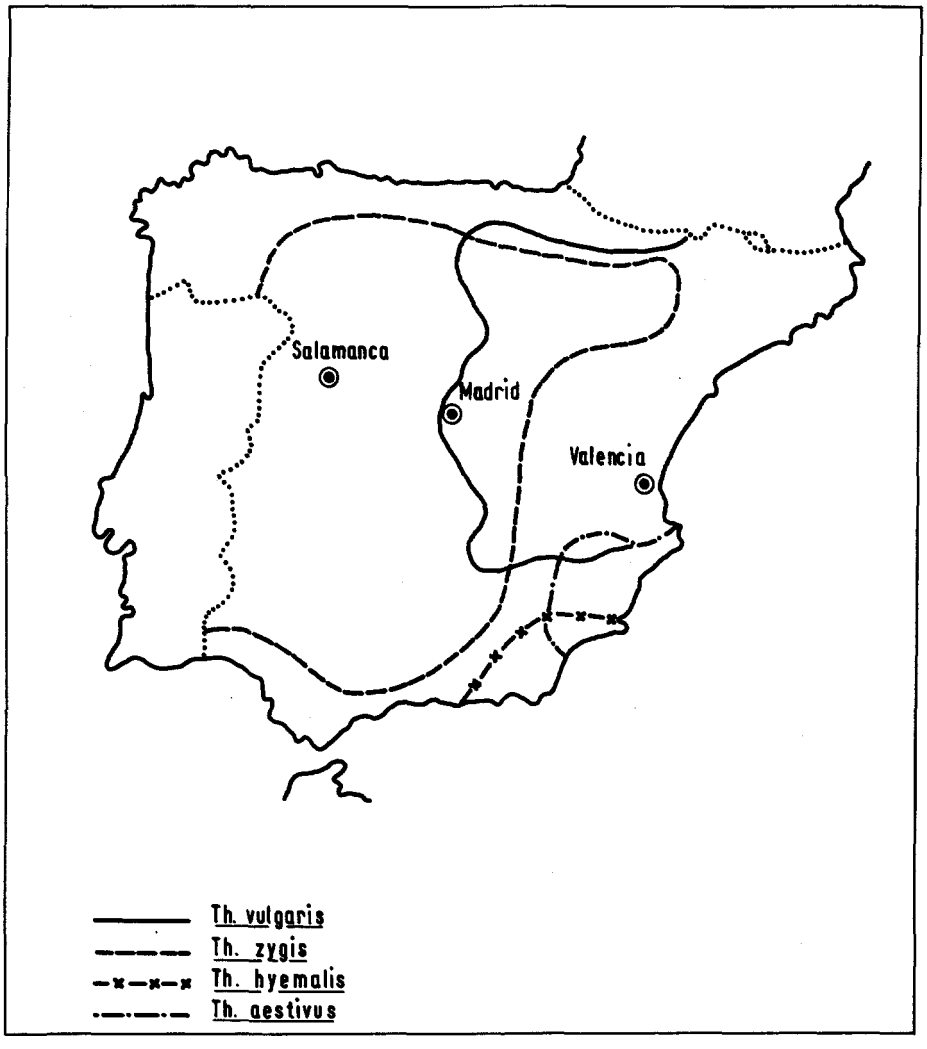

Fig. 1.—Approximate geographical distribution of different *Thymus* taxa.

Europe that in the Iberian Peninsula substitutes *Th. zygis* precisely in the Mediterranean zone extending along the N. E. of the Spanish plateau to Madrid; *Th. aestivus* replaces *Th. vulgaris* in the south of Valencia (Pto. de Albaida, Alcoy, Spain); *Th. hyemalis* appears more to the south, in the subdesertic zones of southeast Spain and replaces *Th. aestivus* in the south of Cartagena (Murcia, Spain) extending throughout the province of Almería (Spain) where it usually occupies zones near the coast.

Flowering time

The flowering season seems to have an intimate relationship with the geographical distribution of the various taxa. The more we approach the subdesertic zones of southeast Spain, the later the flowering becomes. *Th. aestivus* for example, blossoms during the summer months (July, August), while *Th. hyemalis* does so during the winter (December, January).

If we take into acount the fact that much of the flora of the subdesertic regions equally exhibits late flowering (i.e. in winter) we may see in this an adaptive characteristic. Nevertheless, the hereditary nature of this characteristic has been clearly demonstrated by the following experience (Fig. 2): on cultivating the members of these taxa under identical conditions, the differences in precocity are maintained.

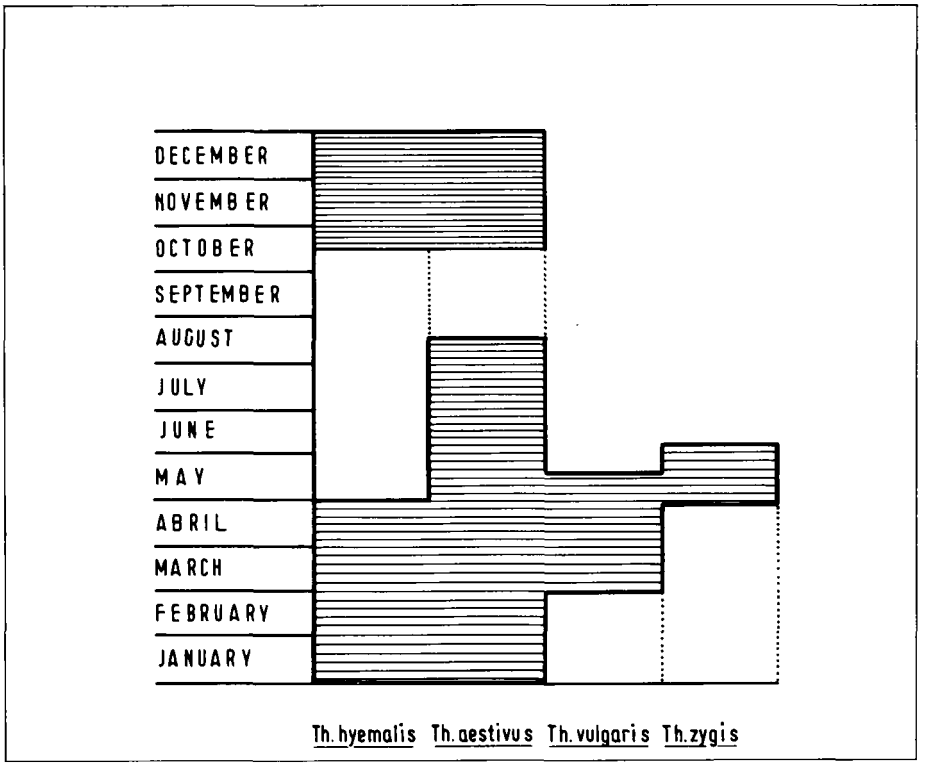

Fig. 2.—Flowering seasons of various taxa; the ruled space indicates the flowering months.

Karyological studies

In the members of *Th. vulgaris* that were analyzed, the number of chromosomes has always been the same $2n = 30$, a count that coincides with those previously made by other authors (VAARAMA, 1947; JALAS, 1948; FAHMY, 1951; BONNET, 1966, etc.) for this species.

In all the populations of *Th. zygis* that were analyzed, we have found $2n = 28$ and $2n = 14$, in somatic and meiosis metaphasic plaques respectively. The number observed does not coincide with that obtained previously by JALAS & POHJO (1965) with $2n = 60$. Considering that the count effected by Jalas was realized on seeds obtained from the Botanical Garden of Coimbra (Portugal), the possibility of an error as to the true origin of the seeds used should not be dismissed. In our case the seeds were personally gathered by us from populations of various origins.

In *Th. aestivus* the number of chromosomes found in the observed plaques has always been well above 60.

In *Th. hyemalis* the number of chromosomes has always been $2n = 58$.

Interspecific hybridizations

In all the crosses effected, we have obtained hybrid F_1 individuals as shown in table I.

TABLE I

mF \ mS	*vulgaris*	*hyemalis*	*aestivus*	*zygis*
vulgaris	(5) 150 / 107	(5) 243 / 123	(2) 30 / 24	(1) 200 / 110
aestivus	(3) 41 / 4	(2) 70 / 5		
zygis				(2) 180 / 51

The intertaxa crosses carried out. The number of crosses is stated by the figure in brackets; the number of seeds by the top figure and the number of plants by the bottom figure.

Upon observing these hybrid individuals during the flowering seasons, we have been able to verify the presence of certain deficiencies in the pollen

grains (Table II). If we exclude from the hybrids the cross *Th. vulgaris* × *Th. aestivus*, the remaining ones presented from 95 % to 100 % infertile pollen and we find the case of *Th. zygis* × *Th. vulgaris* in which the hybrids (F_1) are all male-sterile.

Discussion and conclusions

Judging by the results obtained after morphological, karyological, geographical and flowering times studies, we could consider *Th. aestivus* and *Th. hyemalis* as polyploid forms of *Th. vulgaris* (Fig. 3).

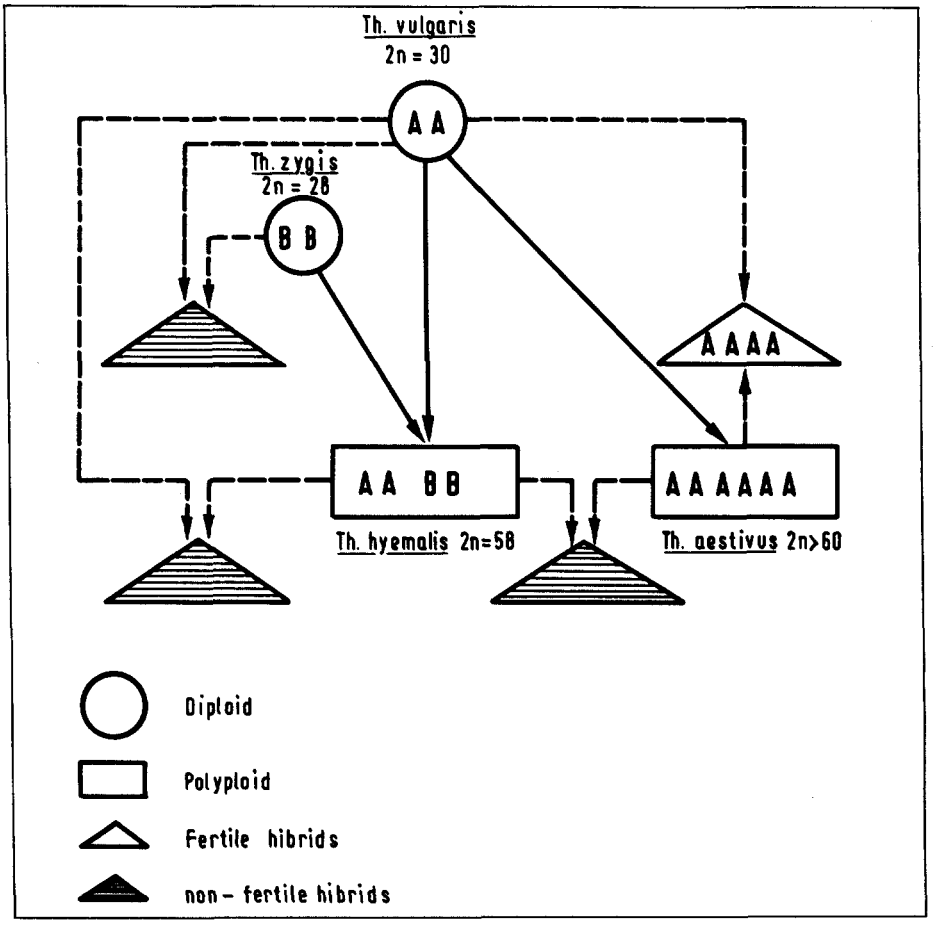

Fig. 3.—A hypothetical scheme illustrating the origin of the species and the interspecific relationships.

Th. aestivus, according to our hypothesis, would be an autopolyploid of *Th. vulgaris* with which it shares an intimate morphological relationship. *Th. aestivus* does not differ morphologically from *Th. vulgaris* except for a bigger plant height and bigger organ size (leaves, flowers). The difference is particulary perceptible in Station E-18 (Pto. de Albaida, Alcoy) where the two forms grow together.

Th. hyemalis would be an allopolyploid (amphidiploid) of *Th. vulgaris* and *Th. zygis* since in its morphological and karyological characters it is intermediate between the two.

Moreover, the chromosome counts effected on *Th. hyemalis* and *Th. aestivus* lead us to belive that the late winter and summer flowering of these taxa and their localization in hot regions are those of the polyploids, a phenomenon already known in the plant world (STEBBINS, 1950).

Regarding the artificial hybridizations, we have obtained hybrid (F_1) individuals in all the crosses effected, a fact which should not surprise us, since all the taxa in question belong to the section *Thymus* Jalas, whose members are considered by all authors to be very similar.

The formation of individuals of the F_2 generation, however, seems obstructed if we consider the results obtained after study of pollen of the F_1 hybrids (Table II). As a result of the interference which arises in the formation of the gametes, the hybrids *Th. vulgaris* × *Th. hyemalis*, *Th. aestivus* × *Th. hyemalis* and *Th. zygis* × *Th. vulgaris*, all present an infertile pollen and particularly the cross *Th. zygis* × *Th. vulgaris*, whose hybrids are all male-sterile (mS). It seems logical to believe that the sterility in the males may be due to a lack of homology among the chromosomes of the crossed taxa (or of the parents used in the crosses) which would produce a defective meiosis in the hybrids as a result of which the gametes will be genotipically unbalanced.

TABLE II

RESULTS OF THE INTERSPECIFIC CROSSES

Crosses	*Hybrids F_1*
Th. zygis x *Th. vulgaris*	all male-sterile
Th. vulgaris x *Th. hyemalis*	100 % infertile pollen
Th. vulgaris x *Th. aestivus*	in winter-quarters - normal pollen in the wild - infertile pollen
Th. aestivus x *Th. hyemalis*	95 % infertile pollen

The relative fertility of the hybrids *Th. vulgaris* × *Th. aestivus* (the pollen is normal under certain circumstances) comes in defense of our hypothesis concerning the origin of *Th. aestivus*. If *Th. aestivus* ($2n > 60$) is an autopolyploid of *Th. vulgaris* ($2n = 30$), it will have the same chromosome dotation as the latter but doubled or tripled. As the number of chromosomes of *Th. aestivus* is greater than 60, it cannot be a tetraploid ($2n = 4x = 60$) but can very well be an hexaploid, with $2n = 6x = 90$ chromosomes. Accordingly,

the hybrid of the interspecific crossing will be a tetraploid ($2n = 4x = 60$), with two identical sets of 30 chromosomes. In this case, there will be no difficulties in the separation of the chromosomes when the gametes are formed which explains why the hybrids of *Th. vulgaris* × *Th. aestivus* can produce normal pollen.

Nevertheless, many other obstacles exist in nature, in addition to the sterility already mentioned. Amongst others, we may point out those that arise from: different flowering seasons, differences in pollinating agents, geographical barriers and different climates. All of them act in the same way, that is to say, they hinder the formation of the hybrid individuals and consequently promote the diversity of the taxa. In effect, in nature, except in isolated cases, a morphological «species gap» exists. The forms that live together preserve their differences and there is no evidence of crossing.

As a conclussion of our hypothesis, we may consider the taxa *Th. zygis*, *Th. hyemalis* and *Th. vulgaris* as different biological species.

As far as *Th. aestivus* is concerned the situation does not appear so clear. The polyploid could be a consequence of the adaptation of *Th. vulgaris* to new environmental conditions or to adverse conditions. In the process of adaptation the polyploid form will become genetically distinct and will remain isolated reproductively from its parent. When this moment arrives and the forms are morphologically distinct, we will be able to recognize two different species. Meanwhile we will assign to *Th. aestivus* the taxonomic category of subspecies: *Th. vulgaris* sub. *aestivus* (L.) Reut., 1868.

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