Heredity (2007) 98, 21–27
© 2007 Nature Publishing Group All rights reserved 0018-067X/07 $30.00
www.nature.com/hdy

ORIGINAL ARTICLE

Genetic interactions between the parasitoid wasp *Leptopilina boulardi* and its *Drosophila* hosts

A Dubuffet[1], S Dupas[2], F Frey[2], J-M Drezen[1], M Poirié[3] and Y Carton[2]
[1]*IRBI, Université François-Rabelais, Parc Grandmont, Tours, France;* [2]*Laboratoire Populations, Génétique et Evolution, CNRS, Gif-sur-Yvette Cedex, France and* [3]*INRA, Université de Nice-Sophia Antipolis, Sophia Antipolis, France*

Coevolutionary arms races between hosts and parasites would not occur without genetic variation for traits involved in the outcome of parasitism. Genetic variations in resistance and virulence have only rarely been described in pairwise host–parasitoid interactions and have never been analysed in multi-species interactions, in contrast to well-characterized plant–pathogen interactions. This paper reports genetic variation in resistance of *Drosophila yakuba* to the parasitoid wasp *Leptopilina boulardi*. The genetic basis and geographic distribution of resistance is analysed. On the basis of these and previous findings, we demonstrate that there are different resistance patterns to the parasitoid species *L. boulardi* in *D. melanogaster* and *D. yakuba*, as well as different specificity levels in the parasitoid species, suggesting complex ecological interactions in the field. This first description of resistance–virulence genetic interactions between a parasitoid and its two host species provides empirical data showing that multi-species interactions may greatly influence coevolutionary processes.
Heredity (2007) **98,** 21–27. doi:10.1038/sj.hdy.6800893; published online 11 October 2006

Keywords: *Drosophila*; parasitoid wasp; resistance; geographical variation; genetic pattern of interaction; coevolution

Introduction

In host–parasite or host–pathogen interactions, antagonistic selective pressures can lead to a coevolutionary arms race based on adaptations and counter-adaptations in each partner. However, this process requires occurrence of genetic variation for traits involved in the final outcome of the interaction (Thompson, 1994; Sorci *et al*., 1997). Such variation has often been described in plant–pathogen interactions (Frank, 1994), but little is known regarding interactions between insect parasitoids and their hosts. In this kind of interaction, where larval stages of the parasitoid are dependent on their arthropod host (Godfray, 1994), reciprocal selective pressures are particularly strong as only one partner can survive infestation.

To defend themselves against parasitoid attacks, host species have set up diverse behavioural and/or immune defences (Gross, 1993; Strand and Pech, 1995). In insects, the most well-known immune defence against parasitoids is the formation of a multi-cellular, melanized capsule around the parasitoid egg. This encapsulation reaction, when successful, leads to parasitoid death and host survival (Carton and Nappi, 1997). To counteract host immune defences, parasitoids have developed various strategies, based mainly on the use of virulence factors (Strand and Pech, 1995; Pennacchio and Strand, 2006). Surprisingly, despite their relevance for the study of coevolution, little data are currently available on host–parasitoid interactions with regard to the genetic bases of resistance and virulence traits (Kraaijeveld *et al*., 1998; Dupas *et al*., 2002).

Variability in both resistance (ability to neutralize the parasitoid egg using immune defences) and virulence (ability to overcome the host immune defences) has been documented in only three host–parasitoid interactions, two of them involving the host species *Drosophila melanogaster* (Kraaijeveld and van Alphen, 1994; Dupas *et al*., 2002) and the other, the pea aphid *Acyrthosiphon pisum* (Henter, 1995; Henter and Via, 1995). In these models, genetic variation for both resistance and virulence has been observed and resistance and virulence components are thus predicted to coevolve under antagonistic selective pressures.

This interpretation is, however, unlikely to explain all resistance and virulence patterns in natural populations (Dupas *et al*., 2002). Indeed, as stressed by Thompson (1999), selective pressures exerted by other parasitoid and host species will also influence the geographic patterns of resistance–virulence in a given pairwise interaction. Unfortunately, data on resistance–virulence traits in complex interactions involving at least one parasitoid and different host species or one host species and its parasitoids are lacking. To estimate potential trade-offs or genetic constraints between resistance and virulence traits, it is now necessary to analyse several pairwise systems involving different interacting host–parasitoid species.

Drosophila species and their parasitoids represent one of the best models to analyse multiple species host–parasitoid interactions and to characterize resistance and

Correspondence: Dr A Dubuffet, Faculté des Sciences et Techniques, Institut de Recherche sur la Biologie de l'Insecte, UMR CNRS 6035, Université François-Rabelais, Parc Grandmont, 37200 Tours, France.
E-mail: dubuffet@univ-tours.fr
Received 4 November 2005; revised 29 June 2006; accepted 12 July 2006; published online 11 October 2006

virulence patterns in the field. Variability in resistance to two different parasitoids, *Asobara tabida* (Kraaijeveld and van Alphen, 1995) and *Leptopilina boulardi* (Dupas *et al*., 2002), has been observed in *D. melanogaster*, and variability in virulence has been reported in the parasitoid species *L. boulardi* against two *Drosophila* host species (Dupas and Boscaro, 1999). The genetic determinism of these traits has, however, only been studied in the *D. melanogaster*/*L. boulardi* pairwise interaction, using crosses between laboratory isofemale lines. In this system, simple genetic determinism was found, with one diallelic locus explaining resistance and one diallelic locus being responsible for virulence (Carton *et al*., 1992; Dupas *et al*., 1998). Interestingly, in tropical Africa, *L. boulardi* can infest several other species of the *melanogaster* subgroup of Drosophilidae, including *D. yakuba* (Carton and Nappi, 1991). This species is widespread on the African mainland (Lachaise *et al*., 1988) and was also described recently on some islands (Lachaise *et al*., 2000). Variability in virulence against *D. yakuba* has been observed in natural populations of *L. boulardi* and is also determined by a single diallelic locus (Dupas and Boscaro, 1999; Dupas and Carton, 1999).

The aim of this work was to quantify genetic variation for resistance in *D. yakuba* in natural populations and to analyse its genetic basis. We compared the genetic systems for resistance to *L. boulardi* in *D. melanogaster* and *D. yakuba*. Besides providing genetic information on a second pairwise host–parasitoid interaction, these data enable us to compare for the first time a parasitoid interaction with two different host species, thereby providing a complete picture of virulence–resistance traits in a multi-species host–parasite system.

Interestingly, combined data show that resistance patterns differ in closely related *Drosophila* host species and that different specificity levels can be observed in a single parasitoid species. Altogether, the *L. boulardi*/*D. melanogaster* interaction can be well explained by a 'gene-for-gene' ('incompatibility') pattern, whereas interaction of *L. boulardi* with *D. yakuba* would be better described by a 'compatibility' pattern. Occurrence of genetically based variations of virulence and resistance in *L. boulardi* interactions with two different *Drosophila* host species makes this model unique for understanding the evolution of complex host–parasitoid relationships and addressing the question of the specificity of resistance and virulence traits.

Materials and methods

Insects

Reference lines of *L. boulardi*: *L. boulardi* isofemale lines IS_y and IS_m have been described by Dupas *et al*. (1998). IS_m (Gif stock, no. 431) derives from a single female collected in the Nasrallah oasis (Tunisia). IS_y (Gif stock, no. 486) derives from a single female originating from a mass culture of a Brazzaville (Congo) population. IS_m parasitoids are highly virulent against *D. melanogaster* but are always encapsulated in *D. yakuba*. On the contrary, parasitism success of IS_y parasitoids in *D. melanogaster* depends on the resistance/susceptibility genotype of the flies. The IS_y line was used by Carton *et al*. (1992) to study the genetics of resistance to *L. boulardi* in *D. melanogaster*.

Both IS_m and IS_y lines were reared on a susceptible *D. melanogaster* strain (Gif stock, no. 1333), at 25°C. After emergence, adults of both lines were kept at 18°C on agar medium with honey.

Reference lines of *D. yakuba*: The isofemale lines 1880-D (R_1 line) and 1907 (R_2 line) were selected from two populations in Tanzania and further used to analyse the genetic basis of resistance. R_1 and R_2 show opposite immune capacities against the IS_y line of *L. boulardi* (see Table 1). These isofemale lines were obtained from a single inseminated founder female collected in the field (David *et al*., 2005) that was used to initiate a full sib line. The sib lines had been maintained in the laboratory for 8 years at the time of experiments, and were thus probably homozygous for all the loci potentially involved in resistance to the parasitoid wasp. The R_1 line was used by Dupas *et al*. (1998) to study the genetics of virulence of *L. boulardi* against *D. yakuba*.

Natural populations of *D. yakuba*: Seven *D. yakuba* populations were collected throughout the species distribution area, in the Afrotropical region, where *D. melanogaster* is also present (Lachaise *et al*., 1988). Several isofemale lines or multi-female strains were obtained from these populations. Occurrence of geographic variation in resistance to *L. boulardi* was tested using either multi-female strains or pools of 6–24 isofemale lines (Table 2). This method was previously shown to preserve the genetic variability of a population (David *et al*., 2005). All *D. yakuba* strains were raised at 25°C using a standard *Drosophila* cornmeal–yeast–agar medium.

Bioassay procedure

For bioassays, batches of 30 second instar *D. yakuba* larvae (48 h old) were submitted to parasitism by three *L. boulardi* females for 4 h. All the bioassays were performed at 25°C. Encapsulation ability was estimated 48 h later by dissecting late third instar larvae. At this time, a melanized capsule is found in resistant larvae, but not in susceptible ones. The encapsulation rate was calculated as the ratio of encapsulated parasitoid eggs to the number of parasitized hosts, using data from only monoparasitized larvae.

Table 1 Collection sites of the *D. yakuba* reference lines used for genetic analyses and percentage of encapsulation of *L. boulardi* eggs from the IS_y and IS_m lines

Strain number (Gif stock)	*Collection site*	*Collection year*	*Status*	*Encapsulation rate of IS_y eggs (number of larvae tested)*	*Encapsulation rate of IS_m eggs (number of larvae tested)*
1880D (R_1)	Tanzania, Udzungwa (south)	1995	Isofemale line	18.8% (101)	100% (89)
1907 (R_2)	Tanzania, Meru (north)	1995	Isofemale line	92.9% (113)	99.4% (157)

Table 2 *D. yakuba* strains collection sites, collection dates, status and percentage of encapsulation of *L. boulardi* eggs from the IS_y and IS_m lines

Strain number (Gif stock)	*Collection site*	*Date*	*Status*	*Encapsulation rate of IS_y eggs (number of larvae tested)*	*Encapsulation rate of IS_m eggs (number of larvae tested)*
1915	Ivory Coast, Taï	1983	Multi-female strain	77.6% (60)	100% (57)
1919	São Tome isle (hybrid zone)	2001	7 isofemale lines	7.5% (53)	100% (77)
1917	São Tome isle (south)	2000	24 isofemale lines	6.0% (84)	100% (74)
1921	Principe Isle	2001	6 isofemale lines	88.9% (54)	100% (46)
1916	Gabon, Lope	1998	14 isofemale lines	92.0% (50)	100% (31)
1920	Kenya, Mt Elgon	1984	Multi-female strain	85.5% (55)	100% (29)
1918	Kenya, Mt Kenya	1984	Multi-female strain	97.9% (47)	100% (32)

Table 3 Percentage of encapsulation of *L. boulardi* IS_y eggs by *D. yakuba* larvae originating from different crosses involving the R_1 and R_2 lines

Crosses	*Mother × father*	*Number of replicates*	*Number of larvae tested*	*Encapsulation rate (mean ± s.e.) (%)*
Parental lines				
1	$R_1 \times R_1$	13	240	12.5 ± 2.1
2	$R_2 \times R_2$	14	299	86.0 ± 2.0
Reciprocal F_1 hybrids				
3	$R_1 \times R_2$	13	383	83.6 ± 1.9
4	$R_2 \times R_1$	13	457	86.4 ± 1.6
Reciprocal F_2 hybrids				
5	$(R_1 \times R_2) \times (R_1 \times R_2)$	11	419	62.5 ± 2.4
6	$(R_2 \times R_1) \times (R_2 \times R_1)$	12	370	60.0 ± 2.6

Genetic analysis

Crossing procedure: Two generations of reciprocal crosses (Table 3) between the R_1 and R_2 lines, respectively susceptible and resistant to the parasitoid IS_y line, were performed. The two parental lines, the two F_1 hybrids and the two F_2 hybrids were tested and compared for their encapsulation ability.

Statistical analysis: The mode of inheritance of *D. yakuba* resistance to the *L. boulardi* IS_y line was assessed using analyses of variance (ANOVA) with the generalized linear model procedure, assuming binomial error term distribution. Analyses were performed according to the method described by De Belle and Sokolowski (1987), which was previously used to analyse the genetic determinism of resistance of *D. melanogaster* to the parasitoid species *L. boulardi* and *A. tabida* (Carton *et al*., 1992; Benassi *et al*., 1998). The following comparisons were made (cross numbers refer to those described in Table 3): difference between parental strains (1 vs 2), dominance or additive effects ($1+2$ vs $3+4$), deviation from an autosomal mode of inheritance (3 vs 4).

The single-gene model with complete dominance of the resistance allele was tested using Mendelian analysis with the ratio of susceptible to resistant larvae, as described by Carton *et al*. (1992). The observed and expected ratios of susceptible to resistant were analysed using χ^2 analysis. All statistical analyses were performed using Systat 10 software (SPSS Inc.).

Results

Occurrence of variability in resistance of *D. yakuba* to *L. boulardi*

Two *D. yakuba* isofemale lines, R_1 and R_2, originating from Tanzanian populations, were selected from a range of candidate lines for their high ($92.9 \pm 2.4\%$) and low ($18.8 \pm 3.9\%$) encapsulation levels of eggs from the *L. boulardi* wasp IS_y line, respectively. Both lines encapsulated almost 100% of the eggs of the reference IS_m line (99.4 and 100%, respectively) (Table 1). Based on these results, R_1 and R_2 were considered as susceptible and resistant, respectively, to the IS_y line of *L. boulardi*.

Genetic determinism of resistance

Encapsulation rates of IS_y parasitoid eggs by *D. yakuba* larvae originating from different crosses performed with the R_1 and R_2 isofemale lines, and results of the contrast analysis of variance are presented in Tables 3 and 4, respectively. As expected, the R_1 and R_2 lines differed significantly in their resistance to infestation by IS_y parasitoids ($F = 436.79$, $P < 0.001$). F_1 hybrids exhibited significantly higher resistance than the mean parents, showing that resistance was dominant ($F = 252.74$, $P < 0.001$). Reciprocal F_1 hybrids were not significantly different from each other, excluding the involvement of non-autosomal inheritance ($F = 1.05$, $P = 0.305$). Larvae from the different crosses were classified into two classes (resistant and susceptible) according to their ability to encapsulate IS_y eggs. The single gene model hypothesis was tested by comparing susceptible vs resistant proportions (S:R) with expected Mendelian proportions, using χ^2 analysis. The results (Table 5) were consistent with the hypothesis of a single major segregating locus with two alleles, the resistant one being completely dominant. The locus responsible for resistance of *D. yakuba* to IS_y parasitoids was named Rlb_{ISy} (for resistance to *L. boulardi* of the IS_y type) and the resistant and susceptible alleles, Rlb^{+}_{ISy} and Rlb^{-}_{ISy}, respectively.

Genetic variability in natural populations

Encapsulation rates of the two reference *L. boulardi* lines, IS_y and IS_m, were recorded in seven *D. yakuba*

Table 4 Contrast ANOVA for encapsulation of *L. boulardi* IS_y eggs by *D. yakuba* larvae originating from crosses involving the R_1 and R_2 lines (generalized linear model with binomial error term)

Source	*d.f.*	*MS*	F	P
Model (between crosses)	5	22.38	136.10	<0.001
Contrasts				
1. R_1 vs R_2 parental lines (1 vs 2)	1	71.83	436.79	<0.001
2. Dominance (1+2 vs 3+4)	1	41.56	252.74	<0.001
3. Deviation from an autosomal mode of inheritance (3 vs 4)	1	0.17	1.05	0.305
Error (within crosses)	2162	0.164		

Table 5 Deviation of the proportions of (susceptible vs resistant) (S:R) larvae in the progeny of different crosses from the proportions expected under a one-locus model with dominance of the resistance allele

	Expected S:R proportion under the model	N	*Expected number of S:R larvae under the model*	*Observed number of S:R larvae*	χ^2	P
Parental lines						
$R_1 \times R_1$	1:0	240	240:0	210:30		
$R_2 \times R_2$	0:1	299	0:299	42:257 Allows calculating the misclassification level		
Reciprocal crosses						
F_1						
$R_1 \times R_2$	0:1	383	0:383 Corrected: 51:332	63:320	3.26	0.07 NS
$R_2 \times R_1$	0:1	457	0:457 Corrected: 61:396	62:395	0.02	0.89 NS
F_2						
$F_1 \times F_1$	1:3	789	197:592 Corrected: 302:487	305:484	0.05	0.83 NS

About 12.5% (30 out of 240) of larvae of the 'susceptible' R_1 parental line encapsulate *L. boulardi* eggs, whereas 14% (42 out of 299) of larvae of the 'resistant' R_2 parental line fail to encapsulate these eggs. This constant observed deviation from the expected 0 and 100% encapsulation rates is mainly due to environmental effects. This corresponds to a mean misclassification rate of 13.25%. The expected number of S:R larvae in the progeny of the crosses was thus corrected to include this misclassification factor, according to the method of Carton *et al*. (1992) and Benassi *et al*. (1998). Data were then compared using a χ^2 goodness of fit test. NS: nonsignificant.

populations (Table 2). Figure 1 presents the geographic pattern of resistance to IS_y parasitoids.

A high level of variation in resistance to the IS_y line was observed in the field, which confirmed the results obtained with the Tanzanian R_1 and R_2 isofemale lines: resistance to IS_y in *D. yakuba* shows significant variability. Encapsulation rates varied from 6 to 98% but most populations encapsulated IS_y eggs at a high frequency (from 77.6 to 97.9%). Only populations from the São Tome island were susceptible to IS_y infestation (6.5 and 7.0% of IS_y eggs encapsulated). Resistance was thus found either at a very high or a very low frequency in *D. yakuba* Afrotropical populations. By contrast, there was no variability between *D. yakuba* strains for resistance to the IS_m parasitoid line, with 100% of eggs encapsulated in each strain (Table 2). Despite intense investigations, no *D. yakuba* population susceptible to the IS_m line of *L. boulardi* has ever been found.

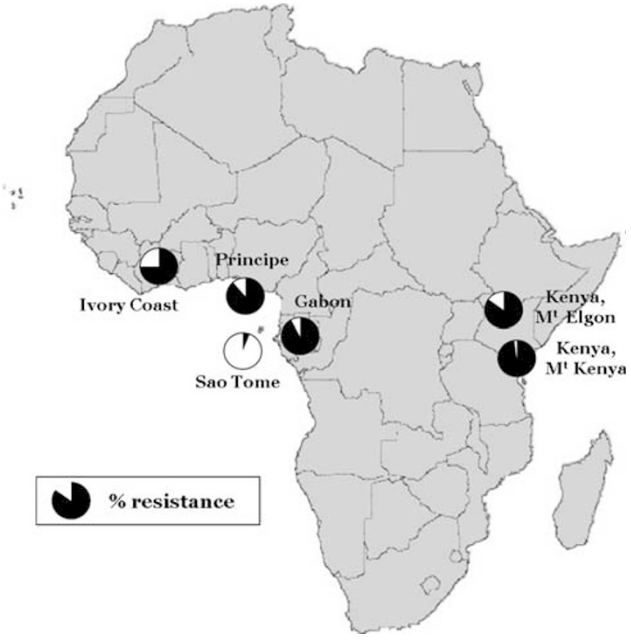

Figure 1 Geographical distribution of *D. yakuba* resistance to IS_y parasitoids in tropical Africa. The resistance level (in black) was estimated from the percentage of IS_y eggs encapsulated by *D. yakuba* larvae.

Discussion

Genetic determinism of parasitoid resistance in *Drosophila*

The present study reports occurrence of variation in the ability of *D. yakuba* to encapsulate eggs of the IS_y line of *L. boulardi*. Using crosses between lines having

contrasted resistance levels, we demonstrate that resistance is determined by a single major locus, which we termed Rlb_{ISy}.

Considering the high number of genes involved in insect immune pathways (Irving *et al*., 2001; Zettervall *et al*., 2004), variation in resistance to parasitoid wasps was expected to be determined by multigenic systems (Sorci *et al*., 1997). However, in all cases in which genetic determinism of resistance to parasitoids has been studied – *D. melanogaster*/*L. boulardi*, *D. melanogaster*/*A. tabida* and now *D. yakuba*/*L. boulardi* – differences between lines resistant and susceptible to a given parasitoid have always been explained by a single diallelic locus. In *D. melanogaster*, two loci, named *Rat* and *Rlb* (labelled as *Rst(2)Lb* in Flybase; ID number: FBgn0016729), localized 35 centimorgans apart, are responsible for resistance to the parasitoids *A. tabida* and *L. boulardi*, respectively (Carton *et al*., 1992; Benassi *et al*., 1998; Poirié *et al*., 2000). The use of isofemale lines, which are not representative of the extent of genetic variation in the field, might favour the recovery of simple genetic systems (Kraaijeveld *et al*., 1998). However, studies by Orr and Irving (1997), dealing with genetic variation of resistance to *A. tabida* in *D. melanogaster* populations from different parts of Europe, also concluded on a simple genetic basis for resistance. The advantage of performing analyses with well-characterized strains is that it allows the precise localization and identification of resistance loci (Hita *et al*., 1999; M Poirié, unpublished data). These loci are expected to contain genes involved in the response to parasitoid attacks, showing enough polymorphism to respond to selection pressures and thus potentially evolving under coevolutionary processes.

The genetic bases of interactions between two different lines of *L. boulardi* and the two host species *D. melanogaster* and *D. yakuba* are summarized in Figure 2. It is the first complete report of genetic interactions between a parasitoid and two different host species.

In the *L. boulardi*–*D. melanogaster* system, the success of parasitism is the rule; failure only occurs when the parasitoid has no virulence alleles (IS_m^-/IS_m^-, IS_y line) and when the host is resistant (at least one Rlb^+ allele, resistant strain) (Dupas *et al*., 2002). This system resembles the 'gene-for-gene' (or 'incompatibility') model of plant–pathogen interactions (Briggs and Johal, 1994). Here one parasitoid genotype (IS_m line) has a 'universal virulence' (Frank, 1994), which means that it can infest all *D. melanogaster* flies, whatever their genotypes.

By contrast, the general outcome in the *L. boulardi*–*D. yakuba* interaction is the encapsulation of the parasite egg; parasitism is successful only if the parasitoid is homozygous for virulence alleles (IS_y^+/IS_y^+, IS_y line) (Dupas *et al*., 1998) and if the host is homozygous for susceptible alleles ($Rlb_{\overline{I}Sy}/Rlb_{\overline{I}Sy}$, R_1 line). This genetic pattern of interactions, different from the one described above, resembles the 'compatibility model' of Briggs and Johal (1994). In this model, parasitoid success would require a specific match between host and parasite 'compatible factors' and each modification of the host target would prevent development of the parasite.

Genetic variations in resistance and virulence have rarely been described in pairwise host–parasitoid interactions and had never been analysed before in multispecies interactions. Our data show that there are different resistance patterns to the parasitoid species

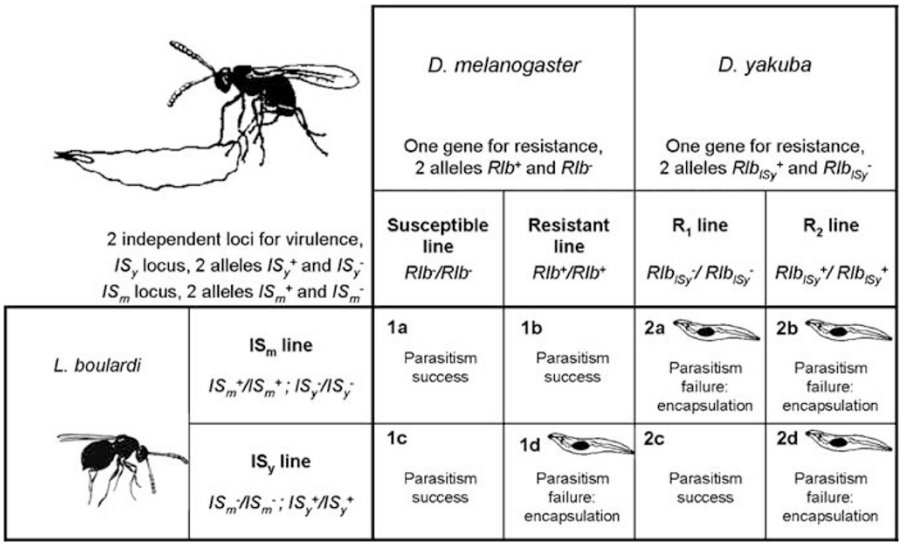

Figure 2 Genetic interactions matrix in the *Drosophila*–*L. boulardi* reference system. The interactions between the reference lines IS_y and IS_m of the parasitoid *L. boulardi* and the reference lines of the host species *D. melanogaster* and *D. yakuba* are illustrated. Resistance in each host species is conferred by one major locus, and two independent loci are responsible for virulence of the parasitoid against the two host species. In the *L. boulardi*–*D. melanogaster* system (cells 1a to 1d), the host alleles Rlb^+ and Rlb^- are responsible for resistance and susceptibility to IS_y infection, and the parasitoid alleles IS_m^+ and IS_m^- are responsible for virulence and avirulence of the parasitoid against the host. Parasitism success is the rule in *D. melanogaster* (cells 1a, 1b and 1c), except when a resistance allele Rlb^+ is present without any virulence allele IS_m^+ (cell 1d). In the *L. boulardi*–*D. yakuba* system (cells 2a to 2d), the host alleles $Rlb_{\overline{I}Sy}^+$ and $Rlb_{\overline{I}Sy}$ are responsible for resistance and susceptibility to the IS_y infection, and the parasitoid alleles IS_y^+ and IS_y^- are responsible for virulence and avirulence of the parasitoid against the host R_1 line. In this system, parasitism failure is the rule (cells 2a, 2b and 2d), except when two virulence alleles IS_y^+ are present with two susceptibility alleles $Rlb_{\overline{I}Sy}$ (cell 2c).

L. boulardi in *D. melanogaster* and *D. yakuba*, suggesting complex ecological interactions in the field.

IS_m eggs are rarely encapsulated in *D. melanogaster* and always encapsulated in *D. yakuba* lines. Despite intense investigations, we have not been able to recover *D. melanogaster* flies that are resistant or *D. yakuba* flies that are susceptible to this parasitoid line. By contrast, encapsulation of IS_y eggs can take place in both host species depending on the genotype of the fly (ie the resistance status of the host strain). Hence, the success of the IS_m line is 'species-dependent', whereas the success of the IS_y line is 'host-genotype-dependent'. We thus report the occurrence of two different levels of host specificity in a single parasitoid species. As suggested in other host–parasite systems, the success or failure of a parasitoid seems to be due to neither parasitoid virulence alone nor host resistance ability alone, but rather determined by complex interactions between host and parasitoid species (Kraaijeveld and Godfray, 2001; Dupas *et al*., 2002; Little *et al*., 2005).

Geographic patterns of resistance of *D. yakuba* to *L. boulardi*

L. boulardi eggs of the IS_m line were encapsulated in all *D. yakuba* strains tested, whatever their geographic origin, thus confirming the absence of variation in resistance to this parasitoid line. Occurrence of variability in resistance to IS_y females, discovered in Tanzanian populations (origin of the R_1 and R_2 lines), was further confirmed using other natural populations. The question remains whether this variability in resistance can also be attributed to the Rlb_{ISy} locus.

A high resistance level was found in Kenya, Gabon, Ivory Coast and in the Principe Island, whereas populations from São Tome were susceptible. Interestingly, resistance to IS_y parasitoids is also high in *D. melanogaster* populations, even in areas where this type of parasitoid is absent (Dupas *et al*., 2002). It is generally postulated that resistance to a pathogen or a parasite is a life-history trait whose polymorphism is maintained by a balance between positive selection forces (the presence of the pathogen) and counter-selecting forces (the cost of maintaining resistance in the absence of the pathogen) (Kurtz *et al*., 2002; Rolff and Siva-Jothy, 2003). In this model, positive selective forces should have acted in every locality where resistance to IS_y parasitoids is observed at high frequencies. So far, *L. boulardi* populations corresponding to the 'IS_y type' (able to develop on *D. yakuba*) have only been found in Congo and, to a lesser extent, in Ivory Coast (Dupas and Boscaro, 1999). If the 'IS_y type' distribution area was the same in the past, other biotic or abiotic pressures might have selected resistance in the majority of *D. yakuba* populations. Whether individuals resistant to 'IS_y parasites' can also resist other parasitoids/pathogens remains to be determined, but preliminary results suggest that they are at least able to resist another parasitoid species found in these localities, *Leptopilina freyae* (Allemand *et al*., 2002; Y Carton, unpublished data). Resistance in *D. yakuba* populations could be considered in this way to be a 'generalist resistance' rather than a 'specific resistance'.

According to the same hypothesis, the absence of resistance in São Tome island could result from the absence of agents selecting resistance in mainland populations, combined with counter-selection of resistance alleles, leading to the loss of resistance. Unfortunately, the questions whether *L. boulardi* parasitoids can be found in São Tome and whether *D. yakuba* resistance is costly remain to be answered. A second hypothesis could be that under a high diversity of attack or a high prevalence of parasites, tolerance or non-investment in immune defence mechanisms is an optimal strategy (Sasaki and Godfray, 1999; Jokela *et al*., 2000). We lack information on the abundance of *D. yakuba* pathogens or parasitoids on this island but considering classical theories in biogeography, this hypothesis seems rather unlikely (MacArthur and Wilson, 1967). Finally, the susceptibility of São Tome populations to *L. boulardi* might be explained by a founder effect having occurred during colonization of the island by a small number of flies (Cariou *et al*., 2001).

Observation of variability in resistance and virulence in host–parasitoid communities and determination of their genetic bases are important in understanding diffuse coevolutionary processes. This can be achieved by analysing and comparing pairwise interactions involving the same parasitoid and different host species or the same host and different parasitoid species. We draw here the first complete picture of resistance–virulence genetic interactions between a parasitoid and its two host species, and suggest that multi-species interactions may indeed greatly influence coevolutionary processes. Deciphering the physiological and molecular bases of these traits will finally help to address the question of specificity in host–parasitoid interactions and will provide precious information on the ongoing coevolutionary arms races.

Acknowledgements

This work was supported by the French GDR 2153. Aurore Dubuffet received financial support from the François Rabelais University. We are grateful to Daniel Lachaise for providing *Drosophila* lines, to Carole Labrousse for technical assistance and to Elisabeth Huguet and Georges Periquet for helpful comments on the manuscript.

References

Allemand R, Lemaître C, Frey F, Boulétreau M, Vavre F, Nordlander G *et al*. (2002). Phylogeny of six African *Leptopilina* species (Hymenoptera: Cynipoidea, Figitidae), parasitoids of *Drosophila*, with description of three new species. *Ann Soc Entomol Fr* **38**: 319–332.

Benassi V, Frey F, Carton Y (1998). A new specific gene for wasp cellular immune resistance in *Drosophila*. *Heredity* **80**: 347–352.

Briggs SP, Johal GS (1994). Genetic patterns of plant–parasite interactions. *Trends Genet* **10**: 12–16.

Cariou ML, Silvain JF, Daubin V, Da Lage JL, Lachaise D (2001). Divergence between *Drosophila santomea* and allopatric or sympatric populations of *D. yakuba* using paralogous amylase genes and migration scenarios along the Cameroon volcanic line. *Mol Ecol* **10**: 649–660.

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

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

Carton Y, Nappi AJ (1997). *Drosophila* cellular immunity against parasitoids. *Parasitol Today* **13**: 218–227.
David JR, Gibert P, Legout H, Petavy G, Capy P, Moreteau B (2005). Isofemale lines in *Drosophila*: an empirical approach to quantitative trait analysis in natural populations. *Heredity* **94**: 3–12.
De Belle S, Sokolowski MB (1987). Heredity of rover/sitter: alternative foraging strategies of *Drosophila melanogaster* larvae. *Heredity* **59**: 73–83.
Dupas S, Boscaro M (1999). Geographic variation and evolution of immune suppressive genes in a *Drosophila* parasitoid. *Ecography* **22**: 284–291.
Dupas S, Carton Y (1999). Two non-linked genes for specific virulence of *Leptopilina boulardi* against *Drosophila melanogaster* and *D. yakuba*. *Evol Ecol* **13**: 211–220.
Dupas S, Carton Y, Poirié M (2002). The genetic dimension of the coevolution of virulence resistance in *Drosophila*–parasitoid wasp relationships. *Heredity* **90**: 84–89.
Dupas S, Frey F, Carton Y (1998). A single parasitoid segregating factor controls immune suppression in *Drosophila*. *J Hered* **89**: 306–311.
Frank SA (1994). Recognition polymorphism in host–parasite genetics. *Philos Trans R Soc London B* **346**: 283–293.
Godfray HCJ (1994). *Parasitoids: Behavioral and Evolutionary Ecology*. University Press: Princeton.
Gross P (1993). Insect behavioral and morphological defenses against parasitoids. *Annu Rev Entomol* **38**: 251–273.
Henter HJ (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 population. *Evolution* **49**: 439–445.
Henter HJ, 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.
Hita MT, Poirié M, Leblanc N, Lemeunier F, Lutcher F, Frey F *et al.* (1999). Genetic localization of a *Drosophila melanogaster* resistance gene to a parasitoid wasp and physical mapping of the region. *Genome Res* **9**: 471–481.
Irving P, Troxler L, Heuer TS, Belvin M, Kopczynski C, Reichhart JM *et al.* (2001). A genome-wide analysis of immune responses in *Drosophila*. *Proc Natl Acad Sci USA* **98**: 15119–15124.
Jokela J, Schmid-Hempel P, Rigby MC (2000). Dr Pangloss restrained by the Red Queen – steps towards an unified defence theory. *Oikos* **89**: 267–274.
Kraaijeveld AR, Godfray HCJ (2001). Is there local adaptation in *Drosophila*–parasitoid interactions? *Evol Ecol Res* **3**: 107–116.
Kraaijeveld AR, van Alphen JJ, Godfray HC (1998). The coevolution of host resistance and parasitoid virulence. *Parasitology* **116**: S29–S45.
Kraaijeveld AR, van Alphen JJM (1994). Geographical variation in resistance of the parasitoid *A. tabida* against encapsulation by *D. melanogaster* larvae: the mechanism explored. *Physiol Entomol* **19**: 9–14.
Kraaijeveld AR, van Alphen JM (1995). Geographical variation in encapsulation ability of *Drosophila melanogaster* larvae and evidence for parasitoid specific components. *Evol Ecol* **9**: 10–17.
Kurtz J, van der Veen IT, Ryder JJ (2002). Ecological immunity of arthropods – a thread of Ariadne? *Trends Ecol Evol* **17**: 204–205.
Lachaise D, Cariou M-L, David JR, Lemeunier F, Tsacas L, Ashburner M (1988). Historical biogeography of the *Drosophila melanogaster* species subgroup. In: Hecht M, Wallace B, Prance GT (eds). *Evolutionary Biology*, Vol 22. Plenum Publishing Corporation: New York, pp 159–225.
Lachaise D, Harry M, Solignac M, Lemeunier F, Bénassi V, Cariou M-L (2000). Evolutionary novelties in islands: *Drosophila santomea*, a new *melanogaster* sister species from São Tomé. *Proc R Soc London B* **267**: 1487–1495.
Little TJ, Hultmark D, Read AF (2005). Invertebrate immunity and the limits of mechanistic immunology. *Nat Immunol* **6**: 651–654.
MacArthur RH, Wilson EO (1967). *The Theory of Island Biogeography*. Princeton University Press: Princeton, NJ.
Orr HA, Irving S (1997). The genetics of adaptation – the genetic basis of resistance to wasp parasitism in *Drosophila melanogaster*. *Evolution* **51**: 1877–1885.
Pennacchio F, Strand MR (2006). Evolution of developmental strategies in parasitic hymenoptera. *Annu Rev Entomol* **51**: 233–258.
Poirié M, Frey F, Hita M, Huguet E, Lemeunier F, Periquet G *et al.* (2000). *Drosophila* resistance genes to parasitoids: chromosomal location and linkage analysis. *Proc R Soc London B* **267**: 1417–1421.
Rolff J, Siva-Jothy MT (2003). Invertebrate ecological immunology. *Science* **301**: 472–475.
Sasaki A, Godfray HCJ (1999). A model for the coevolution of resistance and virulence in coupled host–parasitoid interactions. *Proc R Soc London B* **266**: 455–463.
Sorci G, Møller AP, Boulinier T (1997). Genetics of host–parasite interactions. *Trends Ecol Evol* **12**: 196–200.
Strand MR, Pech LP (1995). Immunological basis for compatibility in parasitoid–host relationships. *Annu Rev Entomol* **40**: 31–56.
Thompson JN (1994). *The Coevolutionary Process*. University of Chicago Press: Chicago.
Thompson JN (1999). Specific hypotheses on the geographic mosaic theory of coevolution. *Am Nat* **153**: S1–S14.
Zettervall C-J, Anderl I, Williams MJ, Palmer R, Kurucz E, Andó I (2004). A directed screen for genes involved in *Drosophila* blood cell activation. *Proc Natl Acad Sci USA* **101**: 14192–14197.