Bulletin of Insectology **57** *(2): 73-77, 2004*
ISSN 1721-8861

Inheritance of morphological mutations in *Trichogramma*

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Abstract

Five morphological mutations were described and studied in *Trichogramma* Westwood (Hymenoptera Trichogrammatidae). The mutations "fertile-antennapedia" changing antennae for legs, "dark-body" affecting the colour of adults and "unfolded" affecting the folding and position of wings were found in *T. turkestanica* Meyer. The mutations "vermilion-eyes" affecting the colour of the eyes and "vestigial-wings" affecting the size and shape of the wings were found in *T. evanescens* Westwood. For each mutation, an atypical strain was constituted, and crosses between individuals with different mutations were preformed. The results were analyzed at the F1 and F2 generation. Each mutation is determined by one recessive allele at one locus and a strong linkage was found between "vestigial-wings" and "vermilion-eyes" alleles in *T. evanesces*. In *T. turkestanica*, the results between "fertile-antennapedia" and "unfolded" individuals are contradictory for the two reciprocal crosses and the possibility of a gene linkage between the genes coding for these atypical phenotypes is discussed. A gene linkage was not detected in all other crosses.

Key words: Insecta, Hymenoptera, *Trichogramma*, parasitoids, gene linkage.

Introduction

Trichogramma Westwood are minute Hymenoptera, parasitoids of insect eggs, especially Lepidoptera. The genus is distributed worldwide and includes numerous species, some of them used in biological control of agricultural pests.

The first morphological mutation discovered in *Trichogramma* ("vermilion coloured eye") was reported by Fukada and Takemura (1943) in an undetermined species. Since then, four other morphological mutations were reported in this genus. Russo and Pintureau, (1980) reported the mutation "antennapedia" in *T. evanescens* Westwood in a strain originating from France and reared on *Ephestia kuehniella* Zeller. The "vestigial" mutation was found in a strain of *T. brassicae* Bezdenko (Pintureau, 1982) originating from France and reared on *E. kuehniella* eggs. The "brown host" mutation in *T. fuentesi* Torre was reported by Rodríguez *et al.*, (1994). This mutation occurs in nature and turns the host egg into brown, being different in comparison to the wild-type phenotype which turns the host egg into black. The "wing-unfold" mutation was reported by Stouthamer and Kazmer, (1994) in a strain of *T. deion* Pinto and Oatman reared on *Trichoplusia ni* (Hübner) eggs. All these mutations were found to be recessive to the wild-type phenotype. In this work, some morphological variations discovered in *T. evanescens* and *T. turkestanica* Meyer are described, and their inheritance and linkage are analysed.

Material and methods

Selection of atypical phenotypic strains

Atypical *Trichogramma* were selected from one strain of *T. evanescens*, viz., strain A2 originating from Monsols (Rhône, France), and from two strains of *T. turkestanica,* viz., strains PB and MB21 originating from Moncarapacho and Mora, respectively (Southern Portugal). All strains were collected from noctuid eggs and reared on irradiated *E. kuehniella* eggs for several generations until the detection of the atypical phenotypes.

All atypical phenotypes were detected in males. Several pairs of atypical males and conspecific wild-type virgin females were placed in glass tubes to mate. Offspring from each pair (F1 progeny) were allowed to cross freely. Individuals of the F2 progeny were isolated and only atypical males were allowed to cross with their wild-type sisters. Individuals of the F3 progeny were again isolated, atypical males and females were selected and mated to establish atypical strains. These strains were maintained at 23ºC on irradiated *E. kuehniella* eggs over at least 3 generations before crossing experiments. The "vestigial-wings" males were maintained mixed with the wild-type females due to "vestigial-wings" female sterility (see results).

Atypical strains were named according to their phenotype with the following abbreviations: VW and VE strains of *T. evanescens* correspond to "vestigial-wings" and "vermilion-eyes" phenotypes, respectively; ATPF, DB, and UF strains of *T. turkestanica* correspond to "fertile-antennapedia", "dark-body" and "unfold" phenotypes, respectively.

Crossing experiments

The inheritance of the atypical phenotypes was studied in strains of *T. evanescens* and *T. turkestanica*. *E. kuehniella* eggs that were parasitised by strains VW, VE, ATPF, DB and UF were isolated and emerging individuals sexed. Conspecific males and females of different phenotypes were paired, and from each pair host eggs parasitised by female wasps were isolated; females with "vestigial-wings" phenotype were not used in these crosses due to their sterility. Emerging individuals (F1 generation) were classified according to their sex and phenotype. Wild-type females from all pairs were allowed to reproduce as virgins to obtain only a male

(haploid) offspring; these offspring (F2 generation) were placed in 75% alcohol and the phenotype of each individual was registered.

Statistical tests

All tests were performed by χ^2 methods adding together all the F2 males issues from each cross type, except the homogeneity test where comparisons were performed between female offspring. The Yates's correction for continuity was applied in all tests.

To test if the phenotype of F1 mothers could determine the viability of some phenotypes in the offspring, leading to different F2 segregation ratios in reciprocal, the F2 phenotypic frequencies were compared between each reciprocal cross. To detect a possible interaction between parental genome and F2 phenotypic segregation, a χ^2 test for homogeneity was performed testing the homogeneity of segregation ratios among hybrid (F1) female's offspring, in each cross type. The deviation from the expected phenotypic segregation ratios of one (1:1) or two (1:1:1:1) simple Mendelien traits was tested, comparing the observed and expected segregation ratio at the F2 generation. The independent assortment of the 4 phenotypes was tested at F2 generation in each cross type with the χ^2test for independence. The fractions of recombinant and non-recombinant phenotypes were also compared to each other.

Results and discussion

Phenotype descriptions

Individuals with the "fertile-antennapedia" phenotype (figure 1) that were discovered in *T. turkestanica* are morphologically similar to "antennapedia" mutants discovered in *T. evanescens* (Russo and Pintureau, 1980). In both cases, individuals have legs in place of the antenna. However, some differences between the two phenotypes were observed. Firstly, in *T. evanescens* the transformation of the antenna into a leg is complete but in *T. turkestanica*, only the funicular and club segments have the shape of a tibia and a tarsus; the first two segments of an antenna remain in the shape of the scape and pedicel. Secondly, the mutants discovered in *T. evanescens* are sterile, whereas in *T. turkestanica* both males and females are fertile. Thirdly, copulation in males and oviposition in females are disturbed in both species but unlike *T. evanescens, T. turkestanica* has a normal locomotory activity.

Individuals with the "dark-body" phenotype differ from the wild-type by a change in the coloration of the adults. Yellow zones of the body change into gray and darker zones into black. Red eyes also change into black.

Individuals with "unfolded" phenotype (figure 2) discovered in *T. turkestanica* are morphologically similar

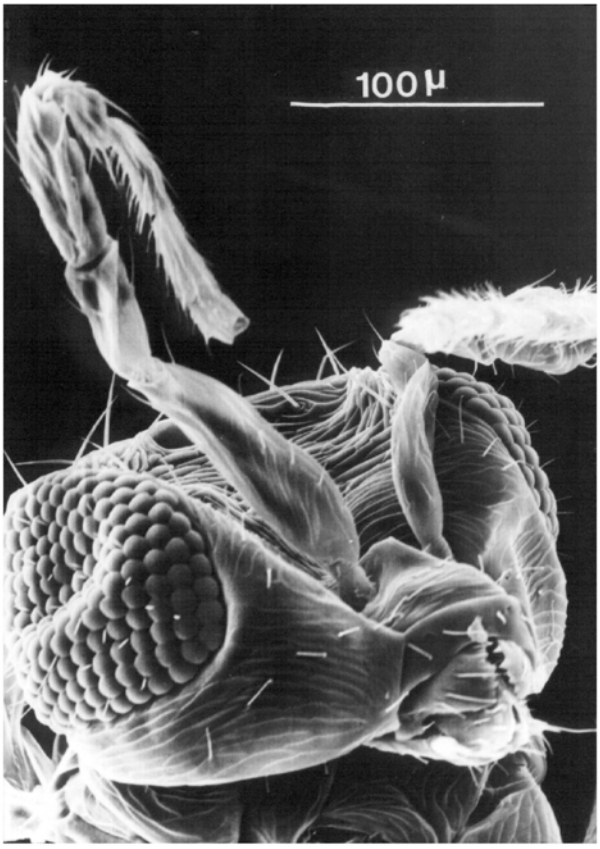

Figure 1. Morphological variants discovered in *Trichogramma*; "fertile-antennapedia" in *T. turkestanica.*

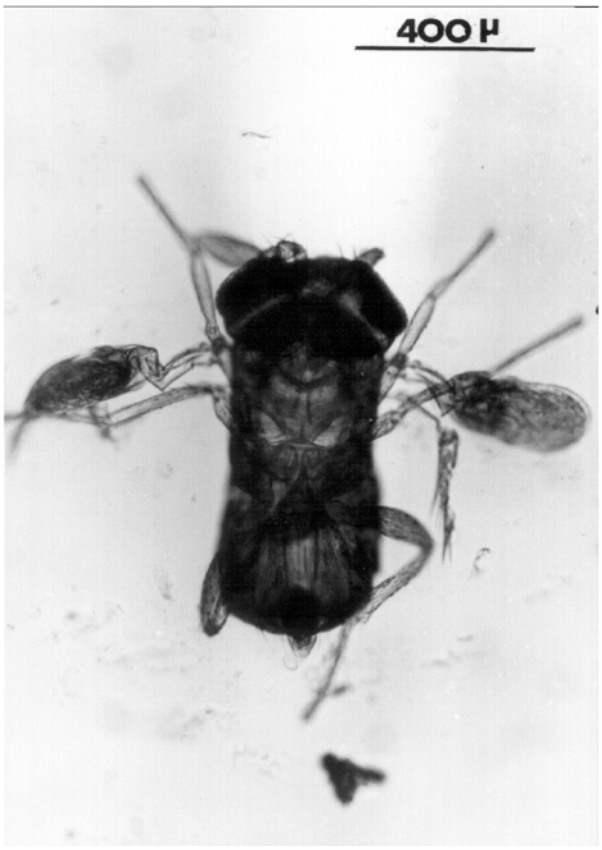

Figure 2. Morphological variants discovered in *Trichogramma*; "unfolded" in *T. turkestanica.*

to "wing-unfold" mutants reported in *T. deion* (Stouthamer and Kazmer, 1994): individuals always have wings perpendicular to the longitudinal axis of the body. Usually, they do not fold their wings, but variations among individuals were observed for this trait. Both males and females have wings partly folded or, rarely, completely folded. The position and the structure of the atypical wings raise difficulties in maintaining the insect equilibrium disturbing the copulation in males and oviposition in females.

Individuals with the "vermilion-eyes" phenotype are similar to "vermilion colored eyes" mutants found in a non identified species by Fukada and Takemura (1943): individuals have scarlet eye colour.

Individuals with the "vestigial-wings" phenotype (figure 3) are similar to "vestigial" mutants found in *T. brassicae* (Pintureau, 1982): males and females have both wing pairs reduced. However, differences in relation to "vestigial" phenotype were observed. Unlike *T. brassicae*, in *T. evanescens*, variations in wing size and shape among atypical individuals were not observed and females are sterile.

Atypical phenotype inheritance and gene linkage

Offsprings of all conspecific *T. evanescens* and *T. turkestanica* pairs (F1 generation) were composed of wild-type females and males holding the same phenotype as their mother. These females, when allowed to reproduce as virgins, produced an F2 male generation with the parental phenotypes and the recombinant phenotypes (table 1). The phenotypic frequencies were never significantly different in reciprocal crosses. Genes coding for atypical phenotypes are therefore present in F1 wild-type females and are recessive in relation to genes coding for the wild-type phenotypes. Loci and recessive alleles (between brackets) were denominated as follows: "fertile-antennapedia" (atpf), "dark-body" (db), "unfolded" (uw), "vermilion-eyes" (ve) and "vestigial-wings" (vw).

Phenotypic frequencies of atypical and wild-type phenotypes in the F2 generation are usually consistent with the segregation ratio of one simple Mendelien trait (1:1), suggesting that a single gene determines the atypical traits (table 1). However, differences in relation to the expected segregation ratio were observed in crosses between "unfolded" and "dark-body" individuals of *T. turkestanica* and one could hypothesise for these traits a multilocus genetic determination. If more than one locus is required to determine these traits, variability in segregation ratio among hybrid (F1) female's offspring can be predicted. Such variability (homogeneity test) was not detected and the distorted F2 segregation ratio is probably caused by differences in phenotypic viability. Also, the occurrence of chromosomal inversions in *T. turkestanica* could explain the distorted segregation ratios.

A F2 phenotypic frequency not compatible with a segregation ratio of two simple Mendelien traits (1:1:1:1) was found in *T. turkestanica* crosses between "fertile-antennapedia" females and "unfolded" males and between "dark-body" and "unfolded" individuals, and in *T. evanescens* between "vermilion-eyes" females and "vestigial-wings" males (table 1). To assign these results to a gene linkage, the χ^2 from independence tests were analyzed. Concerning *T. turkestanica*, only for crosses between "fertile-antennapedia" females and "unfolded" males the independence test was significant. Moreover, a significant difference between the number of recombinant and non-recombinant F2 individuals was detected in these pairs and could confirm a possible gene linkage. In the reciprocal cross a contradictory result was observed: the probabilities from these two tests did not

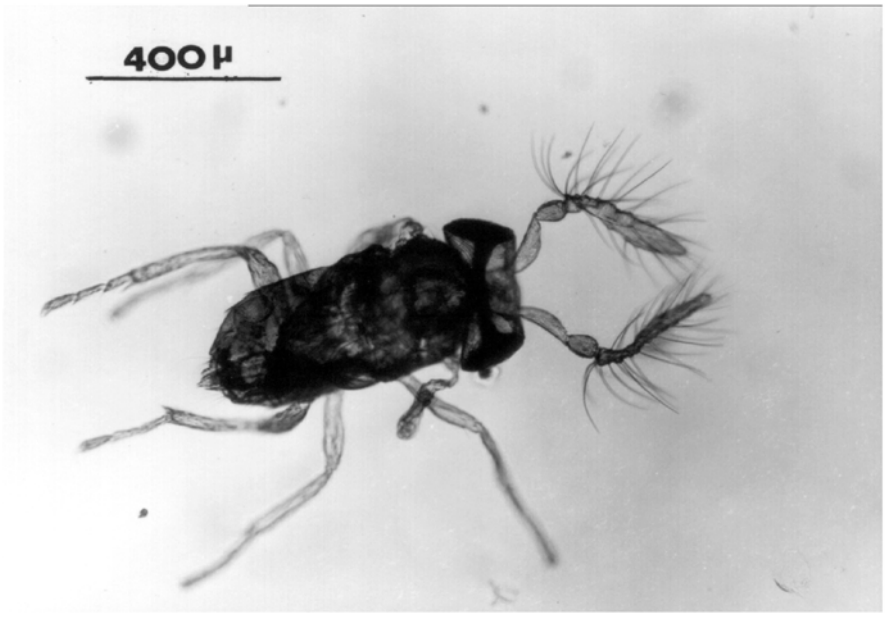

Figure 3. Morphological variants discovered in *Trichogramma*; "vestigial-wings" in *T. evanescens.*

Table 1 Proportions of parental (M, maternal; F, paternal) and recombinants (MF, maternal and paternal; W, wild) phenotypes at F2 generation of crosses with *T. turkestanica* (*T. t.*) and *T. evanescens* (*T. e.*). N, number of individuals observed. Phenotypes - atpf, "fertile-antennapedia"; db, "dark-body"; uf, "unfolded"; ve, "vermilion-eyes"; vw "vestigial-wings".

Cross type			No. of F1 mothers tested	N	F2 phenotypic segregation (%)				χ^2					
					Parental phenotypes		Non parental phenotypes		Mother atypical trait segregation equal 1:1 (a)	Father atypical trait segregation equal 1:1 (a)	Phenotypic segregation equal 1:1:1:1 (b)	Heterogenity among F1 mothers offspring (c)	Independence test (d)	% of rec. and nonrec. offspring equal (a)
Species	M	F			M	F	MF	W						
T. t.	atpf	db	19	1002	22.8	26.5	26.2	24.5	0.4 NS	2.9 NS	3.5 NS	89.4 NS	0.2 NS	0.2 NS
T. t.	db	atpf	19	1078	26.2	23.2	26.2	24.4	2.3 NS	0.1 NS	2.6 NS	80.2 NS	0.1 NS	0.2 NS
T. t.	atpf	uf	14	575	29.2	26.3	20.2	24.3	0.1 NS	2.9 NS	9.9 *	47.9 NS	6.6 *	6.9 **
T. t.	uf	atpf	15	769	28.3	25.1	22.8	23.8	0.4 NS	1.4 NS	5.4 NS	30.7 NS	3.3 NS	3.7 NS
T. t.	db	uf	13	732	30.5	23.1	21.8	24.6	1.6 NS	7.5 **(–)	12.8 **	39.6 NS	3.0 NS	3.7 NS
T. t.	uf	db	21	1450	22.3	28.9	25.2	23.6	3.8 NS	9.9 **(+)	14.6 **	66.7 NS	0.5 NS	0.9 NS
T. e.	ve	vw	22	1691	48.3	49.4	1.2	1.1	0.3 NS	0.7 NS	751.4 ***	42.5 NS	1531.0 ***	1539.0 ***

(a) degrees of freedom = 1; (b) degrees of freedom = 3; (c) degrees of freedom = 3*(Nb. of mothers –1); (d) degrees of freedom = 1; NS P> 0.05; * 0.05 ≥ P > 0.01; ** 0.01 ≥ P > 0.001; *** P ≤ 0.001; (–) deficit of the atypical phenotype; (+) excess of the atypical phenotype.

reach the significance. These contradictory results could be explained if between these two genes exists a very week linkage; in this case, the number of individuals observed in these offsprings could be insufficient to detect it. Or, in fact, the two genes are not linked, and the significant result obtained in the cross between "fertile-antennapedia" females and "unfolded" males might be due to hazard. Again, chromosomal inversions could be responsible for the observed segregation ratios. In *T. turkestanica* pairs between "dark-body" and "unfolded" individuals, neither the independence tests, nor the difference between recombinant and non-recombinant F2 individuals are significant. So, a gene linkage explanation cannot justify the distorted segregation ratios of two simple Mendelien traits (1:1:1:1) obtained in this cross type. On the other hand, differences in a single trait phenotypic segregation ratio (1:1) were detected in both reciprocal crosses suggesting viability differences between the two phenotypes that could explain these results. Again, chromosomal inversions could be responsible for this phenotypic segregation. In *T. evanescens* pairs, both the independence test and the difference between recombinant and non-recombinant F2 individuals are highly significant. This implies a close linkage and justifies the distorted double-trait segregation ratio observed in these pairs.

Due to their haplodiploid sex determination, *Trichogramma* and other hymenopterous parasitoids are useful organisms for genetic analysis. Thus, it is important to obtain gene markers that could be used to study the biology, behaviour, reproduction (Pintureau et al., 1997) or evolution of these insects. The mutations described in this study appeared spontaneously and, taking into account earlier studies where similar variants were reported, it is possible that mutations in *Trichogramma* are more frequent than previously thought. Due to their small size, it is difficult to detect morphological variations and this can justify the scarcity of morphological markers in these parasitoids.

Studies using genetic markers are important not only from the academic standpoint but also have practical implications, namely in biological control. Because some mutations can reduce parasitoid fitness, for biological control it is important to know the frequency with which new mutations arise in *Trichogramma* populations and their evolution in relation to several factors. This is particularly important, if in the course of introductions the size of a population is reduced. Although morphological markers are seldom deleterious, and in this sense they rarely could be used in the field, in the laboratory they can rapidly be noticed in a mixed culture having therefore some advantages in relation to those markers which need more or less complicated analytical methods such as electrophoresis or molecular methods.

Acknowledgements

The JNICT / PRAXIS XXI (Portugal) financed the grant of the first author. This study was supported by grant AIR3-CT94-1433 from European Union. M. del P. Iglesias Calvín was responsible for detection of

"vermilion-eyes" phenotype. We thank I. M. M. S. Silva (Univ. Wageningen) for sending the strain MB 21, B. Delobel (INRA Villeurbanne) for reviewing the manuscript and J. Osório (Univ. do Algarve) for the statistical advice.

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Received May 5, 2004. Accepted September 2, 2004.