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Phenological segregation of insecticide resistance alleles in the codling moth *Cydia pomonella* (Lepidoptera: Tortricidae): a case study of ecological divergences associated with adaptive changes in populations

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Summary

Intrapopulation variability in the seasonal regulation of insect lifecycles has been shown to be due partly to genetic changes. Selection for insecticide resistance in the codling moth *Cydia pomonella* results from allelic substitution at two to three loci in south-eastern French populations of this species. However, such an adaptive process has been associated with an increased heterogeneity in the developmental responses to climatic factors such as temperature. In this paper, we investigate whether such pleiotropic effects of resistance on development induce a significant discrepancy in seasonal regulation in this species. The seasonal changes in a susceptible and two insecticide-resistant homozygous genotypes of *C. pomonella*, as well as their reciprocal F1 progeny, were followed under natural conditions during the reproductive season through the emergence events of adults, within-generation developmental rates and the number of generations. A significant delay in the occurrences of homozygous resistant genotypes resulted from significantly lower pre-imaginal developmental times relative to homozygous susceptible ones. Subsequent assessment of the number of generations indicated significantly higher diapause propensities in carriers of the resistance alleles (37·0–76·2%) than in susceptible homozygotes (6·7%), which mostly pupated towards a third generation of adults. In the light of these findings, pleiotropic effects of adaptive changes might be a crucial source of divergence in seasonal regulation at the population level, involving significant life-history trade-offs. In addition to man-made selective factors during the reproductive season, such an effect on the lifecycle could be a key component in the process of selection for resistance genes in south-eastern France *C. pomonella* populations.

1. Introduction

Strong selective constraints act on populations for an appropriate synchronization of crucial life-history events with annual cycles of both biotic and abiotic factors in the environment (Tauber *et al.*, 1986). At the population level, divergences in the seasonal timing of lifecycle events (phenology) are also likely to occur, owing to intrinsic variability in life-history traits (Dingle & Hegman, 1982; Danks, 1987). Such variation can be an expression of both genetic differences and environmental changes (Taylor & Karban, 1986). Interestingly, processes of adaptation of genomes to local environmental changes have been shown to involve pleiotropic interactions that entail significant qualitative and quantitative changes in life-history characters (Holloway, 1990; Carrière & Roff, 1995). Such interactions are generally regarded as costly pleiotropic trade-offs that might result from interference by the newly selected mechanisms with pre-existing physiological functions, or from re-allocation of resources towards novel major enzymatic systems (Fisher, 1958; Uyenoyama, 1986). Man-made changes are generally assumed to involve rapid evolution through drastic changes of environmental properties (Bradshaw, 1991). In agro-ecosystems, the intensive use of chemicals provided a noteworthy illustration of Bradshaw's assertion through the expanding world-wide problem of resistances in pest populations. As a typical Darwinian process, resistance results from allelic substitution at one or several loci, which is favoured under strong insecticide selection acting on initial genotypic variation (McKenzie & Batterham, 1994). However, many resistance cases have been

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associated with phenotypes displaying a selective disadvantage, compared with susceptible ones in the absence of pesticide (see reviews by Roush & McKenzie (1987) and Coustau *et al.* (2000)), suggesting that such genetic variants have encountered the cost of pleiotropic interactions. Resistance costs have proved to be one of the key elements to be considered in the field of adaptation biology through significant alterations of reproduction (Scott *et al.*, 1997; Campanhola *et al.*, 1991), development (Clarke & McKenzie, 1987) or behaviour (Rowland, 1991; Foster *et al.*, 1999; Berticat *et al.*, 2002). However, few studies have focused on disruptive effects on phenological traits (fitness components associated with life-cycle patterns and phenology) (Carrière & Roff, 1995; Carrière *et al.*, 1995).

The codling moth *Cydia pomonella* (L.) is a cosmopolitan pest that closely matches the distribution of apple trees in both northern and southern hemispheres (Riedl, 1983). This tortricid moth is a multivoltine species with a facultative winter diapause occurring at the last larval instar, and the number of generations increases with southern latitudes (Shel'Deshova, 1967). In south-eastern France, two to three generations can be completed each year, requiring a continuous use of insecticides covering the whole growing season. As a consequence, *C. pomonella* populations have evolved insecticide resistance (Sauphanor & Bouvier, 1995) through the independent involvement of both enhanced non-specific detoxification and target-site modification (Sauphanor *et al.*, 1997; Bouvier *et al.*, 2001). Nevertheless, such resistance mechanisms in this species have recently been shown to have pleiotropic costs, especially slower developmental rates in non-overwintering individuals (Boivin *et al.*, 2001). Within the context of pleiotropic trade-offs associated with the acquisition of adaptive characters, and because development is recognized as a major component of seasonal regulation (Roff, 1980), one would make the assumption that a significant increase in developmental heterogeneity would result in a significant change in phenology. The present study was designed to test this assumption by the use of insecticide-resistance alleles as a pleiotropic source of variation in phenological responses under natural conditions. Using one susceptible and two insecticide-resistant strains of *C. pomonella*, we investigated whether insecticide resistance alleles have a pleiotropic effect on crucial ecological components of the insect lifecycle such as adult emergence patterns, intergeneration developmental rates and diapause propensities at the end of the summer.

2. Methods

(i) *Insects*

C. pomonella larvae were collected in 1995 in two apple orchards, referred to as t and v, located 10 km apart near Avignon in south-eastern France. These orchards were chosen because *C. pomonella* populations displayed a significant decrease of susceptibility to diflubenzuron in orchard t and to deltamethrin in orchard v. 30 and 130 larvae were collected in orchards t and v, respectively, in order to generate the Rt and Rv strains, respectively. Neonate progeny of the ten first generations were selected by exposure to increasing concentrations of diflubenzuron (for the Rt strain) or deltamethrin (for the Rv strain) that induced 50% mortality. The 20 subsequent generations of Rt and Rv were further selected with the same insecticides at a concentration sufficiently high to kill all susceptible larvae. At the end of this selection regime, individuals of Rt and Rv strains were homozygous for resistance alleles at both enhanced cytochrome-P450-dependent monooxygenase (MFO) and glutathione-*S*-transferase (GST) loci (Sauphanor *et al.*, 1997; Bouvier *et al.*, 2002). In addition, individuals from the Rv strain were also homozygous for a resistance allele of the *kdr* gene (Sauphanor *et al.*, 1997; Bouvier *et al.*, 2001). Neonate larvae of the Rt strain expressed a resistance ratio to diflubenzuron that was over 10 000-fold, and neonate larvae of the Rv strain expressed a 80-fold resistance ratio to deltamethrin. Individuals of the Rt and Rv resistant strains were referred to as RtRt and RvRv, respectively.

The field population collected in orchard v was also used to generate a susceptible strain. 80 larvae of the first generation of the Rv strain were grown in the absence of insecticide treatment. The resulting adults were used to create isofemale lines. Progenies of these lines were divided into two groups: one was grown in the absence of insecticide and the other was treated with a concentration of deltamethrin known to kill all susceptible larvae (Sauphanor *et al.*, 1998). We detected 50 isofemale lines for which all the treated progeny died. The untreated progenies of these lines were pooled for generating the susceptible strain (Sv). Further biochemical and molecular analyses performed on individuals of the Sv strain indicated homozygosity for susceptible alleles at the MFO, GST and *kdr* loci. Individuals of the susceptible Sv strain were referred to as SvSv.

The use of a reverted susceptible strain from the field population collected in orchard v increases the chance that any phenotypic differences between the susceptible and the resistant strains are the consequence of the resistance alleles themselves (or of other alleles at linked genes) rather than other genetic variations of the genetic background. Subsequent to their selection process, Rt, Rv and Sv strains were maintained by mass rearing in which the size of the breeding populations of each strain approximated 4000 individuals at each generation, to limit inbreeding effects.

In order to determine the dominance level of the pleiotropic costs, we also generated individuals that were heterozygous at the resistance genes. Such individuals were generated by performing the two reciprocal crosses between the susceptible strain and each resistant strain: SvRt, RtSv, SvRv and RvSv were the progenies of ♂SvSv × ♀RtRt, ♂RtRt × ♀SvSv, ♂SvSv × ♀RvRv and ♂RvRv × ♀SvSv crosses, respectively. Hence, SvRt and RtSv individuals were heterozygous at the MFO and GST loci, whereas SvRv and RvSv individuals were heterozygous at the MFO, GST and *kdr* loci.

(ii) *Thermal thresholds required for* C. pomonella *development*

Assessment of the developmental responses of poikilothermic organisms to a thermal environment requires one to establish the temperature range within which insect development occurs. Therefore, neonate larvae of each genotype (SvSv, RtRt, RvRv, SvRt, RtSv, SvRv and RvSv) were introduced into individual plastic cups (20 × 20 × 20 mm) containing an artificial diet (Stonefly Industries, Texas, USA). The pre-imaginal developmental rate r_T (1/adult emergence time) was determined at six different temperatures (14·5 °C, 20 °C, 23 °C, 26 °C, 31 °C and 34 °C) under a long photoperiod (18 hours light, 6 hours dark). Hilbert and Logan's (1983) nonlinear model was fitted to the data in order to estimate values of: (i) the lower developmental threshold (T_o) defined as the temperature below which the developmental rate equals zero; and (ii) the upper developmental threshold (T_{opt}), defined as the temperature at which the developmental rate is optimum. The model is given by

$$r_T = \Psi[T^2/(T^2 + D^2) - \exp((T_m - T)/\Delta T)] \qquad (1)$$

where T is the deviation in the observed temperature from T_o, T_m is the deviation in the lethal maximum temperature threshold from T_o, ΔT is the width of the high-temperature boundary area, Ψ and D are arbitrary parameters to be estimated. In this equation, r_T is approximately equal to zero when T_o equals the observed temperature, so that T_o represents the lower developmental threshold. T_{opt} was calculated from the temperature at which the derivative of r_T in Eqn 1 equals zero.

(iii) *Phenological responses under natural conditions*

During the summer of 2000, 300 G1 neonates of each genotype (SvSv, RtRt, RvRv, SvRt, RtSv, SvRv and RvSv) were introduced into individual plastic cups containing an artificial diet and exposed at 25 °C under a natural photoperiod in order to induce diapause. At the end of the summer, the diapausing G1 larvae were submitted to naturally fluctuating winter conditions under an outside open shelter. During the spring of 2001, emergence of the G1 moths was recorded daily and the G1 overwintering mortality was estimated for each genotype. G1 moths were used to generate the second generation of larvae. To recover all the different genotypes, we performed the following crosses: ♂SvSv × ♀SvSv, ♂RtRt × ♀RtRt, ♂RvRv × ♀RvRv, ♂SvSv × ♀RtRt, ♂RtRt × ♀SvSv, ♂SvSv × ♀RvRv, and ♂RvRv × ♀SvSv. For each type of cross, 10–25 virgin pairs of moths were individually crossed in egg-laying cages maintained under natural conditions, each containing a sugar solution as food. The first ten G2 neonates of each pair were fed in individual plastic cups, and maintained under the outside open shelter. Emergence of the G2 moths was recorded daily, allowing the calculation of the pre-imaginal developmental time of the G2 larvae of each genotype. G2 moths were used to generate the third generation using the procedure as described above for the G1 moths. G3 neonates hatched in the middle of the summer 2001. At the end of the larval development, G3 larvae either entered into diapause or pupated to generate G3 adults. Emergence of the G3 moths was recorded daily, allowing the calculation of the pre-imaginal developmental time of the G3 larvae of each genotype. The proportion of viable G3 larvae that did not pupate at the end of the summer was used to estimate the diapause propensity of each genotype.

Emergence patterns of G1, G2 and G3 moths were characterized and compared between genotypes using their 50 % frequency of occurrence. The 50 % emergence dates and pre-imaginal developmental times were further converted into required numbers of accumulated degree-days (DD) for a critical representation of physiological times. Therefore, records of minimum and maximum temperatures under the open shelter were compiled daily, and accumulated DD data were obtained by using the single sine curve method (Baskerville & Emin, 1969; Zalom *et al.*, 1983) with horizontal cut-off; both the lower (T_o) and upper (T_{opt}) developmental thresholds were estimated for each genotype using Eqn 1. January 1st was used as the starting date for DD accumulation.

(iv) *Statistical analysis*

Goodness of fit of Hilbert & Logan's (1983) nonlinear model to our data set was estimated using the sum of squares error (SSE). Following Huet *et al.* (1996), comparisons of both the lower and the upper thresholds between genotypes were made using the 95 % confidence intervals (CI) for T_o and T_{opt}. Overwintering mortality and diapause propensities were compared using 0·05 threshold χ^2 tests, with one degree of freedom (Statview, SAS Institute, USA). Comparisons of both developmental rates and thermal constant values were made using 0·05 thresholds from analyses of variance (ANOVA) (Statview, SAS Institute, USA). Mean comparisons were made using a 0·05 threshold

Table 1. *Parameters of Hilbert and Logan's (1983) model of pre-imaginal development in homozygous susceptible (SvSv) and insecticide-resistant (RtRt and RvRv) genotypes, and in reciprocal heterozygotes (SvRt, RtSv, SvRv and RvSv) of* Cydia pomonella

Parameter estimate[a]	Genotype SvSv	RtRt	RvRv	SvRt	RtSv	SvRv	RvSv
Sum of squares error[b]	$1{\cdot}33\times10^{-5}$	$8{\cdot}91\times10^{-6}$	$8{\cdot}67\times10^{-6}$	$1{\cdot}88\times10^{-5}$	$1{\cdot}72\times10^{-5}$	$1{\cdot}49\times10^{-5}$	$1{\cdot}62\times10^{-5}$
Ψ	0·057	0·051	0·051	0·057	0·056	0·055	0·056
D	12·99	12·81	12·82	13·23	12·74	12·73	12·83
T_m	26·98	26·03	26·03	26·85	26·86	26·83	26·85
ΔT	1·26	0·66	0·65	1·15	1·16	1·12	1·15
T_o (95 % CI)[c]	9·70 (8·12–11·28)	9·71 (8·24–11·18)	9·70 (8·18–11·22)	9·73 (8·14–11·29)	9·71 (8·21–11·21)	9·70 (8·20–11·20)	9·71 (8·13–11·29)
T_{opt} (95 % CI)[c]	31·35 (29·28–33·42)	32·25 (29·37–35·13)	32·21 (29·29–35·13)	32·04 (29·12–34·96)	31·98 (29·14–34·78)	32·06 (29·21–34·91)	32·01 (29·11–34·91)

[a] These parameters were estimated by nonlinear least squares with the model fit to data.
[b] This parameter estimates the goodness of fit of the model.
[c] In these rows, the estimates have overlapping 95 % confidence intervals (95 % CI).

protected least significant different Fisher test. Estimates of 50 % emergence events and their 95 % confidence intervals were obtained using the probit computer program (Raymond *et al.*, 1993).

3. Results

(i) *Thermal thresholds required for* C. pomonella *development*

As expected, developmental responses to temperatures ranging from 14·5 °C to 34 °C were found to be nonlinear and asymmetric around an optimum (T_{opt}). The data were plotted along with Hilbert & Logan's (1983) nonlinear model, and the SSE calculated for each genotype indicated that the model provided acceptable fits to our data set (Table 1). An overlapping of the 95 % CI of both T_o and T_{opt} indicated that neither of these two estimates differed significantly between genotypes (Table 1). Conversely, developmental rates were significantly lower in both RtRt and RvRv genotypes than in SvSv (Table 2). Heterozygotes were statistically indistinguishable from SvSv genotypes (Table 2), suggesting that the developmental cost was recessive. There was no evidence for any interaction between both of the genotype and temperature factors ($F_{30,2925}=1{\cdot}225$, $P=0{\cdot}299$).

(ii) *Phenological responses under natural conditions*

The overwintering mortality varied between 19·9 % and 25·9 % but did not differ significantly among genotypes (Table 3). This suggested that the presence of insecticide resistance alleles did not influence the mortality level during the overwintering period. The emergence patterns were significantly different between genotypes. In the first generation, RvRv moths emerged significantly later than RtRt, SvSv and heterozygous moths (Table 4). During the emergence of the second generation, SvSv, SvRt and RtSv moths emerged earlier than RtRt, RvRv, SvRv and RvSv moths (Table 4). Finally, RtRt, RvRv and heterozygous G3 moths emerged significantly later than the susceptible ones (Table 4).

The length of pre-imaginal development was calculated for both the second and the third generations. For each genotype, the mean DD values were not significantly different between the two generations (Table 5). The only exception was the RvRv larvae, which displayed a higher DD value at the second generation (533·7) compared with the third generation (491·2) ($F_{13,1135}=42{\cdot}16$, $P<0{\cdot}0001$). The mean DD values of the G2 and G3 larvae of both the Rt and Rv strains were higher than those of the Sv strain (Table 5), suggesting that the resistance alleles increased the length of pre-imaginal development. Such a pleiotropic cost was recessive, because heterozygous

Table 2. *Pre-imaginal developmental rates* (*1/number of days to adult emergence*) *in homozygous susceptible* (*SvSv*) *and insecticide-resistant* (*RtRt and RvRv*) *genotypes, and in reciprocal heterozygotes* (*SvRt, RtSv, SvRv and RvSv*) *of* Cydia pomonella*, at six different temperatures*

Temperature (°C)	Genotype SvSv	RtRt	RvRv	SvRt	RtSv	SvRv	RvSv	$F_{(\text{d.f.})}$	*P*
14·5	0·0094^{a}	0·0086^{b}	0·0086^{b}	0·0096^{a}	0·0096^{a}	0·0096^{a}	0·0096^{a}	690·79 (6, 580)	<0·0001
20	0·0209^{a}	0·0190^{b}	0·0192^{b}	0·020^{a}	0·0210^{a}	0·0209^{a}	0·0209^{a}	367·16 (6, 536)	<0·0001
23	0·0296^{a}	0·0269^{b}	0·0269^{b}	0·0299^{a}	0·030^{a}	0·0295^{a}	0·0297^{a}	32·42 (6, 329)	<0·0001
26	0·0332^{a}	0·0304^{b}	0·0303^{b}	0·0325^{a}	0·0325^{a}	0·0326^{a}	0·0326^{a}	140·67 (6, 582)	<0·0001
31	0·0424^{a}	0·0384^{b}	0·0386^{b}	0·0423^{a}	0·0425^{a}	0·0421^{a}	0·0424^{a}	99·19 (6, 569)	<0·0001
34	0·0370^{a}	0·0364^{b}	0·0363^{b}	0·0375^{a}	0·0372^{a}	0·0373^{a}	0·0373^{a}	2·58 (6, 329)	0·0186

^{a,b} In each row, mean developmental rate values sharing the same letter do not differ significantly using a protected least significant difference Fisher test ($P>0{\cdot}05$).

Table 3. *Overwintering mortality percentages in homozygous susceptible* (*SvSv*) *and insecticide-resistant* (*RtRt and RvRv*) *genotypes, and reciprocal heterozygotes* (*SvRt, RtSv, SvRv and RvSv*) *of* Cydia pomonella

Genotype	Overwintering G1 larvae	Spring-emerging G1 moths	Overwintering mortality (%)	χ^2	*P*
SvSv	200	159	20·50	–	–
RtRt	106	83	25·90	0·060	0·806 (NS)
RvRv	190	148	22·10	0·150	0·699 (NS)
SvRt	124	97	21·77	0·075	0·784 (NS)
RtSv	126	101	19·89	0·021	0·885 (NS)
SvRv	114	89	21·92	0·089	0·765 (NS)
RvSv	119	92	22·68	0·213	0·644 (NS)

The overwintering mortality in SvSv genotypes was compared with those measured in RtRt, RvRv and heterozygotes using χ^2 tests with one degree of freedom (non-significant (NS) $= P>0{\cdot}05$).

Table 4. *Adult emergence patterns in homozygous susceptible* (*SvSv*) *and insecticide-resistant* (*RtRt and RvRv*) *genotypes, and in reciprocal heterozygotes* (*SvRt, RtSv, SvRv and RvSv*) *of* Cydia pomonella. *Emergence patterns were expressed in accumulated degree days* (*since 1st January*) *and were characterized by their 50% frequency of occurrence*

	First generation (G1 moths)		Second generation (G2 moths)		Third generation (G3 moths)	
Genotype	*n*	Emergence 50% (95% CI)	*n*	Emergence 50% (95% CI)	*n*	Emergence 50% (95% CI)
SvSv	159	376·3^{b} (370·3–382·0)	145	1058·7^{c} (1047·2–1069·7)	211	1653·7^{c} (1645·0–1662·7)
RtRt	83	382·2^{b} (379·8–403·7)	108	1151·4^{b} (1140·0–1162·7)	88	1809·9^{b} (1785·9–1834·1)
RvRv	148	485·8^{a} (465·4–485·8)	101	1272·3^{a} (1216·3–1330·9)	34	1890·9^{a} (1855·0–1927·4)
SvRt	97	374·8^{b} (363·0–386·9)	43	1062·1^{c} (1036·8–1085·2)	81	1754·9^{b} (1719·9–1790·6)
RtSv	101	373·3^{b} (368·6–380·0)	72	1060·7^{c} (1032·6–1084·5)	45	1768·8^{b} (1748·7–1787·5)
SvRv	89	379·1^{b} (373·6–384·8)	117	1158·8^{b} (1147·3–1170·2)	40	1771·1^{b} (1750·4–1790·4)
RvSv	92	374·3^{b} (368·7–379·9)	144	1121·3^{b} (1097·5–1145·5)	31	1746·2^{b} (1719·9–1770·2)

$^{a\text{–}c}$ For each generation, estimates of 50% emergence sharing the same letter indicate the overlapping of their 95% confidence intervals (95% CI).

and susceptible homozygous genotypes had similar DD values (Table 5). These results were congruent with the recessive cost of resistance found in the laboratory experiments conducted simultaneously at different temperatures (Table 2).

At the end of the summer, the outcome of the G3 larvae indicated a strong, significant difference in diapause propensities between genotypes (Fig. 1). Only 6·7% of the homozygous susceptible larvae entered diapause, whereas this fraction reached 65·8% and 76·2% for the RtRt and RvRv larvae, respectively. Compared with their homozygous parental strains, heterozygous larvae displayed intermediate diapause propensities (Fig. 1).

Table 5. *Thermal constants in degree-days* (*DD*) *of pre-imaginal development under natural conditions in second* (*G2*) *and third* (*G3*) *generation larvae of homozygous susceptible* (*SvSv*) *and insecticide-resistant* (*RtRt and RvRv*) *genotypes, and in reciprocal heterozygotes of* Cydia pomonella

	G2 larvae		G3 larvae	
Genotype	*n*	Mean DD	*n*	Mean DD
SvSv	145	461·6[a]	211	461·0[a]
RtRt	108	527·1[c]	88	525·2[c]
RvRv	101	533·7[c]	34	491·2[b]
SvRt	43	458·8[a]	76	469·2[a]
RtSv	71	458·4[a]	45	464·3[a]
SvRv	117	471·2[a]	40	466·3[a]
RvSv	38	466·6[a]	32	469·2[a]

[a–c] Mean DD values sharing the same letter do not differ significantly (Protected Least Significant Difference Fisher Test, $P > 0·05$).

4. Discussion

(i) *Phenological segregation of insecticide resistance alleles*

Our data provided evidence for a significant difference in developmental traits involved in seasonal regulation between susceptible and insecticide-resistant strains of *C. pomonella*. Field sampling procedures might result in bottlenecks that severely affect genetic variability in the collected population. Thus, we cannot exclude the possibility that the observed variation in the phenological traits could be due to other alleles that were fixed independently of the resistance selection process by genetic drift. Drift effects could also occur in such reduced laboratory populations during the selection process of the resistant strains as a consequence of the inbreeding associated to their maintenance (Falconer, 1981; Georghiou, 1972). Nonetheless, given the size of the breeding populations used in the mass rearing of both the Rt and Rv strains at each generation, we postulate that our results might support the pleiotropy hypothesis for such divergence between the genotypes of *C. pomonella*.

Both resistant homozygous genotypes were associated with a significant increase in thermal constants of pre-imaginal development. Although preliminary laboratory investigations did not indicate any significant developmental cost in overwintering individuals (Boivin *et al.*, 2001), RvRv genotypes displayed a significantly delayed spring emergence relative to SvSv genotypes, but also to RtRt ones. Additionally to the same metabolic resistance mechanisms as those found in Rt, the Rv strain expresses the nerve insensitivity *kdr*-type mutation (Sauphanor *et al.*, 1998; Bouvier *et al.*, 2001). Resistance conferred by target site modifications has given well-known cases of deleterious pleiotropic effects, principally with mutations in enzymes involved in the nervous system (Chevillon *et al.*, 1997; Lenormand *et al.*, 1999; Berticat *et al.*, 2002). *Kdr*-type mutations in the peach–potato aphid *Myzus persicae* (Sulzer) have already been associated with a reduced responsiveness to environmental stimuli such as alarm pheromones (Foster *et al.*, 1999). In *C. pomonella*, thresholds of perception of environmental cues are known to be part of the diapause termination process (Riedl, 1983). This suggests that target-site modification could have pleiotropic effects on other non-developmental phenological traits, which were not estimated in the present study. Although the adult spring emergence of RtRt moths was statistically indistinguishable from that of SvSv moths, the significantly higher requirement of heat units for pre-imaginal development in both G2 and G3 larvae entailed a significant divergence in the emergence patterns of both G2 and G3 moths. The G2 and G3 emergence patterns of both SvRv and RvSv moths were found to be significantly higher than those of SvSv moths, probably because G2 and G3 heterozygotes were generated by crossing the delayed RvRv parents with the later SvSv ones only. Conversely, both the relative synchronization of both SvSv and RtRt spring emergences and the recessive developmental cost precluded any significant delay in the G2 emergence patterns in both the SvRt and RtSv progeny. However, the delayed occurrence of G2 RtRt adults relative to SvSv ones was sufficiently delayed to entail a significant divergence in G3 emergences in both the SvRt and RtSv progeny. It should be pointed out that phenological traits in heterozygotes issued from crosses between two heterozygous parents were not considered in the present work because of our inability to distinguish such genotypes from their homozygous counterparts. Nevertheless, data on both the emergence patterns of G1 moths and pre-imaginal development suggested that the seasonal change in heterozygotes would be similar to that of susceptibles.

In *Culex pipiens* L. mosquitoes, temporal variation in resistance frequencies in overwintering sites in the absence of insecticide was attributed to migration effects rather than to the expression of pleiotropic costs of resistance genes (Gazave *et al.*, 2001). By describing the seasonal patterns of both susceptibility and resistance, the present work suggests that life-history trade-offs resulting from adaptive changes might also be crucial for explaining variation in the temporal dynamics of organisms at the population level.

(ii) *Pleiotropy of insecticide resistance and the life cycle of* C. pomonella

C. pomonella is a short-day insect, in which the number of generations has been shown to be strongly

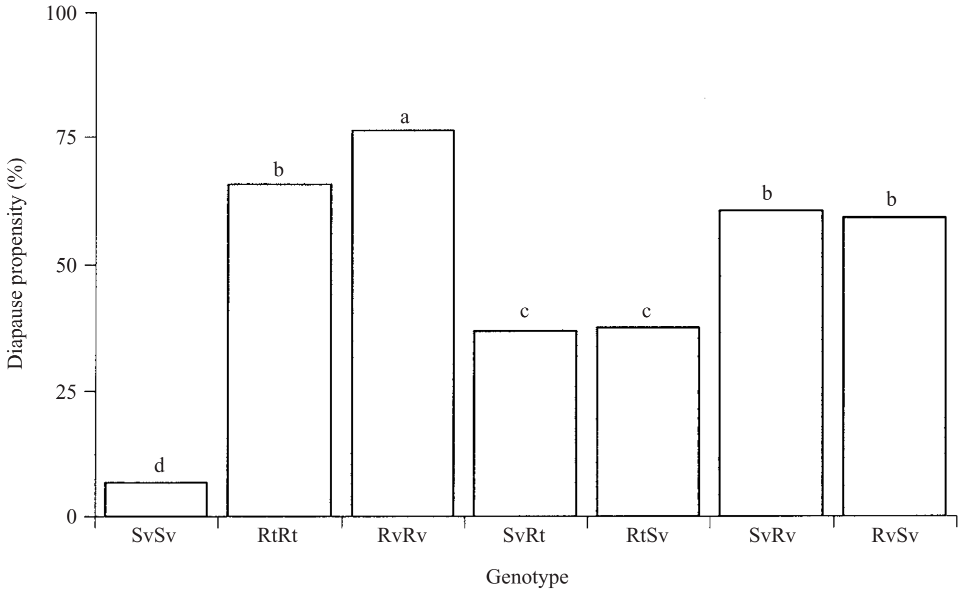

Fig. 1. Diapause propensity in homozygous susceptible (SvSv) and insecticide-resistant (RtRt and RvRv) genotypes, and in reciprocal heterozygotes (SvRt, RtSv, SvRv and RvSv) of third-generation larvae of *Cydia pomonella*. Bars sharing the same letter do not differ significantly using a χ^2 test with one degree of freedom ($P > 0{\cdot}05$).

controlled by the decrease in day length (Peterson & Hammer, 1968; Riedl & Croft, 1978). The magnitude of this trait is thus expected to depend on developmental time relative to the average length of the reproductive season, defined as the available time between diapause termination in overwintering larvae and diapause-inducing photoperiods. As a consequence of both a later spring emergence and lower within-generation developmental rates, the phenological delay measured in resistant genotypes might have increased the proportion of G3 larvae that were exposed to the diapause-inducing photoperiods. This resulted in higher diapause propensities in resistant genotypes than in earlier and faster susceptible ones. Carrière *et al.* (1995) described a positive correlation between physiological resistance and higher diapause propensities across insecticide-treated populations of *Choristoneura rosaceana* (Harris) and suggested that diapause was likely to respond to selection on insecticide resistance because of pleiotropic effects of the resistance alleles. Our results provide crucial insights on the physiological mechanisms that would underlie such a phenomenon, by describing the patterns of seasonality of resistance alleles from spring emergence in overwintering insects to diapause induction in the middle of summer. In consequence, the intermediate response in the diapause trait in heterozygotes might be due mainly to historical factors such as delayed phenological occurrences of the resistant parental genotypes, rather than to a co-dominant expression of pleiotropic effects of resistance on diapause.

Pleiotropic consequences of insecticide resistance genes are generally regarded as a physiological cost that might put the carriers of such genes at a selective disadvantage in terms of population dynamics and competitive abilities (Minkoff & Wilson, 1992; Boivin *et al.*, 2003), when compared with their susceptible counterparts. Nonetheless, our results suggest that pleiotropy of resistance on developmental components could confer an adaptive advantage by resulting in an increase in diapause propensities in the G3 progeny of *C. pomonella*. In multivoltine species, facultative diapause entails life-history trade-offs between survival and reproduction (Hopper, 1999). Early emerging individuals might benefit from more suitable conditions for reproduction than later emerging ones, in which the increasing cost of reproductive failure as the season advances might principally select for entering diapause. The decision to enter diapause or not might thus depend on the probability of survival to the diapause stage before the occurrence of lethal environmental conditions (Carrière *et al.*, 1995). In major agricultural pests such as *C. pomonella*, this probability is tightly linked to the occurrence of human activities from the end of flowering to the harvest. In this context, the cost of reproductive failure in pupating genotypes might be particularly pronounced if harvest or adverse climate conditions occur before the diapause stage is achieved in the progeny. But one would expect substantial inter-annual variation in both the benefits and costs associated with the phenological response displayed by a given genotype, because of the close climate-dependence of the life-cycle with regard to annual temperatures. Although overwintering survival costs associated with insecticide resistance have been described as particularly severe (95% in *Lucilia cuprina* (McKenzie, 1990); 70% in *C. pipiens* (Chevillon *et al.*, 1997); 71% in *Pectinophora gossypiella* (Carrière *et al.*, 2001)), our study indicated that the change in diapause propensity resulting from selection for resistance might not be significantly counterbalanced by any overwintering survival disadvantage in *C. pomonella*. Under the climate conditions that occurred in 2001, this suggests that early-diapausing resistant genotypes could contribute more efficiently to the subsequent spring generation than susceptible pupating genotypes. Selection for insecticide resistance in *C. pomonella* could thus be favoured by man-made chemical selection during the reproductive period on the one hand, but also by a higher diapause incidence associated to pleiotropic effects on the other hand. Further investigations focusing on the regulating factors of the diapause strategy could provide critical information about the ecological components of the fitness of resistance genes in French populations of *C. pomonella*.

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