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Mini Reviews

Superparasitism reconsidered: is it an adaptive competition? The example of *Diadromus pulchellus*

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Key words. Superparasitism; endoparasites; *Diadromus pulchellus;* adaptive competition.

With superparasitism, several larvae of solitary entomophagous endoparasites can be found in the same host, and yet only one of them develops. The distribution of eggs can range from uniform distribution to an apparently random distribution or to a contagious distribution, which generates superparasitism.

Diadromus pulchellus WSM. is an Ichneumonid solitary endoparasite of *Acrolepiopsis assectella* pupae. Analysis of its egg distribution is possible because egg-laying is followed by withdrawal of the ovipositor. The size of eggs permits their precise count in host pupae. With 5 hosts of the same age, the females conspicuously concentrate their eggs in a limited number of hosts (table 1). An analysis of the distribution of the eggs reveals a statistically significant concentration compared to a random distribution[1,2]. Likewise, field parasitization by *D. pulchellus* shows a tendency to a contagious distribution. But this evidence is indirect because the hosts are not dissected: on 90 collected hosts, Rousse[3] found 36 stung pupae with on average 2.78 stings per pupa. The same type of distribution has been observed for example in *Pleolophus basizonus*[4]. It is very surprising that females do not avoid superparasitism in spite of their complex sensorial equipment[3] and that wasps constantly probe the pupa's surface with their antennae. The females reject hosts affected by virosis, and systematically select the younger hosts[1]. The presence of

Table 1. Parasitized, superparasitized hosts and fecundity of *D. pulchellus* in experiments conducted in the years 1977 and 1980

	Number of experiments	Available hosts N	Eggs laid	Parasitized hosts n	n/N	Superparasitized hosts n′	n′/n
1977	950	4750	2797	1846	0.39	666	0.36
1980	836	4180	2840	2034	0.48	806	0.28

hemolymph on the surface of the pupa, caused either by a sting for feeding or an egg-laying, does not inhibit drilling and oviposition; the females even make new stings followed by egg-laying within a minute after having withdrawn their ovipositors following release of an egg.

The first instar larvae are powerfully equipped for the elimination of competitors. The larvae of the 'mandible type' always have a solitary development[5]. Salt[6] has stressed: 'sickle-shaped mandibles (of the first instar) clearly suitable for fighting (are) not suitable for feeding on a fluid pabulum or necessary for feeding on soft internal tissues... If we can accept morphological evidence of this sort, fighting for possession of the host is widely practised in the parasitoid hymenoptera, and is commonly limited to the first instar'. Whatever number of eggs were deposited in a host, larval fights of the first instar eliminate, at hatching, all the supernumerary larvae of *D. pulchellus*.

Thus, the behavior and also the morphology of the first instar larvae can be considered as adaptations allowing an active competition and the removal of superparasitism. It is therefore necessary to examine whether superparasitism only results in a waste of progeny, or whether it can present an adaptive advantage for the population.

The adaptive value of superparasitism

An endoparasite's habitat is automatically protected by the host's tissues. Even though the host provides a habitat with decreasing homeostasis as it is being consumed[7], it remains nevertheless highly protective and considerably limits mortality during development. Thus for 962 eggs laid separately in pupae by fertilized females of *D. pulchellus*, 865 adults were obtained, i.e. mortality was only 10%. Thus, nearly all the eggs of endoparasites can, in the absence of superparasitism, give rise to adults. The males (which emerge from hosts before the females) of *D. pulchellus* are mature at emergence, wait until females appear and inseminate them. As the progeny is not significantly reduced by any selection pressure, the only mechanisms that could prevent certain individuals from reproducing would be either the sterility of adults or larval competition.

The first mechanism to consider is the sterility of males. Effectively, the males of *D. pulchellus* – whose haploidy increases the frequency of genotypes disadvantaged by the expression of all their alleles – are more frequently sterile than females. The progeny of 153 inseminated females (with examination of the contents of the spermatheca at the end of the experiment) was examined (table 2). The spermatheca contents of 21 females without daughters showed that only abnormal sperm was present in 13 individuals; in 8 other females a mixture of normal and abnormal sperm was found. The large number of matings of 1 female observed in the field within a few hours after emergence[3], must however reduce the probability of the spermatheca being replete with sterile sperm only.

The second type of compensatory system could be the introduction of larval competition. The stage at which the males died can be precisely determined in the case of eggs laid by virgin females. In the same way, it is possible to determine differential mortality during pupation or shortly before the emergence of adults in the case of eggs laid by inseminated females[3].

Table 3 shows the differential mortality for a) solitary male larvae coming from eggs of virgin females, b) solitary larvae (sex unknown) coming from eggs of inseminated females, and c) 2 male larvae in a single host.

The use of a probabilistic model to analyze the data summarized in table 3 has shown:

α) 0.13 (embryo and L1) mortality in isolated male offspring, 0.000 (embryo and L1) mortality in isolated female offsprings,

β) 0.023 larval mortality in isolated male offspring 0.018 larval mortality in isolated female offspring;

γ) 0.022 pupal mortality in isolated male offspring; 0.000 pupal mortality in isolated female offspring.

Mortality of males and females is significantly different in all 3 comparisons (at 5% level). Thus, male mortality is significantly higher than that of females, even in the absence of any larval competition.

In the absence of competition, the differential mortality can be explained since the males are haploid and all detrimental mutations and thus genic combinations are expressed in the phenotype. This analysis is akin to that of Stebbin's who considers that a more frequent sterility in the heterogametic sex is related to hemizygosity[8]. A differential mortality has been observed by Smith and Shaw[9] and Pickering[10].

Higher male mortality, however, is not sufficient to remove from sexual competition partly or totally sterile males. In our studies of *D. pulchellus* including dissection of thousands of host pupae, it has never been possible to find 2 second instar larvae in the same host.

Table 2. Inseminated females with progeny

Number of females inseminated	Females with progeny N	Females with progeny (nymphs and adults) n	n/N	Females without daughters n'	n'/N
153	153	132	0.86	21	0.14

Table 3. Comparison of mortalities during the development of *D. pulchellus*, *a* isolated male egg (from a virgin female); *b* isolated egg (sex unknown) from an inseminated female; *c* 2 male eggs (from a virgin female)[3]

	Eggs laid	Larvae	Pupae	Dead males before emergence	Dead females before emergence	Adults Males	Females	Frequency of total mortality (egg-larvae-pupae) per host
a	1005	874	854	8	–	846	–	0.158
b	962	891	885	20	0	405	460	0.101
c	616	304	295	6	–	289	–	0.077

Table 4. *D. pulchellus:* evaluation of development, *b* of an egg by a mated female; *d* after successive layings of an egg by a mated female and an egg by a virgin female

	Parasited pupae	Eggs laid	Larvae	Pupae	Pre-emergent mortality		Adults obtained		Male frequency	Mortality frequency per host
					Males	Females	Males	Females		
b	962	962	891	885	20	0	405	460	0.47	0.101
d	961	1922	870	824	13	0	366	445	0.44	0.156

Therefore, competition between first instar larvae always results in elimination of all but one larva.

The competition between 2 male larvae (c) can have consequences for the winner since it slightly increases the frequency of pupal mortality[3, 11]. Lastly the higher survival rate of females relative to males gives a slight majority of females in the isolated progeny of mated females (b) (frequency of females 0.53).

From these elements it is possible to examine in *D. pulchellus* the consequences of competition with a larva from a mated female. The very short time between the 2 successive egg-layings cannot have affected the results[3]. The comparison of sex-ratio (table 4) between the adults obtained from solitary eggs of mated females (b) and those obtained from 2 eggs, one of mated females and one of virgin females (d), shows that the frequency of males among the progeny has not been increased by adding an egg of a virgin female in the pupa. The frequency of males resulting is quite similar in these 3 treatments (0.47 and 0.44). In both experiments, no dead females (pupae or adults) were found in the hosts. Therefore, in the flight between 2 larvae of *D. pulchellus* of different sexes, the female larva is the winner.

The arena provided by the inner environment of the host supplies these endoparasites with a remarkable testing place for the incorporation of new alleles in the genic pool. In *D. pulchellus,* the mutation rate per genome per generation is about 0.05; therefore, larval competition has at its disposal the necessary materials to test new alleles as soon as they are expressed in males. The haplodiploid system allows the mutations and genic combinations to be tested in males whose phenotypes present characteristics similar to those of diploid heterozygotes.

Competition between male larvae is less efficient than competition between male and female larvae. The high adaptive value of the heterozygotic genotype of females, proved by their very low mortality and their very low sterility, makes of them controls in the competition. Only the males with exceptional phenotypes, which have integrated beneficial mutations, can rise above this adaptive threshold and defeat female larvae in intersexual competition. Thus, the more frequent the fights are between larvae of different sex, the more rarely will deleterious mutations become integrated into the gene pool. The larval competition appears to be an indispensable element of the haplodiploid system. The presence of haploid males allows the mutated alleles to be tested; intersexual larval competition allows elimination of all those whose adaptive value does not reach the standard value.

In Hymenoptera, males can be considered as an 'extension' of the gametophyte, since, in them also, there is no dominance. Larval competition can be compared to competition between pollen tubes; but the realization of an 'overlapping model', integrating the selection pressures occuring during haploid and diploid phases, according to Mulcahy's terms[12], can be much more easily imagined in Hymenoptera than in Angiospermae, in which the phenotypes of the gametophyte and sporophyte are widely different. Mulcahy's thesis[12] about the coherence of the adaptive values of different successive phenotypes in the life cycle is very credible. Without such coherence, no species undergoing metamorphoses could exist.

Thus superparasitism appears as an important element in the maintenance of a high adaptive value of hymenopteran populations and favoring the permanent re-evaluation of the individuals' fitness. This possibility implies a high polymorphism in populations of entomophagous Hymenoptera. Now the study of many behavioral traits in *D. pulchellus* shows a very high variation[13], and so probably a high polymorphism. In fact, a precise study of allozyme polymorphism in *D. pulchellus* indicates that 30% of the studied loci segregate for 2 or more alleles[14].

Thus, the ecological adjustments would be made more easily by the haplodiploid system. Actually the changes of niches (attack of new hosts) or even of habitats (exploitation of phytophagous insects living on different plants) are very frequent in entomophagous Hymenoptera. Likewise the haplodiploid system associated with a behavior favorable to larval competition may have been the key factor leading to the great radiation of the Ichneumonidae family, about 2000 species.

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Full Papers

Structure of the porcine thyrotropin receptor: a 200 kilodalton glycoprotein heterocomplex

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Summary. We have determined that the porcine thyroidal TSH receptor is a glycoprotein heterotetramer composed of two $M_r \sim 35{,}000$ (ε) covalently linked subunits which interact noncovalently with two copies of δ (M_r 66,000) chains.
Key words. Thyrotropin receptor; glycoprotein; δ and ε subunits; electrophoresis.

Introduction

Thyrotropin (TSH) is a glycoprotein hormone composed of two noncovalently associated subunits[31,32]. Interest in the structure of the TSH receptor is to a large measure related to the fact that the hyperthyroidism of Graves' disease, which has a prevalence of 1%, is associated with spontaneous antibodies against this receptor[14,26,37].

Many attempts have been made to elucidate the receptor structure[4–6,10,11,17,22,34,38,41]; none have yielded unequivocal evidence as to its molecular mass nor its organization. Estimates of molecular mass varied from 15,000 to 500,000 daltons[14]. One group[4] suggested that two $M_r \sim 50{,}000$ subunits linked by disulphide bonds made up the receptor, whereas Koizumi et al.[22] suggested that affinity purified bovine TSH receptor included M_r 38,000 and 66,000 subunits. The present study took advantage of the knowledge that the receptor was an acidic glycoprotein[6].

We report here that the porcine thyroidal TSH receptor is a glycoprotein heterotetramer. It is composed of two $M_r \sim 35{,}000$ (ε) covalently linked subunits which interact noncovalently with two copies of δ (M_r 66,000) chains.

Methods

Partial purification of TSH receptor

Partially purified plasma membranes were prepared from porcine thyroids as previously described[19]. Membrane pellets thus obtained were resuspended in 50 mM Tris-HCl pH 7.5, protein concentration adjusted to 3 mg/ml and homogenized with an equal volume of 0.2 M lithium-diiodosalicylate (LIS) in 50 mM Tris-HCl, pH 7.5. The homogenate was stirred for 1 h at 4 °C and the insoluble material was removed by centrifugation at 113,000 × g for 45 min at 4 °C. The supernatant was dialyzed against three changes of 20 mM Tris-HCl pH 8.0 at 4 °C and then centrifuged at 131,000 × g for 45 min. 20 g of thyroid tissue usually yielded 0.7–1.3 mg of solubilized membrane proteins. All steps of thyroid plasma membrane isolation were carried out in the presence of 2 mM phenylmethylsulfonylfluoride. 20–25 ml of membrane lysate were applied to a DEAE-Sephacel column (1.5 × 20 cm), bed volume 20 ml equilibrated with 20 mM Tris-HCl, pH 8.0. After washing the column with three bed volumes of equilibrating buffer, it was eluted with 30 mM Tris-HCl, 1 M NaCl, pH 8.0 until all the protein material, as determined by absorbance at 280 nm was removed. Fractions containing protein were pooled and desalted by filtration through Sephadex-G-25M (4 × 40 cm) column. Material from two such columns was applied to a new DEAE-Sephacel column which was then eluted with a 0.0–1.0 M linear gradient of NaCl in 20 mM Tris-HCl, pH 8.0 at a flow rate of 17 ml/h. Fractions collected at 10-min intervals were appropriately pooled and concentrated using cx-10 immersible millipore filter (Millipore Corp. Bedford, MA, USA). Pooled fractions were designated I–VIII (fig. 1a) and tested for their capacity to bind ^{125}I-bTSH.

In order to further resolve the fraction which contained this activity (peak VIII), 400 µg–3 mg of fraction VIII in 200 µl of 20 mM Tris-HCl pH 8.0 were applied to a