spermatogenesis with seminiferous tubules lined mainly by Sertoli cells and spermatogonia.
In females there was no noticeable effect of 1 µg thyroxine/100 g b.wt, whilst after 2 µg there was slight suppression of follicular development. The highest dose gave marked suppression of development, with atresia, reduction in follicular size and some evidence of fibrosis (figs 3 and 4).
Treatment caused no obvious effects on the histology of pituitary or adrenal glands, either in males or females, while thyroid cell size was suppressed by increasing dose; the thyroid glands could not be located after the 4-µg treatment.
Discussion. Treatment of the male birds in this experiment with thyroxine caused significant increases in weight gains and carcass weight, but prevented normal development of the testes. Ovarian development was also inhibited in female birds. These effects on the gonads show that thyroxine can prevent normal development, as well as causing atrophy in mature birds, which was previously shown by Wheeler and Hoffman[5] amongst others. Shaffner[6] noted a reduction in fertility in cockerels, and a decline in egg production in hens, by feeding thyroprotein. The review of Maqsood[2] shows that equivalent results have been found in mammals.
More recently, Jallageas and Assenmacher[3] have shown that seasonal hyperthyroidism accounts for the onset of the regressive phase of the annual sexual cycle in domestic ducks, thus supporting the idea that endogenous thyroid hormones affect reproduction so that the effects of exogenous treatment in this experiment are likely to be of physiological significance. It is unlikely, however, that the highest dose of thyroxine used in the work reported here was within the physiological range as it had severe effects on the thyroid glands. While it is highly unlikely that they atrophied completely, they were so much reduced in size and/or changed in color that they could not be detected by the naked eye.
A significant stimulation of growth of chickens by thyroxine was observed in experiment 1, which was similar to the results of Singh et al.[7]. Thyroid hormones are known to be synergistic with growth hormone in the control of growth in mammals, and it is possible that chickens do not normally produce sufficient thyroxine to support maximal growth rates. Hyperthyroidism causes glycogen depletion of the liver[9] which accounts for the hyperglycemia and reduced liver weights observed in experiment 1.
Although the results reported here do not offer conclusive proof that thyroid secretions are responsible for the timing of sexual maturation, there is clearly need to follow up this possibility with further work in this area.

1 Present address: College of Veterinary Medicine, University of Baghdad, Iraq.
2 Maqsood, M., Biol. Rev. *27* (1952) 281.
3 Jallageas, M., and Assenmacher, I., Gen. comp. Endocr. *22* (1974) 13.
4 Mori, G., and George, J.C., Acta anat. *101* (1978) 304.
5 Wheeler, R.S., and Hoffmann, E., Poult. Sci. *27* (1948) 685.
6 Shaffner, C.S., Poult. Sci. *27* (1948) 527.
7 Singh, A., Reineke, E.P., and Ringer, R.K., Poult. Sci. *47* (1968) 212.
8 Snedecor, G.W., and Cochran, W.G., Statistical Methods. Iowa State University Press, 1980.
9 Raheja, K.L., Snedecor, J.G., and Freedland, R.A., Comp. Biochem. Physiol. *29B* (1971) 237.

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Diagnostic alleles and systematics in termite species of the genus *Reticulitermes* in Europe

J.-L. Clément

University P.-M. Curie, Laboratoire d'Evolution. R.C.P. 317-645 E.R.A. 620, 105, boulevard Raspail, F-75006 Paris (France), 28 March 1983

Summary. It was possible to separate species and complexes of species in the *Reticulitermes* genus of Western Europe using esterase 3 and acid phosphatase 2.

Electrophoretic techniques sometimes help taxonomists to differentiate between sibling species on the one hand, and semi-species on the other, morphologically very close in natural populations[1]. In European termites of the *Reticulitermes* genus, morphology gives little information for species discrimination[2]. Research on natural hybridization in the sympatric area is very difficult with classical types of observation (morphology and biometry), but species can be isolated according to their sexual pheromone proportions[3] and cuticular compounds[4].
Luykx[5] described in 1981 an enzymatic polymorphism in one species and we know[6,7] that in Western Europe, enzymatic polymorphism is very marked in *Reticulitermes*. Two loci (esterase 3 and acid phosphatase 2) have diagnostic alleles and allow species discrimination.
Esterase 3. 10 loci were distinguished by electrophoresis at 300 V for 4 h at 5 °C in 12.5% hydrolyzed starch at pH 8.25 on Poulik gel buffer[7] or in a 7.5% acrylamide gel at pH 8.9 in tris-glycine or borate electrode buffer (pH 8.6). Worker termites were homogenized in distilled water. An equal mixture of α naphtyl sodium acetate (Sigma) and β naphtyl sodium acetate (Sigma) with fast blue R.R. salt (Sigma) on sodium phosphate buffer (0.2 M) discriminated different loci at pH 6.25. Esterase 2 and 6 were pink colored, esterase 7 and 9 red, esterase 1, 3, 4, 5, 8 and 10 black. 7 loci were polymorphic (fig. 1). Esterase 3 is the most polymorphic and overlaps other enzymes. These systems were inhibited by n-propanol (13%) during the staining procedure (fig. 2).
Acid phosphatase 2. 2 loci appeared (ACPH1 and ACPH2) when workers were homogenized in distilled water. α-Naphthyl acid phosphate (Sigma), 5% n-Propanol and Fast Garnet GBC salts mixed with sodium acetate buffer (0.2 M) at pH 5 were used in the staining procedure. 2 other loci (PAC1 and PAC2) were revealed when termites were homogenized in 1‰ triton X100.
A single pair of reproducers (winged or neotetenic one) was discovered in 6 societies out of 104, and allowed a formal genetic study of the enzyme genes.
A chi-square test confirmed the existence of a monomeric autosomal and co-dominating locus for each enzyme. 8 alleles coded for esterase 3 and 5 for ACPH 2 (table 1).
104 societies from Western Europe were studied. These

Table 1. Formal genetics of esterase 3 and acid phosphatase 2 in 6 natural colonies

Colonies	Est 3 Genotypes of reproductors ♂	♀	Genotypes of larvae Established	Expected	χ^2	ACPH 2 Genotypes of reproductors ♂	♀	Genotypes of larvae Established	Expected	χ^2
LC 10B1	DE	DE	DD: 3 DE: 5 EE: 1	2.25 4.5 2.25	1.28	AA	AA	AA: 9	9	0
LC 13C9	DD	DD	DD: 28	28	0	AB	AA	AB: 10 AA: 5	7.5 7.5	1.66
LC 6A8	DD	DE	DD: 8 DE: 6	7 7	0.28	AB	BB	AB: 7 BB: 7	7 7	0
EY9	DE	DE	DD: 8 DE: 27 EE: 7	10.5 21 10.5	3.47	AB	AB	AA: 6 AB: 20 BB: 9	8.75 17.5 8.75	1.23
CHE	AE	AD	AA: 7 AD: 5 AE: 11 DE: 4	6.75 6.75 6.75 6.75	4.26	AA	BB	AB: 27	27	0
ORE	AB	AB	AA: 8 AB: 14 BB: 5	6.75 13.5 6.75	0.70	AA	AA	AA: 27	27	0

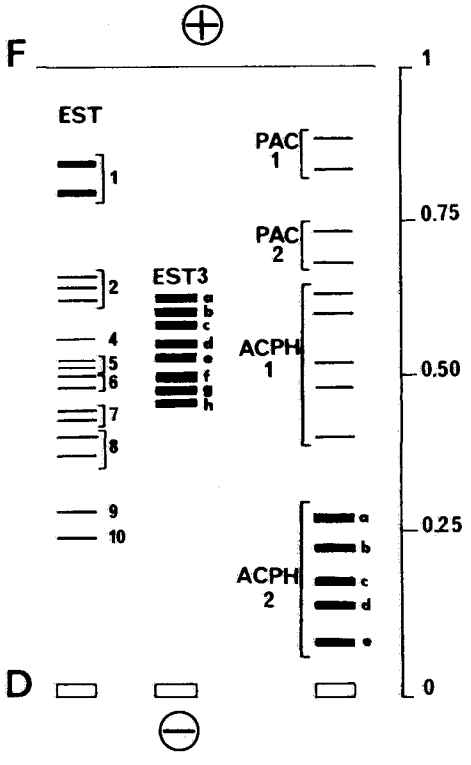

Figure 1. Electrophoretic pattern of esterase and acid phosphatase with their different alleles in Starch gel (Sigma ref. S 4501, Lot No. 37C-0126).

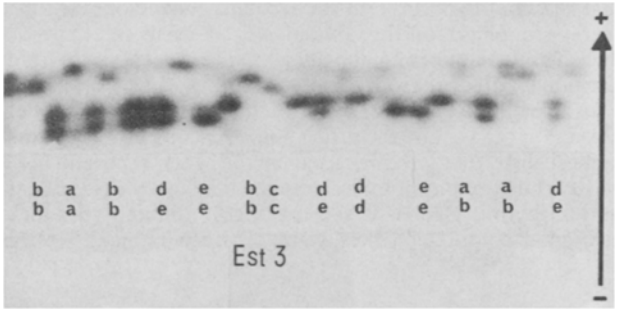

Figure 2. Some esterase phenotypes observed in *Reticulitermes lucifugus* complex esterase 2, 4 and 5 are inhibited.

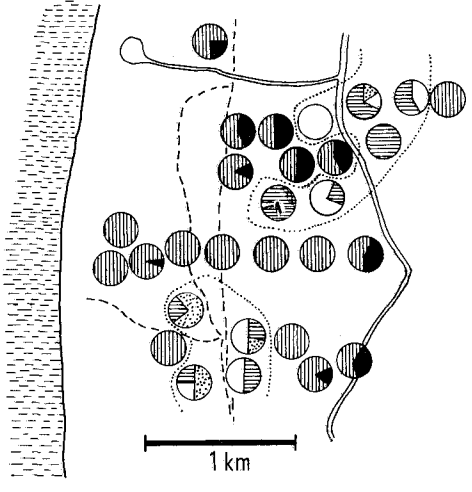

Figure 3. Distribution of esterase 3 genotypes. Est 3 allele frequencies are given as segments of circles for colonies, in the sympatric zone: pine forest from La Coubre, Charente Maritime.
-vertical hatching: allele D
-black: allele E
} *R. (lucifugus) grassei*
-dotted: allele F
-white: allele G
-dash: allele H
} *R. santonensis*

2 enzymes and their diagnostic alleles allowed us to separate *Reticulitermes (lucifugus) grassei* and *Reticulitermes santonensis* free from hybrids in the sympatric area (fig. 3) although hybridization was possible with winged sexual individuals under laboratory conditions when the development was accelerated or decreased[9].

Three species and subspecies of the *lucifugus* complex can be clearly separated using enzymatic alleles; *grassei* in South Western France, *banyulensis* in Eastern Spain and Catalunia, *lucifugus* in Italy and *balkanensis* in Yugoslavia

Table 2. Esterase 3 and acid phosphatase 2 alleles for different species (bracket means a low allele frequency)

	Enzymes EST 3								ACPH 2				
Species \ Alleles	A	B	C	D	E	F	G	H	A	B	C	D	E
santonensis, France						+	+	+				+	+
(lucifugus) grassei, France, North-Spain				+	+				+	+			
(lucifugus) banyulensis, East-Spain	+	+	(+)						+	+			
(lucifugus) grassei × *banyulensis*, South-West Spain, Portugal	+	+	+	+	+				+	+			
(lucifugus) lucifugus, Italy	+	(+)	+						+	+			
(lucifugus) balkanensis, Yougoslavia, Greece	+	+									+		

and Greece (fig. 4 and table 2). Intermediate populations in the circular overlap[5] *(banyulensis x grassei)* have 5 alleles for esterase 3. The true *banyulensis* and *lucifugus* species can be recognized by their different allelic frequencies of esterase 3 (table 2). In Italy *[(lucifugus) lucifugus]* the C allele is present at a 0.56 frequency and in Eastern Spain at a 0.15 frequency and the B allele is present at 0.05 in Italy and at 0.36 in Eastern Spain. Differences between *Apis mellifera ligustica* in Italy and *A.m. mellifera* in France[10] are very similar.

In *Reticulitermes*, a study of the enzymatic polymorphism is the only method for discriminating between European species using workers, but the specific level separating these species was studied using mixiologic analysis: sexual attraction[2] and social group recognition.

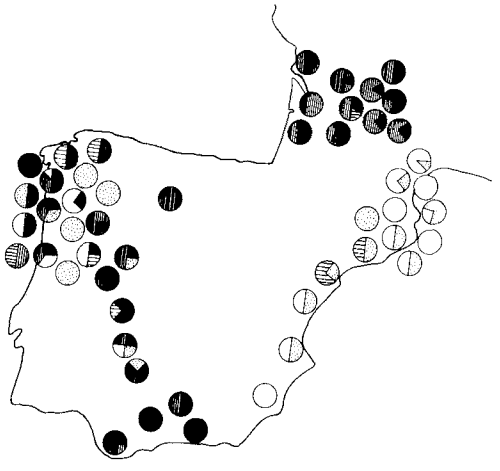

Figure 4. Distribution of esterase 3 genotypes from the Iberian Peninsula. Est 3 allele frequencies are given as segments of circles for populations: -white: allele A, -dotted: allele B, -horizontal hatching: allele C; -vertical hatching: allele D, -black: allele E.

1 Pasteur, G., and Pasteur, N., Mem. Soc. zool. Fr. *40* (1980) 99.
2 Clément, J.L., Anns Soc. ent. Fr. *14* (1978) 131.
3 Parton, A.M., Howse, P.E., Baker, R., and Clément, J.L., in: Biosystematics of social insects, p. 193, Eds P.E. Howse and J.L. Clément. Academic Press, London 1981.
4 Clément, J.L., Revue Biol. Ecol. Médit. *3* (1980) 157.
5 Luykx, P., Heredity *46* (1981) 315.
6 Clément, J.L., in: Biosystematics of social insects, p. 49. Eds P.E. Howse and J.L. Clément. Academic Press, London 1981.
7 Johnson, F.M., Kanapi, C.G., Richardson, R.H., Wheeler, M.R., and Stone, W.S., Stud. Genet. *18* (1966) 517.
8 Clément, J.L., Ann. Sci. nat., Zool., *13* (1979) 251.
9 Clément, J.L., Biol. Behav. *7* (1982) 55.
10 Badino G., Celebrano, G., and Manino, A., Experientia *38* (1982) 540.

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Chromosome arrangement throughout mitosis and interphase in *Allium sativum* (Liliaceae)

M. D. Sosa and F. Cortés

Departamento de Citología e Histología Vegetal y Animal, Facultad de Biología, Sevilla (Spain), 11 April 1983

Summary. Allium sativum (garlic) root-tip chromosomes were subjected to a C-banding procedure. In addition to the nucleolar bands reported previously in this species, bands which are telomeric or close to the telomeres have been detected in some pairs. This has allowed us to analyze the arrangement of chromosomes during interphase.

Due to the uncoiled and extended condition of the greater part of the chromosomes during interphase, the chromosomal arrangement at this stage is always more difficult to investigate than that during mitosis. Nevertheless, it can be studied by preferential staining of constitutive heterochromatin or by cytological detection of a specific replication pattern (mainly late replication) after labeling of DNA. In both cases, a comparison between the distribution of chromocenters observed at interphase and the position of chromosome regions preferentially stained at mitosis can be carried out.

According to a number of reports, the spatial arrangement of chromosomes during interphase is quite similar to that observed at telophase[1-8], and this situation has been compared with the attachment of the bacterial chromosome to a specific region of the cell membrane[9].

In a cytovariety of *A. sativum*, Ghosh and Roy[4] reported C-bands in only 2 submetacentric nucleolar chromosomes,