to arrangements (st and *I*) of chromosome 5. On the other hand, cross B (strain 4s) yields only two genotypes, strongly suggesting an association of the PGM alleles (95 and 100) with arrangements (st and *s*) of chromosome 4. Since both crosses are mutually exclusive we can safely assign the PGM locus to the linkage group of chromosome 4.

Backcross C was performed to estimate the relative distance of the PGM locus from the breakage point of inversion *s* of chromosome 4. Recombination data indicate that the PGM locus is outside the inversion 4*s* at 15.62 morgans from one of its breakage points. Considering that the map distance from the proximal breakpoint of the inversion *s* (4F1c) to the centromere is smaller than the recorded percentage of recombination, it is most probable that the locus of PGM is situated within the cytological interval A–B of chromosome 4 (the region A corresponding to the telomere).

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Tandem gene duplication and fixed heterozygosity in the parasitic wasp, *Trichogramma marylandense*[1]

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Summary. All males and females of the parasitic wasp *Trichogramma marylandense* exhibit the 5-band ME phenotype normally found in heterozygous individuals. Since no diploid males were found and all males are hemizygous at both PGI and PGM loci, it is concluded that the permanent heterozygosity at this ME locus in *T. marylandense* is the result of tandem gene duplication.
Key words. Trichogramma; malic enzyme; gene duplication; fixed heterozygosity.

The existence of duplications of genetic loci was inferred in *Drosophila* by Sturtevant[2] early in this century. Roberts and Baker[3] postulated that the four esterase loci in *Drosophila montana* are evolutionarily related through a process of duplication of the original locus. Costa et al.[4] reported a duplication of the esterase 6 locus in a wild population of *D. melanogaster* and there is evidence of duplicate genes for alcohol dehydrogenase in *D. mojavensis*[5,6]. Duplication of the hexokinase locus in dipterans has also been suggested[7]. However, studies in enzyme gene duplication are mostly limited to the vertebrates, especially in fishes[8]. Here I report, to the best of my knowledge, the first published case of enzyme gene duplication in Hymenoptera.

Trichogramma are minute wasps that parasitize eggs of many Lepidoptera and a few species of Diptera and Coleoptera. They have been widely used in biological control projects in various parts of the world. The genetic basis of electrophoretic variations of malic enzyme (ME; E.C. 1.1.1.40), phosphoglucose isomerase (PGI; E.C. 5.3.1.1.) and phosphoglucomutase (PGM; E.C. 2.7.5.1.) in *Trichogramma* was established by progeny analyses[9]. It was found that ME allozymes in this group of insects function as a tetramer and are controlled by fast and slow alleles at a single locus, with heterozygotes exhibiting a 5-band phenotype. Both PGI and PGM have a single locus and each has four codominant alleles. However, PGI is a dimer and PGM is a monomer in *Trichogramma*.

As in other hymenopterous insects, *Trichogramma* males are haploid and females diploid. Therefore, only females can be heterozygous for any locus. However, regardless of the sexes, all wasps from two cultures of *Trichogramma marylandense* collected in Beltsville, Maryland, showed the 5-band ME phenotype. A chromosome number of n = 2SM + 2T + 1A has been reported in *Trichogramma*[10]. Although diploid males were found in a strain of *Trichogramma* from Japan[11], no diploid males have been found in these two cultures. This rules out the possibility that these male wasps with the 5-band ME phenotypes are diploid and heterozygous at this ME locus.

Gene duplication by polyploidization has been promoted as a major evolutionary phenomenon in vertebrates[12,13]. However, since no diploid males were found, the possibility of polyploidization as the cause of this ME gene duplication can be ruled out. This is further supported by allozymic studies of both PGI and PGM[9]. Although variations in PGI and PGM were found in these two cultures of *T. marylandense* with heterozygous females showing 3-band phenotype in PGI and 2-band phenotype in PGM, all males are hemizygous (with only one-band phenotype) at both loci. Therefore, the permanent heterozygosity at this ME locus in both diploid females and haploid males in this *Trichogramma* species is the result of tandem gene duplication. These two cultures have been established for more than 20 generations and still no segregation of the 5-band genotype was observed, indicating that these two loci are tightly linked.

Gene duplication and fixed heterozygosity have been reported in the diploid plant *Clarkia franciscana*[14] and the cultivated soybean *Glycine max*[15]. The present finding of ME gene duplication could facilitate the study of the evolution of Hymenoptera in general and the genus *Trichogramma* in particular. As to the origin of ME gene duplication in this species, we can only speculate at this stage. It could result from unequal crossing-over between two homologous chromosomes in a heterozygote during meiosis, or from duplication during replication followed by a mutation in one of the genes. In either case, if this duplication means a selective advantage, every member of the population would eventually become homozygous for this duplication which would result in the fixed heterozygosity observed in this species.

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Effects of phthalimide on growth and alkaloid formation of *Datura metel* L.

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Summary. When administered to *Datura metel* L. by foliar or root application, the new plant growth regulator phthalimide exercises a favorable effect on the vegetative growth and also stimulates the formation of tropane alkaloids.
Key words. Datura metel; phthalimide; vegetative growth; alkaloid content.

Recently, phthalimide and its related compounds have been reported to be a novel group of chemicals which influence a variety of growth and development processes in many plant species[1]. *Solanaceae* in particular (*Solanum esculentum* Mill. and *S. tuberosum* L.) are very responsive. However, no information is recorded on the influence of phthalimides upon medicinal plants. In this context, in the course of research to study the morphophysiological effects induced by new plant growth regulators in *Sollanaceae* synthesizing tropane alkaloids, we tested phthalimide (PTL) on *Datura metel* L. The investigation presented here was made to discover how far PTL could influence the growth and the synthesis of secondary products in this important solanaceous plant.

Materials and methods. Uniform size seedlings of *Datura metel* L. (25 days old) were employed in this study. The method of seed germination and the soil composition are described in a previous publication[2]. PTL, employed in water solution as potassium salt, was administered by foliar spraying and/or by root immersion. Foliar treatment was made by spraying PTL solutions (700 and 1400 µg/ml) containing 0.6% Tween 20 by a small pressure pump at a rate of 10 ml per plant. The plants received two sprays at 10 and 20 days after transplanting. Root immersion treatment was carried out by dipping the organs for 5 sec in a solution containing 4000 µg/ml of PTL immediately before transplanting. 50 pots were used with 10 samples for each concentration of PTL as well as a control at the onset of the experiment, which was carried out in the greenhouse of the Pharmaco-Biological Department of the University of Messina (Italy).

At 30 days after transplanting, the plants were harvested and the following parameters were recorded: plant height, stem diameter, number of leaves, dry weight, alkaloid content. Height measurements were taken from the soil line to the highest apex. Stem diameter was determined by measuring the circumference of the main stem at the soil line, at the top below the first main 'Y' branch, and midway between these two points. The average of these three readings was taken as the stem diameter. For fresh and dry weight measurements, the plants were divided into leaf-tops, stem and root portions and the fresh weight of each portion was determined immediately. Dry weight as a percentage of fresh weight was determined after drying for 48 h in a forced-air drier at 48.5 °C[3]. For alkaloid estimation, samples from dry plant organs were powdered (to 60 mesh). Alkaloids were extracted with a mixture of chloroform and peroxide-free ether (1:3). The extract was evaporated to dryness on a water-bath and the residue dissolved in a few milliliters of chloroform and 0.02 N sulphuric acid. The excess of acid was titrated with 0.02 N sodium hydroxide using methyl red mixed solution as indicator. The total alkaloid content, expressed as hyoscyamine, was determined from the expression given in the European Pharmacopoeia[4] and the results were calculated on a dry weight basis.

Results and discussion. As seen in the table, PTL-treatment exercised a marked stimulatory action upon the vegetative growth of *D. metel.* The effect was observed when PTL was administered both by foliar and root applications. At harvest, all treated plants were taller than the controls (fig. 1) and showed a marked internode elongation. The greatest increase in height occurred in plants sprayed with 1400 µg/ml of PTL. This concentration also induced the major increase in stem diameter. In the presence of PTL the number of leaves was greater, and the stimulatory effect was weakly higher when the chemical was administered throughout the roots. However, in the PTL-treated plants no difference was noted with regards to the size and morphology of the leaves, or the formation of lateral buds and shoots. Furthermore, under our experimental conditions PTL was completely non-toxic for *Datura metel* plants. This conclusion is supported by the observations of increase in dry weight. In fact, from data in the table it can be observed that PTL exercised a favorable effect also on the increase in dry weight in all the tested organs of *Datura metel* plants. Moreover, also in this case the major increase occurred when a solution containing 1400 µg/ml of PTL was sprayed on the leaves.

Effect of phthalimide on vegetative growth of *Datura metel* L.[1]

Treatments (µg/ml)	Stem length cm	% of control	Stem diameter cm	% of control	Leaves No.	% of control	Dry material[2] Roots g	% of control	Stems g	% of control	Leaf-tops g	% of control
Foliar spraying												
Control	29.35 ± 0.9	–	0.65 ± 0.03	–	14.34 ± 0.5	–	15.53 ± 0.2	–	13.19 ± 0.2	–	14.22 ± 0.4	–
700	40.12 ± 1.5	136.69 ± 9.2	0.84 ± 0.02	129.23 ± 9.1	17.63 ± 4.0	122.94 ± 7.1	17.32 ± 0.2	111.53 ± 2.7	15.36 ± 0.3	116.45 ± 4.0	18.05 ± 0.6	117.58 ± 6.8
1400	48.66 ± 1.9	165.79 ± 11.5	0.92 ± 0.05	141.54 ± 14.2	18.47 ± 0.7	128.80 ± 9.4	20.07 ± 0.1	129.33 ± 2.3	18.30 ± 0.3	138.74 ± 4.6	16.72 ± 0.5	126.93 ± 7.8
Root immersion												
Control	27.06 ± 0.5	–	0.53 ± 0.02	–	14.26 ± 0.5	–	15.08 ± 0.1	–	12.80 ± 0.2	–	13.88 ± 0.3	–
4000	36.28 ± 1.2	134.07 ± 6.9	0.85 ± 0.03	134.92 ± 9.1	18.58 ± 0.6	130.29 ± 9.2	19.21 ± 0.2	127.39 ± 2.2	16.65 ± 0.3	130.08 ± 4.4	15.75 ± 0.5	113.47 ± 6.1

[1] Values are mean ± SE of four determinations. [2] Calculated as % fresh weight.