Systematic and genetical problems revised in two closely related species of *Trichogramma, T. embryophagum* and *T. cacoeciae* (Hym., Trichogrammatidae)

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*Systematic and genetical problems revised in two closely related species of Trichogramma, T. embryophagum and T. cacoeciae* (Hym., Trichogrammatidae).— Several systematic and genetical problems are dealt with in *Trichogramma embryophagum* and *T. cacoeciae*. The difficulty in finding the description of *T. embryophagum* and the poor descriptions of both species have induced nomenclatural problems which are treated here. The particular reproductive system of these species led authors to hypothesize about the origin of *T. cacoeciae* and the sex determination of *Trichogramma*. Alternative hypotheses are proposed. New electrophoretic results in *T. cacoeciae* are reported and interpreted taking into account the mode of reproduction. New geographical data are also provided.

Key words: Systematics, Genetics, *Trichogramma*, Thelytoky, Wolbachia, Sex determination.

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**Introduction**

All 38 European species of the genus *Trichogramma* Westwood are minute parasitoids of Insect eggs. *Trichogramma embryophagum* (Hartig) and *T. cacoeciae* Marchal, two closely related species belonging to the *pretiosum* group (PINTUREAU, 1994a), exhibit different reproductive systems: thelytokous parthenogenesis in *T. cacoeciae* and in some individuals of *T. embryophagum*, and bisexual reproduction in most *T. embryophagum* individuals. The determining factors of thelytoky are different in the two species, one of them being associated with microbes. The diploidisation processes were recently clarified and hypotheses were proposed to explain the origin of *T. cacoeciae* (non-microbe-associated thelytoky) and the sex determination in *Trichogramma* (PINTO & STOUTHAMER, 1994; STOUTHAMER & KAZMER, 1994). Here alternative hypotheses are proposed.

Both species are poorly described and systematic problems appear. In this work a nomenclatural restatement is carried out including a discussion about synonymies, and the geographical data are updated.

Although *T. cacoeciae* is electrophoretically well known, this is not the case for *T. embryophagum*. New electrophoretic results are reported and interpreted taking into account the recent data about diploidisation processes.

**Nomenclatural problems**

The library of the “Ecole nationale du génie rural, des eaux et des forêts” in Nancy (France) holds all the publications of T. Hartig (648 pages published between 1837 and 1839). A thorough search to find the description of *Encyrtus embryophagus* Hartig, a species belonging in fact to the genus *Trichogramma*, was carried out there. An indication of the species was found but there was no description whatsoever. This species was therefore proclaimed *nomen nudum* (PINTUREAU, 1987, 1990).

QUEDNAU (1960) and SOROKINA (1977) described two forms of *T. embryophagum*, one bisexual which could correspond to the Hartig form, and the other, thelytokous, which could correspond to the form studied by TELENGA (1958, 1959). HOCHMUT & MARTINEK (1963) also considered that the *T. embryophagum* of Hartig was bisexual. Nevertheless, N. V. Kurdjumov, cited by TELENGA (1958), saw Hartig’s individuals in the Berlin Museum (before its destruction) and declared that the Hartig collection of *T. embryophagum* shows only females. PINTUREAU (1987, 1990, 1994a) accepted the latter opinion. After acceptance of the two forms at the species level, two different interpretations appeared:

- the thelytokous form named *T. telengai* Sorokina, and the bisexual form named *T. embryophagum* Hartig (SOROKINA, 1987, 1993);

The description of *T. embryophagum* (Hartig) was recently found in HARTIG (1838) in the second footnote of page 250. It is very short: “Geflügel; überall, ausser die grossen Augen mattgelb. Männchen mit braunem Hinterleibstüden und pinselförmig behaarten Fühlern. Länge 1/5-1/4 Linie” [winged; all the body dull yellow except the large eyes. The male has brown tergites and spindle shaped antennae provided with setae. Length: 1/5 to 1/4 of line (0.4 to 0.5 mm)]. The host is *Dendrolimus pini* L. (Lep.: Lasiocampidae) and the locality of capture Charlottensburg (Berlin suburbs).

*Trichogramma embryophagum* (Hartig) is not a *nomen nudum*, and the male description indicates it does not correspond to *T. cacoeciae* which is a thelytokous species. It corresponds to *T. embryophagum* Quednau considered by PINTUREAU (1987, 1990, 1994a) who agrees with the interpretation by SOROKINA (1987, 1993).

However, two divergences persist with SOROKINA (1987, 1993). The first concerns the thelytokous form identified as *T. cacoeciae*, which she named *T. telengai* (Pintureau, 1990) (PINTUREAU, 1990) (table 1). The second concerns *T. bezdencovii* Bezdenko described in 1968. SOROKINA (1992) wrote “*T. bezdencovii* is very close to *T. embryophagum* (Hartig). In structure of the male genitalia these species are virtually identical”, but this author opted “for maintaining the name *T. bezdencovii*, at
least as a preliminary decision". SOROKINA (1993) figured very similar male genitalia of T. embryophagum and T. bezdenkovi, but the two "species" appear to be separated by some couplets in her key (SOROKINA, 1993: 63-64). The diagnostic characters cited are variable and difficult to use: length of longest seta on male flagellum =1.1 to 1.6 or 1.7 to 1.8 the antennal width; small differences in male genitalia (length and width of median ventral projection, length of aedeagus in relation to length of apodemes); shade of colour. Trichogramma telengai is at the beginning of the key (p. 17) because it is separated by the mode of reproduction (thelytoky) which is not a reliable diagnostic character (PINTUREAU, 1987, 1993c; STOUTHAMER et al., 1990b). On the other hand, PINTUREAU (1987, 1990) synonymised T. embryophagum and T. bezdenkovi (table 1), and the two species T. embryophagum and T. cacoeciae appear at the same page, in connected couplets, in the key of PINTUREAU (1994a).

The type or syntypes of T. embryophagum (Hartig) were destroyed. PINTUREAU (1987, 1990) considered a drawing by QUEDNAU (1960) as the lectotype of the species. With the new interpretation, a neotype is necessary. Unfortunately, nobody possesses individuals from the Berlin region to designate this type.

Reproductive system

Trichogramma cacoeciae always shows thelytokous parthenogenesis, whereas T. embryophagum often has bisexual reproduction and sometimes thelytokous reproduction (PINTUREAU, 1994b). In Trichogramma, thelytokous parthenogenesis is often due to an intracellular microorganism, Wolbachia trichogrammae Louis & Pintureau (HURST et al., 1990; STOUTHAMER et al., 1990a; 1993; ROUSSET et al., 1992; LOUIS et al., 1993; PINTUREAU et al., 1993; STOUTHAMER & WERREN, 1993). This is the case in T. embryophagum, but not in T. cacoeciae where Wolbachia are not present and thelytoky is probably genetic (STOUTHAMER et al., 1990b, 1993; PINTUREAU et al., 1993; PINTO & STOUTHAMER, 1994; PINTUREAU, 1994b). According to STOUTHAMER et al. (1990b) and PINTO & STOUTHAMER (1994), hybridization is suspected to be the cause of permanent thelytoky in T. cacoeciae, but parental species were not identified. In this species, the normal meiosis is replaced with a mitotic process, whereas in microbe associated thelytoky, females show normal meiosis and the diploid number of chromosomes is restored by fusion of the products of the first mitotic division (STOUTHAMER et al., 1993; PINTO & STOUTHAMER, 1994; STOUTHAMER & KAZMER, 1994).

Although speciation by hybridization exists in animals (BULLINI, 1985) and thelytoky induced by hybridization has been reported from different Trichogramma species (NAGARKATTI, 1970; PINTUREAU & BABAILLET, 1981; PINTUREAU et al., 1982), often without maintenance of thelytoky for more than one generation, it is not clear that hybridization explains the particular reproduction of T. cacoeciae. Hybrid species are characterized by a high heterozygosity never found in this Trichogramma species (PINTUREAU, 1993a). It is thus possible that a fragment of DNA from an intracellular microorganism (perhaps different from Wolbachia trichogrammae, since effects on meiosis are not the same) was incorporated into the host genome (PINTUREAU et al., 1993). Such an incorporation was hypothesized to explain the presence of the f factor, a mobile genetic element which may be derived from a bacteria, in some feminized individuals of the isopod Armadillidium vulgare Latreille (UCHAULT & MOQUART, 1993; HURST, 1993).

In microbe-associated thelytoky, complete homozygosity does not explain sex determination by the multiple locus model (SNELL, 1935). According to this theory, a diploid individual becomes a female when at least one sexual locus is heterozygous. STOUTHAMER & KAZMER (1994) hypothesized that in such cases of thelytoky, the genetic balance theory (CUHNA & KERR, 1957) could explain sex determination. A more complex alternative hypothesis can be drawn up on the basis of the multiple locus model: incorporation of a Wolbachia DNA fragment, not in the nucleus of the host cells but in the cytoplasm (mitochondria?), where it could mime a sexual gene of Trichogramma. This factor could induce heterozygosity at a sexual locus. Wolbachia would therefore act not only on diploidisation, but also as a sexual allele (different from the Trichogramma alleles). Nevertheless, another assumption has to be
Table 1. List of the European species belonging to the *Trichogramma pretiosum* group. 
*Lista de las especies europeas pertenecientes al grupo Trichogramma pretiosum.*

<table>
<thead>
<tr>
<th>Species</th>
<th>Synonyms</th>
<th>Secondary homonyms</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. cacoeciae</em> Marchal</td>
<td><em>T. neustadt</em> Xie &amp; Zhu</td>
<td><em>T. telengai</em> Sorokina <em>T. flavum</em> Marchal</td>
</tr>
<tr>
<td><em>T. daunala</em> Dugast &amp; Voegelé</td>
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<td></td>
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<tr>
<td><em>T. embryophagum</em> (Hartig)</td>
<td></td>
<td><em>T. bezdenconii</em> Bezdenko</td>
</tr>
<tr>
<td><em>T. euproctidis</em> (Girault)</td>
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<tr>
<td><em>T. ingrircum</em> Sorokina</td>
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<tr>
<td><em>T. leptoparameron</em> Dyurich</td>
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<tr>
<td><em>T. mirabile</em> Dyurich</td>
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<td><em>T. misiae</em> Kostadinov</td>
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<tr>
<td><em>T. oleae</em> Voegelé &amp; Pointel</td>
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<tr>
<td><em>T. pelovi</em> Kostadinov</td>
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<td><em>T. piniperda</em> Wolff</td>
<td><em>T. pini</em> Meyer</td>
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<tr>
<td><em>T. savalense</em> Sorokina</td>
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<tr>
<td><em>T. semblidis</em> (Aurivillius)</td>
<td></td>
<td><em>T. schuberti</em> Voegelé &amp; Russo</td>
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<tr>
<td><em>T. silvestre</em> Sorokina</td>
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<tr>
<td><em>T. sorokiniae</em> Kostadinov</td>
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<tr>
<td><em>T. zeirapherae</em> Walter</td>
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</tbody>
</table>

made since an arrhenotokous line derived by antibiotic treatment from an isofemale line of thelytokous *Trichogramma* produces female offspring from fertilized eggs (Stouthamer et al., 1990b; Stouthamer & Kazmer, 1994). The incorporated DNA fragment, insensitive to the treatment, would only act on diploid individuals (a homozygous individual “2n + factor” would become female, and an individual “n + factor” would become male). There is a resemblance between this theory, based on the multiple locus model, and the genetic balance theory, but a completely homozygous individual (2n) without factor would become male according to the first, and female according to the second theory.

A new theory explaining the sex determination, the imprinting hypothesis, was described in 1992 (summarized by Cook & Crozier, 1995). If this theory is accurate in *Trichogramma* associated to Wolbachia, one only has to assume that symbiotes, inducing the diploidisation, also prevent the females from imprinting the sex loci.

**Esterases studied by electrophoresis**

In the *Trichogramma* genus, the commonly used esterase system provides diagnostic characters to determine species (Klausnitzer et al., 1983; Pintureau, 1993a, 1993b). A strain named Cal, originating from Haut-Rhin, France (numerous individuals collected in 1992), was sent in April 1994 by J. Daumal (INRA-Antibes) and reared as five isofemale lines (A to E) from July 1994. To confirm the specific identity of this strain, esterases were
analysed by electrophoresis in December 1994.

The technical procedure was described in Pintureau (1993a). One homogenate consisted of 20 females chosen at random in a line. Three homogenates per line were analysed. Controls consisted of known strains of T. cacoeciae and T. brassicae Bezdenko.

Cal strain belongs to T. cacoeciae and no intra-line variation was detected. Lines A, B, D, and E did not show differences (8 bands), but line C had another pattern (only five bands). The five bands in line C are similar to bands stained and interpreted in 35 strains from Bulgaria, France, Germany, Morocco, Switzerland, Turkey, Ukraine and former Yugoslavia (Pintureau, 1987, 1993a, 1993b; Pintureau & Keita, 1989): four loci with one allele each (table 2).

Lines A, B, D and E showed four similar bands (0.24, 0.45, 0.48, 0.51). The band 0.07 is lacking but four other bands (0.10, 0.12, 0.40 and 0.43) are present. Band 0.12 is known in two strains from The Netherlands and Ukraine (Est 10.12); band 0.10 is very similar to band 0.12 and to band known as Est 10.10 in one strain of T. cacoeciae (Parisian region) and in several other species like T. evanescens Westwood; bands 0.40 and 0.43 are very similar to bands 0.45 and 0.48 of T. cacoeciae and to bands known as Est 5'0.40-0.43 in T. dendrolimi Matsumura and T. principium Sugonjaev & Sorokina (Pintureau, 1987, 1993a). Therefore in lines A, B, D and E, all individuals had two alleles at the locus Est 1 and two alleles at the locus Est 5' (table 2).

Such a polymorphism has rarely been found in T. cacoeciae. A polymorphism was only noted in one strain from Germany and in one strain from The Netherlands at the locus Est 1 (0.06 and 0.07 or 0.06 and 0.12 alleles, respectively), and in one strain from Turkey at the locus Est 3 (0.06 and 0.28 alleles) (Pintureau, 1987, 1993a, 1993b). In these three strains, no heterozygous genotypes were detected, but only the two kinds of homozygous genotypes. In the same works, another phenomenon was described in 6 strains from France, Morocco and Switzerland: permanent heterozygosity at the Est female 1 locus with alleles 0.65 and 0.68. The simultaneous presence of two alleles at the locus Est 1 and two alleles at the locus Est 5' in all replicates of lines A, B, D and E probably results from the same phenomenon.
Individuals are thus all homozygous and it is possible that two duplications and mutations occurred in strain 326 (Est 3 and Est female 1) and strain 327 (Est 3 and Est 5'). Such a duplication is known in a bisexual strain of an American species for the malic enzyme gene (Hung, 1985).

In *T. embryophagum*, few electrophoretic analyses were carried out. Klaunitzer et al. (1983), Walter (1985) and V. V. Soumenkova et al. (pers. comm.) reported some results on esterases. The species appears closely related to *T. cacoeciae*.

**Geographic distribution**


The geographical distribution of *T. cacoeciae* is wider than that of *T. embryophagum* in Eurasia, since it includes Eastern Asia. Pintureau (1987, 1990) cited Belorussia, Bulgaria, P. R. of China, former Czechoslovakia, Denmark, France, Germany, Greece, Italy, Kizghizia, Latvia, Moldavia, Morocco, The Netherlands, Poland, Russia, Switzerland, Turkey, Ukraine, Uzbekistan and former Yugoslavia. In addition, Sorokina (1993) cited Estonia, Kazakhstan and Lithuania. No countries but several regions can be added. In France, 13 départements were listed (Ain, Alpes-de-Haute-Provence, Bouches-du-Rhône, Corrèze, Creuse, Drôme, Essonne, Haute-Garonne, Hautes-Alpes, Hauts-de-Seine, Var, Vaucluse and Yvelines), and two are new: Haut-Rhin (host: *Lobesia botrana* Denis & Schiffermüller, Lep. Tortricidae; material sent by J. Pizzol), and Rhône (Monsols and Vénissieux; host: *Noctua pronuba* L., Lep. Noctuidae). In Germany, three Länder were listed (Baden-Württemberg, Hesse and Saxony), and one is new: Rhenish Palatinate (Bernkastel-Kues, material sent by S. A. Hassan). In Italy, three regions were listed (Abruzzi, Emilia-Romagna and Tuscany), and one is new: Valle d'Aosta (Torgnon at 1800 m altitude; host: *Cydia strobilella* L., Lep. Tortricidae; material sent by E. Brockerhoff). The species is also reported on *Lobesia botrana* in Italy (material sent by S. Maini). In Switzerland, no canton was justified, and one is now known: Valais (Martigny and Tseuzier, host: *Cydia strobilella* and *Strobilomyia anthracina*, Dipt. Anthomyiidae; material sent by E. Brockerhoff). The species is also reported on *Eupeocilia ambiguella* Hb. (Lep. Tortricidae) in Switzerland (material sent by IOBC, Leg. Remund).

**Acknowledgements**

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**Resumen**

Se tratan algunos problemas sistemáticos y genéticos que existen entre *T. embryophagum* y *T. cacoeciae*. La dificultad en encontrar la descripción de *T. embryophagum* y las pobre descriptores existentes de ambas especies han inducido algunos problemas de nomenclatura que se tratan en este artículo. El particular modo de reproducción de estas especies conduce a los autores a hipotetizar sobre el origen de *T. cacoeciae* y sobre la determinación del sexo en *Trichogramma*. Se proponen hipótesis alternativas. Se dan e interpretan nuevos datos electroforéticos de...
T. cacoeciae teniendo en cuenta su modo de reproducción. También se dan nuevos datos geográficos.

References


