A Single Parasitoid Segregating Factor Controls Immune Suppression in *Drosophila*

S. Dupas, F. Frey, and Y. Carton

Encapsulation has evolved as an efficient mechanism whereby an insect host can survive infection by parasitoids This ability is controlled by a major gene in *Drosophila melanogaster* hosts. The parasitoid *Leptopilina boulardi* (Hymenoptera Eucoilidae) can suppress the *Drosophila* immune reaction by injecting viruslike particles. Analysis of Mendelian crosses between strains of *L. boulardi* of opposite immune suppressive abilities indicated that the trait is controlled by a single chromosomal factor with semidominant effect. We developed a method to test the monogenic hypothesis. The range of possible genotypic values in back-crosses was studied using various progeny that were genotypically homogenous. These could be obtained because of the arrhenotokous mode of reproduction. The progeny groups were divided into two clusters according to the major gene classification and the hypothesis of another unlinked genetic factor was rejected. Lastly, there was a residual progeny effect within the major groups, indicating that minor genes are also present. This study rules out the polygenic effect for a trait governing the interaction between the insect and parasitoid. It demonstrates that the gene-for-gene model commonly found in plant-parasite interactions may also explain natural variations in insect-parasitoid traits.

Insect parasitoids are highly virulent parasites that invariably kill their host before they reproduce. They are therefore useful tools for studying genetic conflicts between species and for determining whether coevolution is induced by such severe ecological antagonism. Coevolution, or reciprocal evolution between species, assumes that there is genetic variation in both partners for traits involved in the interaction (Boulétreau 1986). Recently Henter (1995) and Henter and Via (1995) demonstrated the existence of genetic variation in host resistance and parasitoid virulence in a sympatric aphid-parasitoid interaction. However, the genetic and physiological mechanisms involved were not elucidated. Unfortunately the mechanisms underlying reciprocal evolution cannot be understood unless the genetic and molecular factors involved are identified. This is demonstrated by the tremendous development of coevolutionary theory that followed the identification by Flor (1951) of the genetic models governing plant-pathogen systems. Insect-parasitoid interactions are themselves too complex to allow the development of a similar genetic model to explain the overall success of the parasitoid.

But genetic models can be built to describe specific traits in the interaction between *Drosophila* and its parasitoids. A major gene determining the foraging behavior of *Drosophila* (de Belle and Sokolowski 1992) was shown to be related to susceptibility to parasitoid infection (Carton and Sokolowski 1992). To survive infection, hosts must destroy the intruder by encapsulation (Carton and Nappi 1991, 1997). This is a cell-mediated process in which host hemocytes aggregate and form a multicellular sheath or capsule around the parasitoid egg. Parasitoids have developed several strategies to evade encapsulation. Some parasitoids obtain passive protection by masking the egg with host-like antigens (Strand and Pech 1995), while others actively suppress the host immune system by injecting viruses called polyDNA viruses along with their eggs (Lavine and Beckage 1995; Strand and Pech 1995). The genes of the virus are expressed in the host's hemocytes (Li and Webb 1994), but they cannot replicate outside the parasitoid's calyx cells and are genetically transmitted along with the parasitoids' chromosomes (Stoltz 1990). The parasitoids of *Drosophila* from the genus *Leptopilina* also inject viruslike particles

From the Laboratoire Populations, Génétique et Evolution, CNRS 91198, Gif-sur-Yvette Cedex, France. Thanks to Frederic Hospital and Pierre Capy for their useful comments on the manuscript and to Nancy Beckage and Anthoni Nappi for their help in the improvement of the manuscript. This work was supported by an ACC program (MESR, France) and by an E.C. program (CEE-AIR3-CT9-1433). Address correspondence to Dr. Dupas at the address above or e-mail: dupas@pge.cnrs-gif.fr.

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that may contain DNA into the host (Dupas et al. 1996). These particles cause specific alterations in host hemocytes (Rizki and Rizki 1990b).

As noted by Beckage (1993), most studies on parasitoid virulence factors have been aimed at determining the genes and molecules involved, without any reference to their variation and potential for evolution in natural population. However, the intensity of immune reactions varies tremendously over an evolutionary time scale, especially in newly created parasitic associations, and many authors have suggested that this evolution is due to reciprocal selective pressure on both parasitoid and host (reviewed in Boulétreau 1986). A genetic variation in the encapsulation rate has been found in both partners of the *D. melanogaster-L. boulardi* association in natural populations (Carton and Nappi 1991). Variation in the host is determined by a major dominant gene (Carton et al. 1992), but variation in the parasitoid is presently not well understood. Walker (1959) studied the ability of *L. heterotoma* to counteract the *Drosophila* immune reaction and thereby demonstrated that the trait is governed by a dominant autosomal factor I (inhibitor). Recently, inbred lines of the wasp *L. boulardi* were examined for their content of viruslike particles and their ability to suppress the *D. melanogaster* immune reaction (Dupas et al. 1996). It was found that the ability of these lines to suppress the host immune response varied and was correlated with the morphology of the viruslike particle carried by the female wasp. Since the particles are believed to contain DNA, the question arises of whether they are responsible for the extra-chromosomal transmission of immune-suppressive ability, or whether they are transmitted within the chromosomes of the parasitoid as are polyDNA viruses. The present study was therefore carried out to assess the genetic components that determine the variation in the ability of the *Leptopilina* parasitoid to counteract the host immune response. We first attempted to determine whether the viral genome contributed to the genetic transmission of the trait, and contracted a genetic model to describe parasitoid encapsulation rate that includes both the host and the parasitoid factors.

Two inbred lines of the parasitoid were used, one from an immune suppressive (IS) population and one from a non-immune suppressive (NIS) population (Russo et al. 1996). We performed two generations of reciprocal crosses between them. The crosses were analyzed to estimate the extra-chromosomal components, the degree of dominance, and the number of genetic factors involved. Single-gene determinism and the presence of residual polygenic variation were tested for using a method to determine the array of possible genotypic values in the backcross. Most of genetic variation in the trait could be attributed to a single chromosomal region of the parasitoid chromosomes having a semidominant effect. We presume that this genetic factor responsible for host immunosuppression is also present in the genome of the symbiotic virus which is injected into the host during infection.

Material and Methods

Origin of the Strains

Two isofemale lines of *L. boulardi* differing in their capacities to suppress the *D. melanogaster* immune reaction were used for crossing. The immune suppressive line (IS, or G431 strain) was propagated from a single female collected in the Nasrallah oasis in Tunisia and maintained for 8 years in the laboratory. The nonimmune suppressive line (NIS, or G486 strain) has been maintained for 6 years and propagated from a single female originating from a mass culture of a Brazzaville (Congo) strain collected in 1977. The infection experiments were performed using two resistant *D. melanogaster* strains (R940 and R445) that were selected for their ability to form hemocytic capsules around the eggs of *L. boulardi.* The selection procedure has been described in Russo et al. (1996).

Bioassay

The parasitoid virulence of each female was estimated by calculating the proportion of the eggs that escaped encapsulation in a host strain which was normally totally resistant against *L. boulardi* NIS strain. The factors responsible for evading encapsulation may have different origins, including embryonic cells and several maternal tissues such as the maternal accessory glands and ovaries (Lavine and Beckage 1995; Strand and Pech 1995). Only factors from the accessory gland have been described for *L. boulardi* (Dupas et al. 1996). Virgin females were used in our genetic study, so that whenever the factors were produced by the egg, their mean activity represented the genotype of the female, since the eggs are produced by arrhenotokous parthenogenesis in virgin females. Strains of *D. melanogaster* R940 and R445 that were inherently resistant to *L. boulardi* (NIS) were used as hosts. In each experiment, 40 second instar host larvae were infected by one virgin parasitoid female. The larvae were dissected 48 h later to estimate the rate of immune suppression, defined as the number of eggs developing without encapsulation compared to the total number of eggs deposited by the wasp female.

Genetic Crosses

Two generations of crosses (Table 1) were performed to yield seven lines of progeny: two parental strains, two reciprocal F_1 hybrids, two back crosses to each parental strain, and one F_2 hybrid. Because of arrhenotoky, the results of the F_2 progenies are equivalent to that of backcrosses of F_1 to the F_1 mother's line. Data from the F_2s were therefore included in their homologous backcross progeny. The parasitoids were reared on a host strain susceptible to *L. boulardi* to avoid selection for immune suppressive ability (S22; Russo et al. 1996). The results of the crosses were compared using the method of de Belle and Sokolowski (1987) to assess the hereditary components involved.

Statistical Analysis

For statistical tests, rates of host immune suppression were normalized with the arcsine function suitable for binomial rates (r/n) designated for a moderately large n (angle = $\arcsin\sqrt{(r + 3/8)/(n + 3/4)}$; Rao 1951). Kolmogorov-Smirnov tests for normality were performed on transformed data. No significant deviation from normality was observed (Table 1). The mode of inheritance of the trait was determined by contrast analysis of variance (ANOVA) using the method of de Belle and Sokolowski (1987). The crosses contrasted are shown in brackets and described in Table 1. Each cross has a coefficient of ($\times 1$) unless shown otherwise.

1. IS versus NIS (1 versus 2) tested the genetic differentiation between parental strains.
2. F_1s (3 versus 4) tested deviation from a chromosomal mode of inheritance (permanent or transient maternal effect).
3. IS versus F_1 [1($\times 2$) versus 4 + 5] tested the hypothesis of dominance.
4. IS + NIS versus F_1 (1 + 2 versus 4 + 5) tested the semidominant hypothesis.
5. NIS versus F_1 [3($\times 2$) versus 4 + 5] tested the recessive hypothesis.

Table 1. Mean immune suppression rates of eggs of *L. boulardi* females from crosses between immune suppressive (IS) and nonimmune suppressive (NIS) strains of *L. boulard* (NS = nonsignificant)

Cross number	Mother × Father	Number of females tested	Number of eggs dissected	Rate of imune suppression (mean ± SE) (%)	Arcsine transformed data (radian)	Kolmogorov-Smirnov test for normality on transformed data (d)
Parental strains						
1	IS × IS	12	248	99.8 ± 0.2	1.43 ± 0.01	0.171 (NS)
2	NIS × NIS	17	492	3.6 ± 1.4	0.21 ± 0.02	0.170 (NS)
Reciprocal F_1 hybrids						
3	IS × NIS	12	267	53.7 ± 4.1	0.74 ± 0.04	0.081 (NS)
4	NIS × IS	10	223	56.3 ± 4.9	0.72 ± 0.05	0.181 (NS)
Backcrosses						
5	(IS × NIS) × IS	30	440	25.9 ± 13.9	1.06 ± 0.06	0.118 (NS)
6	(IS × NIS) × NIS	10	198	84.9 ± 11.3	0.38 ± 0.08	0.179 (NS)
Backcrosses with homogenous genotype (see Figure 1)						
7	IS × F_2 (IS × NIS)	9	260	97.1 ± 1.3	1.38 ± 0.03	0.197 (NS)
8	IS × F_2 (IS × NIS)	5	63	100 ± 0.0	1.39 ± 0.02	0.338 (NS)
9	IS × F_2 (IS × NIS)	10	324	41.9 ± 3.1	0.70 ± 0.03	0.252 (NS)
10	IS × F_2 (IS × NIS)	6	168	51.9 ± 3.3	0.80 ± 0.03	0.104 (NS)
11	IS × F_2 (IS × NIS)	5	77	50.1 ± 2.8	0.79 ± 0.03	0.113 (NS)
12	IS × F_2 (IS × NIS)	6	85	100 ± 0.0	1.40 ± 0.01	0.221 (NS)
13	IS × F_2 (IS × NIS)	7	65	86.7 ± 3.5	1.18 ± 0.07	0.328 (NS)

Number of Segregating Factors

The number of genetic factors with additive effects that contribute to the expression of a quantitative trait can be estimated by applying Wright's formula from Lande (1981):

$$n = (\mu_2 - \mu_1)/(8 \times \sigma_S^2) \qquad (1)$$

Where μ_1 and μ_2 are the phenotypic means of the parental strains and σ_S^2 is the extra genetic variance segregating in the F_2 population beyond that in the F_1 hybrid. In our case, since F_1 males originated from the development of a unfertilized haploid ovocyte, the F_2 generation was similar to the backcross generation. Lande (1981) provided a formula using differences in phenotypic variance between backcrosses, F_1 hybrids, and parental populations (see below):

$$\sigma_S^2 = \sigma_{B_1}^2 + \sigma_{B_2}^2 - \left(\frac{1}{2}\sigma_{P_1}^2 + \sigma_{F_1}^2 + \frac{1}{2}\sigma_{P_2}^2\right). \qquad (2)$$

Skewness in the estimation originating from environmental differences between phenotypic variances are minimized by weighting their contributions by the Mendelian ratios in F_2. The standard error of n corresponding to this estimation method was calculated according to the formula provided by Lande (1981). The actual number of genes may be underestimated by this method if they are linked or if there is dominance and epistasis (Lande 1981).

Test of the One Gene Model Using Genetically Homogenous Progenies

The arrhenotoky of *L. boulardi* and the associated haplodiploid strategy of sex determination permitted the constitution of sets of progeny composed of individuals that were genetically homogeneous and recombined for their paternal chromosome. The cross procedure is described in Figure 1. Seven F_2 males were backcrossed to the IS female parent. The immune suppressive abilities of 5–10 females per fam-

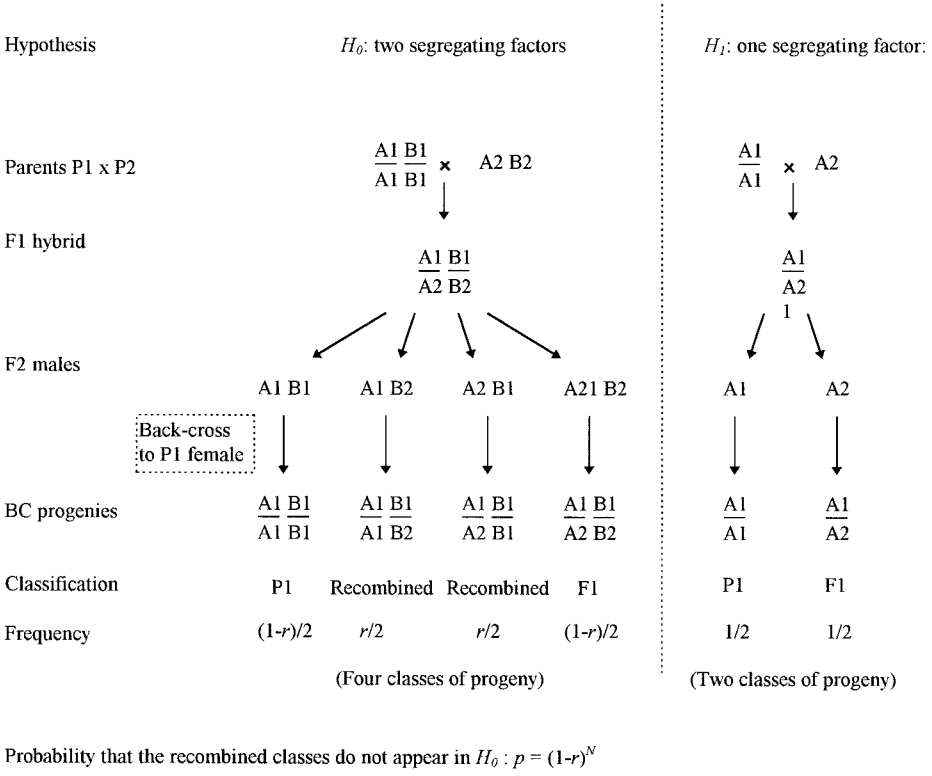

Figure 1. Cross procedure to obtain back-cross families with genetically homogenous progeny. The effect of recombination on the trait is expressed as the difference between them and the nonrecombinant progeny (F_1 and P_1). Principle of the test of the H_0 hypothesis with two segregating loci (A1/A2 and B1/B2). The P value of the test is the probability that the recombined classes do not appear in the backcross progeny if we assume H_0 (H_0 is rejected whereas it is true). This is given by the frequency of parental haplotypes in the F_2 male population under H_0 (r = recombination rate, N = number of progeny measured).

Table 2. Contrast ANOVA for the stability of the encapsulation rate in parental strains during the course of experiments (null hypothesis = no effect of the series of experiment; NS = not significant)

Source	*F*	df Effect	df Error	*P*
R (R940)/IS (G431)	.921	2	17	.424 (NS)
R (R940)/NIS (G486)	.380	7	9	.818 (NS)

Table 3. Student's *t* tests for the stability of immune suppressive ability of parental parasitoid strains against the resistant strain of *D. melanogaster* R445 after 7 years of rearing (*n* = number of parasitoid eggs investigated; NS = not significant)

Infection experiments (host strain/ parasite strain)	Rate of immune suppression (%) [mean ± SE (*n*)]		*t* tests		
	1989	1996	*t*	df	*P*
R445/G431 (IS)	100.0 ± 0.0 (187)	99.8 ± 0.4 (364)	1.07	13	.30 (NS)
R445/G486 (NIS)	12.2 ± 2.0 (286)	14.8 ± 5.8 (121)	.59	20	.56 (NS)

ily were determined (crosses 7 to 13 in Table 1). Each progeny was genetically homogenous for IS genes because the F_2 fathers were haploid and the P_1 mothers were homozygous. Furthermore, since the chromosomes from the F_2 fathers were recombinant chromosomes, all the possible genetic values of an ordinary backcross ($F_1 \times P_1$) could be obtained. This original cross procedure was a useful tool for determining the array of possible genetic values in a backcross and inferring the number of additive segregating factors. P_1 (cross 1, i.e., the IS strain) and F_1 (crosses 3 and 4), crosses that corresponded to the unrecombined genotypes, were also associated with the dataset to give a total of nine families. The families were classified by the unweighted pair group method with arithmetic averages (UPGMA) to determine whether they fell into the major groups corresponding to the genotypic values of the major gene. This technique of classification was used by Thompson and Mascie-Taylor (1985) to detect major segregating factors for frequency of veingap in *Drosophila* with crosses involving isofemale strains. Each cross was assigned to a major group, and discriminant analysis was performed to estimate the posterior probability that it belonged to the other major group. Finally, the residual polygenic variance effect within each major group was tested. All the statistical techniques were performed using the STATISTICA 5 software package.

Results

Genetic Stability of the Host and Parasitoid Strains

The stability of the immune suppressive rates of the parental strains was tested over a period of 2 years during the course of the experiments. ANOVA was performed from one series of experiments to another (Table 2). There was no significant variation in the encapsulation rate, suggesting that the encapsulation rates were genetically fixed by the two partners. This hypothesis was tested for the parasitoid strain by infecting another host strain (R445; resistant to *L. boulardi*) over a longer period. There was no change in either parental strain over a 7 year breeding period (Table 3). In keeping with this stability, we accepted the hypothesis that the parental strains were homozygous for IS genes.

Estimation of the Immune Suppressive Ability of the Various Crosses

The mean immune suppressive abilities of the crosses are given in Table 1 and the contrast ANOVAs are given in Table 4. The genetic difference between the two parental strains was confirmed by the significant difference observed between them (contrast 1; $P = 10^{-6}$). The lack of significant difference between reciprocal F_1 hybrids (contrast 2; $P = .60$) indicated that the trait was inherited chromosomally (without transient or permanent extrachromosomal factors). Finally, among the different models of dominance considered in contrasts 3, 4, and 6, the best fit was obtained for the semidominant model ($P = .04$; contrast 5); the dominant and recessive models were rejected ($P = 10^{-6}$; contrast S4 and 6).

Estimation of the Number of Loci Involved in Immune Suppression

The quantitative method from Lande (1981) was used to estimate the number of genetic factors with equal effect segregating in the F_2 generation. The estimate $n \pm SE = 1.336 \pm 0.385$ suggested that the trait was determined by a single factor.

Test of the One-Gene Model

The cross procedure used to obtain the recombinant progeny of homogenous genotype is described in Figure 1. The values of recombined genotypes which might differ from the F_1 and P1 values if recombining factors were present could be estimated with standard errors. The UPGMA classification of the progeny shown in Figure 2 (also including P_1, F_1) clearly separated them into two clusters corresponding to F_1 and P_1 values, and each family could be easily assigned to one particular cluster (Table 5). This structure agreed with the one-gene model of variation in the backcross. The Wilks' lambda of 0.12 obtained in discriminant analysis attests that most of the variation between progeny is explained by the classification (Thompson and Mascie-Taylor 1985). The posterior probabilities that an offspring belonged to the other major group were always negligible (*P* value always less than 10^{-4}; Table 5). However, ANOVA (Table 6) demonstrates that there was a family effect, at least within one of the two major clusters. This indicates that minor segregating factors also had significant effect. The probability *P* that such a major gene classification was obtained if two genetic factors had major effects was equal to the probability that no recombination occurred between them in any of the cross. This is given by

$$P = (1 - r)^N, \qquad (3)$$

where *r* is the recombination rate and *N* is

Table 4. Contrast ANOVA of immune suppressive ability of females from five crosses between immune suppressive (IS) and nonimmune suppressive (NIS) *L. boulardi* strains

Source of variation	df	SS	MS	*F*	*P*
Model (between crosses)	4	12.318	3.079	233.3	10^{-6} ***
1. Genetic differentiation between IS and NIS strains	1	10.268	10.268	777.9	10^{-6} ***
2. Deviation from a chromosomal mode of inheritance	1	0.004	0.004	.283	.60 NS
3. Deviation from the dominant model	1	3.492	3.492	264.6	10^{-6} ***
4. Deviation from the semidominant model	1	0.056	0.056	4.243	.04*
5. Deviation from the recessive model	1	2.751	2.751	208.4	10^{-6} ***
Error (within crosses)	50	0.660	0.013		
Total	54	12.978	0.240		

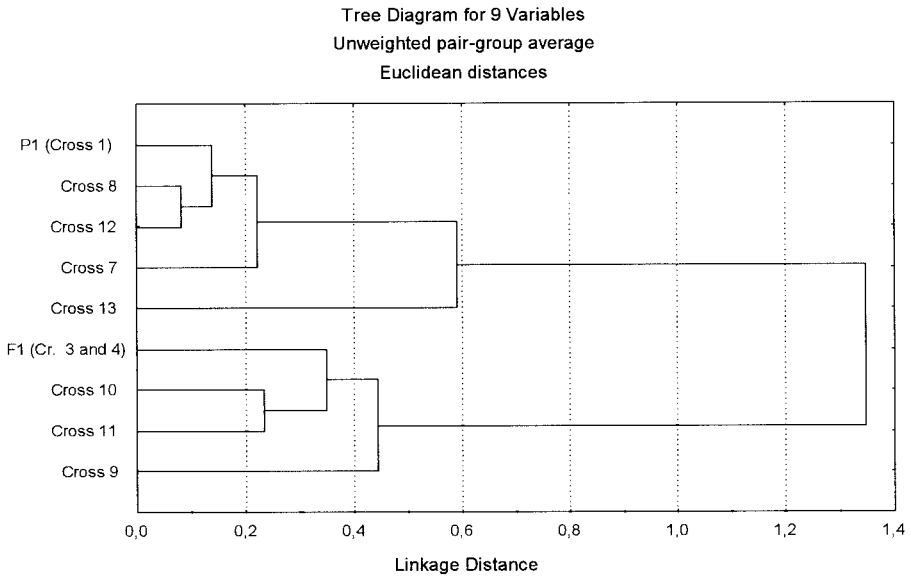

Figure 2. Dendrogram showing the degree of similarity between backcross progeny with homogenous genotype and P_1 (cross 1 only, i.e., the virulent parental strain) and F_1 (crosses 3 and 4 mixed since there was no significant difference between these reciprocal F_1 hybrids) progeny. Cluster analysis were carried out by the unweighted pair group method with arithmetic averages. Cluster analysis assesses the degree of similarity among pairs of quantitative comparisons. The comparisons developed here are between pairs of crosses, such as those in Table 1.

the number of experiments (see Figure 1). Using this formula, we rejected the hypothesis that there was another unlinked major factor with $P = (1 - 0.5)^6 = .01$. We also deduced from Equation (3) the maximum size of the chromosomal region containing most of the genetic variation involved in the trait. Considering that $1 - P$ is the degree of confidence and r, the recombination rate between the limits of the region, we have

$$r = 1 - p^{1/N}. \quad (4)$$

In our experiment, the size of the region was $r = 1 - 0.05^{1/7} = 34.8$ cM with 95% confidence.

Discussion

In a previous article (Dupas et al. 1996) we identified viruslike particles in the *L. boulardi* accessory gland that were capable of influencing the suppression of immune reaction in a *D. melanogaster* host. This led us to consider the possibility of extrachromosomal genetic transmission of immune suppression through the viral genome. This study indicates that this type of transmission does not occur. The factors that determine immune suppression are not vertically transmitted along with the viruses, but within the parasitoid chromosomes. The genetic transmission of the polyDNA virus genome had been studied by Stoltz (1990) in both braconid and ichneumonid parasitoids. He demonstrated that the extrachromosomal viral DNA plays no role in the genetic transmission of virus-DNA RFLP polymorphism. This study extends to cynipid parasitoids the fact that viruses do not participate in the host-parasitoid interaction as a genetically independent entity. PolyDNA viruses belong to the parasitoid genome from an evolutionary point of view, and can be considered to be simple tools used by parasitoids to modify the physiology of their hosts. The tests on Mendelian ratios do not help to determine the number of genes because of the semidominance of the genetic factors. We therefore used the method of Wright, which assumes quantitative inheritance, to estimate the minimum number of segregating factors. Our results are consistent with an oligogenic model ($n \pm SE = 1.336 \pm 0.385$). We then developed a method that allowed us to determine the array of possible genotypic values in a backcross so as to specify the components of this model. Most of the observed variation could be explained by a single segregating factor.

Table 5. Classification of progeny groups with homogenous genotype into P_1 or F_1 groups and probabilities of misclassification

Cross number (Table 1)	1	3 + 4	7	8	9	10	11	12
Classification of the progenies into P_1 or F_1 group	P_1	F_1	P_1	P_1	F_1	F_1	F_1	P_1
Probability of misclassification	10^{-6}	10^{-6}	10^{-6}	10^{-6}	10^{-6}	10^{-6}	10^{-6}	10^{-6}

The probability of misclassification of a progeny is the probability a posteriori of the other classification (P_1 when F_1 and vice versa) for a new progeny having the same value. The crosses are described in Figure 1 and Table 1.

Table 6. Progeny effect within P_1 and F_1 groups

Group	df Effect	df Error	F	P level
P_1	4	30	9.11	10^{-5} (***)
F_1	3	39	.68	.56 (NS)

The progeny are composed of individuals with homogenous genotypes and the groups are deduced from the dendrogram in Figure 1.

Immune suppressive ability was also found by Walker (1959) to be dominant in a related species, *L. heterotoma.* The most common type of immune suppression involves either altering the appearance of the host hemocytes which is correlated with inhibition of their capacity to adhere or spread, or with complete apoptosis of hemocytes leading to their destruction (Lavine and Beckage 1995). A specific class of *Drosophila* hemocytes lose their ability to spread in insects infected by *L. boulardi* (Rizki et al. 1990). The changes that occur in morphology of hemocytes after *L. heterotoma* infection require modifications in microtubule assembly (Rizki and Rizki 1990a). It is therefore likely that the *L. boulardi* gene product responsible of immune suppression influences interaction between the viruslike particles of the wasp and a system that regulates the cytoskeleton in the host hemocytes. Nevertheless, the single-factor inheritance shown in this study indicates that the physiological mechanism is simple. This appears to contrast with the other systems studied to date, in which the patterns seem to be complex. The infected *Campoletis sonorensis* host contains a complex of parasitoid ovarian proteins (Webb and Luckhart 1996) and at least 12 viral transcripts have been detected (Blissard et al. 1986). *Cotesia rubeculata* has developed a passive protection system using calyx fluid proteins (Asgari and Schmidt 1994) which is coupled to suppression of the host immune system by two main transcripts (Asgari et al. 1996). The single-factor inheritance in *L. boulardi* should therefore be interpreted cautiously; the trait may be controlled by a cluster of genes that cannot be dissected by Mendelian genetic methods. We do not know

to date whether the viral genes in *C. sonorensis* that are expressed in the host are associated in a cluster or dispersed among the parasitoid chromosomes (Soldevila and Webb 1996). The other interpretation of the result is that only one or a few of the factors that are injected into the host by *Leptopilina* or *Campoletis* are detected in the crosses because only some can suppress the immune system of a particular host strain.

This study indicates that the encapsulation of the parasitoid *L. boulardi* by its host *D. melanogaster* may be governed by a gene-for-gene relationship, since the effect of the major gene for resistance variation in the host demonstrated by Carton et al. (1992) is suppressed by a semidominant genetic factor in the parasitoid. Encapsulation only occurs following the infection of the R strain of *D. melanogaster* by the NIS strain of *L. boulardi.* The three other interactions involving the susceptible (S) strain of *Drosophila* or the IS strains of parasitoid lead to successful parasitoid development (Russo et al. 1996). Intuitively, and as demonstrated by theoretical models, if we assume that the immune reaction is blocked by a simple gene-for-gene mechanism in the field, then genetic evolution will tend toward the fixation of the resistant and virulent genes. This may lead to the disappearance of the immune reactions in field situations. This absence of encapsulation was observed by Carton and Nappi (1991) for the *L. boulardi-D. melanogaster* system in most, but not all, the localities studied. The trade-off between immune suppression and other fitness-related traits must be included in models built to explain the local appearance of encapsulation. According to this trade-off hypothesis, immune suppressive ability is lost when host resistance is low. Population cage experiments are presently under way to document this question of the cost for parasitoid virulence.

References

Asgari S, Hellers M, and Schmidt O, 1996. Host haemocyte inactivation by an insect parasitoid: transient expression of a polydnavirus gene. J Gen Virol 77:2653–2662.

Asgari S and Schmidt O, 1994. Passive protection of eggs from the parasitoid *Cotesia rubeculata,* in the host *Pieris rapae.* J Insect Physiol 40:789–795.

Beckage NE, 1993. Games parasites play: the dynamic roles of proteins and peptides in the relationship between parasite and host. In: Parasites and pathogens of insects, vol. 1 (Beckage NE, Thompson SN, and Federici BA, eds). San Diego: Academic Press; 25–57.

Blissard GW, Vinson SB, and Summers MD, 1986. Identification, mapping and in vitro translation of *Campoletis sonorensis* virus mRNAs from parasitized *Heliotis virescens* larvae. J Virol 160:120–134.

Boulétreau M, 1986. The genetic and coevolutionary interactions between parasitoids and their hosts. In: Insect parasitoids (Waage J and Greathead D, eds). Orlando, Florida: Academic Press; 169–200.

Carton Y, Frey F, and Nappi AJ, 1992. Genetic determinism of the cellular immune reaction in *Drosophila melanogaster.* Heredity 69:393–399.

Carton Y and Nappi AJ, 1991. The *Drosophila* immune reaction and the parasitoid capacity to evade it: genetic and coevolutionary aspects. Acta Oecologica 12:89–104.

Carton Y and Nappi AJ, 1997. *Drosophila* cellular immunity against parasitoids. Parasitol Today 13:218–227.

Carton Y and Sokolowski MB, 1992. Interactions between searching strategies of *Drosophila* parasitoids and the polymorphic behavior of their host. J Insect Behav 5:161–175.

de Belle S and Sokolowski MB, 1987. Heredity of rover/sitter: alternative foraging strategies of *Drosophila melanogaster* larvae. Heredity 59:73–83.

Dupas S, Brehelin M, Frey F, and Carton Y, 1996. Immune suppressive virus-like particles in a *Drosophila* parasitoid: significance of their intraspecific morphological variations. Parasitology 113:207–212.

Flor HH, 1956. The complementary genetic systems in flax and flax rust. Adv Genet 8:29–54.

Henter H, 1995. The potential for coevolution in a host-parasitoid system. II. Genetic variation within a population of wasps in the ability to parasitize an aphid host. Evolution 49:439–445.

Henter H and Via S, 1995. The potential for coevolution in a host-parasitoid system. I. Genetic variation within an aphid population in susceptibility to a parasitic wasp. Evolution 49:427–438.

Lande R, 1981. The minimum number of genes contributing to quantitative variation between and within populations. Genetics 99:541–553.

Lavine MD and Beckage NE, 1995. Polydnaviruses: potent mediators of host immune dysfunction. Parasitol Today 11:368–378.

Li X and Webb BA, 1994. Apparent functional role for a cysteine-rich polydnavirus protein in suppression of the insect cellular immune response. J Virol 68:7482–7489.

Rao CR, 1951. Advanced statistical methods in biometrical research. New York: John Wiley.

Rizki RM and Rizki TM, 1990a. Microtubule inhibitors block the morphological changes induced in *Drosophila* blood cells by a parasitoid wasp factor. Experientia 46:311–315.

Rizki RM and Rizki TM, 1990b. Parasitoid virus-like particles destroy *Drosophila* cellular immunity. Proc Natl Acad Sci USA 87:8388–8392.

Rizki TM, Rizki RM, and Carton Y, 1990. *Leptopilina heterotoma* and *L. boulardi*: strategies to avoid cellular defense response of *Drosophila melanogaster.* Exp Parasitol 70:466–475.

Russo J, Dupas S, Frey F, Carton Y, and Brehelin M, 1996. Insect immunity: early events in encapsulation process of parasitoid (*Leptopilina boulardi*) eggs in reactive and nonreactive strain of *Drosophila.* Parasitology 112:135–142.

Soldevila AI and Webb BA, 1996. Expression of polydnavirus gene under polydnavirus promoter regulation in insect larvae infected with baculovirus recombinants. J Gen Virol 77:1379–1388.

Strand MR and Pech LL, 1995. Immunological basis for compatibility in parasitoid-host relationships. Annu Rev Entomol 40:31–56.

Stoltz DB, 1990. Evidence for chromosomal transmission of polydnavirus DNA. J Gen Virol 71:1051–1056.

Thompson JN and Mascie-Taylor CGN, 1985. Detection of simple polygenic segregation in a natural population. Proc Natl Acad Sci USA 82:8552–8556.

Webb BA and Luckhart S, 1996. Factors mediating short- and long-term immune suppression in a parasitized insect. J Insect Physiol 42:33–40.

Walker I, 1959. Die Abvehrreaktion des Wirtes *Drosophila melanogaster* gegen die zoophage Cynipide *Pseudocoila bochei Weld.* Rev Suisse Zool 66:569–631.

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Corresponding Editor: Ross MacIntyre