Physiological Entomology

Physiological Entomology (2011) **36**, 253–260 DOI: 10.1111/j.1365-3032.2011.00793.x

Evaluating reproductive fitness and metabolic costs for insecticide resistance in *Myzus persicae* from Chile

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Abstract. The development of insecticide resistance in pest insects is an increasing problem for agriculture, forestry and public health. Aphids are ubiquitous herbivorous insects, with approximately 4700 known species, of which less than 5% exploit the agricultural environment successfully. Of these, the peach-potato aphid *Myzus persicae* Sulzer is recognized as one of the most important pests worldwide because it has acquired resistance to many insecticides. Although resistance to insecticides provides important benefits for pests in agricultural fields that are treated with insecticides, it may be associated with fitness (or other) costs in environments that are insecticide free. In the present study, the fitness and energy costs that might be experienced by *M. persicae* in an insecticide-free environment when carrying at least one insecticide resistance mutation (IRM), or by having an increased production of esterases, are evaluated. The study investigates whether genotypes that have an IRM also have enhanced esterase production, whether there is any metabolic cost associated with insecticide resistance, and whether there are any fitness costs associated with insecticide resistance and metabolic expenditure. The intrinsic rate of increase, standard metabolic rate (i.e. a measure of maintenance costs) and constitutive esterase activity are determined for 30 different multilocus genotypes carrying (or not carrying) at least one of the two most frequent insecticide resistance mutations (MACE and *kdr*/super-*kdr*) that occur in Chile. The results show that genotypes carrying at least one IRM have higher levels of total esterase activity than genotypes without an IRM, that there is no evidence of an energy cost associated with total esterase activity or IRM, and no evidence for a reproductive fitness cost associated with total esterase activity, IRM or metabolic rate. The results agree with previous studies showing linkage disequilibrium between insecticide resistance mechanisms, although they contrast with those of studies that report fitness costs associated with insecticide resistance in *Myzus persicae*.

Key words. Energy costs, *Myzus persicae*, reproductive fitness, standard metabolic rate.

Introduction

A major postulate in evolutionary physiology is the allocation principle, which is a driving force of an important part of research in physiological ecology (Sibly & Calow, 1986).

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The allocation principle indicates that, as energy inputs are limited, individuals with higher maintenance costs will have less energy available to allocate to growth, reproduction and/or other aspects of performance. The main prediction of this principle is that natural selection should maximize the residual energy available, and therefore higher maintenance costs will be associated with lower fitness if no compensation in other functions is available. Although this principle is effectively an untested dogma in evolutionary physiology, Czarnoleski *et al*. (2008) provide one of the first examples of direct evidence to support it. By selecting for larger body sizes of the land snail *Helix aspersa* (i.e. more energy allocated to growth), they show a negatively correlated response in the energy metabolism of juveniles (i.e. less energy is available for maintenance and performance).

The development of insecticide resistance in pest insects is an increasing problem for agriculture, forestry and public health (McKenzie, 2001; Onstad, 2008). Several mechanisms are reported to confer insecticide resistance. These include: behavioural evasion, which allows insects to avoid insecticides; thickening of the cuticle, which reduces insecticide penetration; increased sequestration or excretion of insecticides; increased activity of detoxifying enzymes (metabolic resistance); and modifications of insecticide target-sites that reduce or abolish insecticide sensitivity (Mutero *et al*., 1994; Ffrench-Constant *et al*., 2004; Onstad, 2008). Target-site modifications are produced by point mutations that provide a specific resistance mechanism (e.g. by decreasing enzyme activity or diminishing the efficiency of the enzyme as in the case of the modified acetylcholinesterase; MACE) (Fournier, 2005), or by target-site insensitivity as a consequence of knockdown mutations (*kdr*) affecting the voltage-gated sodium ion channels (Brooke, 2008). Insecticide resistance conferred by point mutations (insecticide resistance mutation, IRM) is reported for many insect species, indicating that these mechanisms are widespread among insect pests and have appeared independently during the evolution of insecticide resistance (Andreev *et al*., 1999; Anstead *et al*., 2005; Ffrench-Constant *et al*., 2007).

Aphids (Hemiptera: Aphididae) are ubiquitous herbivorous insects, with approximately 4700 known species, of which less than 5% exploit the agricultural environment successfully (van Emden & Harrington, 2007). Nonetheless, the economic impact of aphids as crop pests is immense, and can be explained by the interplay of several factors. In particular, the production of telescoping generations allows the rapid build up of large population sizes that cause damage to crops through direct feeding and/or by transmission of plant viruses. Additionally, some aphid species have developed several insecticide resistance mechanisms, transforming them into worldwide pests (Foster *et al*., 2007a). Among these, the peach-potato aphid *Myzus persicae* Sulzer is recognized as one of the most important pests worldwide (Blackman & Eastop, 2000; van Emden & Harrington, 2007). This species uses over 400 host plant species around the world, causing damage through feeding and transmission of plant viruses (Blackman & Eastop, 2000). Although several insecticides are used to control *M. persicae*, resistance has developed to many of them, either through metabolic or target site mutation mechanisms (Foster *et al.,* 2007a). In particular, *M. persicae* is reported to exhibit three mechanisms of insecticide resistance through target site mutations. These are MACE, which confers insensitivity to dimethyl carbamates, pirimicarb and triazamate (Moores *et al*., 1994; Foster *et al*., 2000); changes in voltage-gated sodium channels, known as knockdown resistance (*kdr*) and super-*kdr*, which confer resistance to pyrethroids and dichlorodiphenyltrichloroethane (Martínez-Torres *et al*., 1999; Anstead *et al*., 2005); and changes to the γ-aminobutyric acid receptor (*Rdl*), which gives resistance to cyclodienes (Anthony *et al*., 1998). Additionally, *M. persicae* shows resistance to organophosphates, and a secondary resistance to pyrethroids, by overproducing esterase-based detoxifying enzymes (Foster *et al*., 2007a). More recently, a resistance to neonicotinoids has developed through over-expression of the cytochrome P450 monooxygenase gene *CYP6CY3* (Philippou *et al*., 2009; Puinean *et al*., 2010).

Although it is clear that resistance to insecticides (either through IRM and/or metabolic change) provides important benefits to individuals of a pest population in agricultural fields that are sprayed with insecticides, it is known also that this resistance is associated with fitness costs, such as a reduction in reproductive performance, longer development times and a reduction in body size in several insect species in environments that are free of insecticides (Roush & McKenzie, 1987; Carrière *et al*., 1994; Berticat *et al*., 2008). Evidence of fitness costs associated with IRM is also reported for *M. persicae*. For example, individuals that carry *kdr* mutations show a reduced response to alarm pheromone, and aphids with MACE resistance show a lower reproductive performance compared with susceptible individuals (Foster *et al*., 1999, 2000, 2003). Fitness costs are suggested to be a consequence of trade-offs in energy between traits underlying insecticide resistance and fitness-related traits such as reproduction, development time and adult body size (Roush & McKenzie, 1987; Ghadamyari *et al*., 2008; Fenton *et al*., 2010). Nevertheless, empirical evidence for this reallocation is lacking because the relationships between insecticide resistance, fitness and energy costs have not been investigated in any pest species. Given that, in Chile, *M. persicae* makes widespread use of several native and exotic weeds far from agricultural fields as secondary host plants, the species probably experiences a fluctuating exposure to insecticides. In the present study therefore, the reproductive fitness and energy costs that might be experienced by *M. persicae* in an insecticide-free environment when carrying an IRM or having an increased production of esterases, are evaluated. In particular, the study addresses whether genotypes having an IRM also have enhanced esterase production; whether there are any energy costs associated with the insecticide resistance mechanisms; and whether there are fitness costs associated with these resistance mechanisms and their associated energetic expenditure. Thus, the intrinsic rate of increase (i.e. a measure of reproductive performance), the standard metabolic rate (i.e. a measure of maintenance costs) and the level of constitutive esterase activity are determined for 30 different multilocus genotypes carrying (or not carrying) at least one of the two most frequent IRMs (MACE and/or *kdr*/super-*kdr*) that are found in Chile (Fuentes-Contreras

et al., 2007). Aphids are sampled in different crops and weeds across a north–south transect to investigate a large number of clones that will have been subjected to different insecticide management regimes and may be carrying IRM in different agoecosystems.

Materials and methods

Collection sites and maintenance

During spring 2008 and summer 2009, 94 individual aphids were sampled from sites next to roads and from agricultural fields along a north–south transect ranging 1830 km from Copiapó (27°S latitude) to Puerto Montt (41°S latitude), encompassing the whole distributional range of the species in Chile (information regarding sampling locations is provided in the Supporting information, Table S1). Aphids were collected mainly from *Brassica rapa*, *Solanum tuberosum* and *Capsicum annuum*, because these were the most frequent host plant species at the sampling locations. To reduce the chance of sampling from the same parthenogenetic colony, aphids that were separated by at least 20 m were collected. Parthenogenetic colonies were established on Blackman boxes (Blackman, 1971) that contained seedlings of *Capsicum annuum* var. *grossum* (cv. Resistant). Each colony deriving from a single adult wingless female collected in the field (Table S1). Colonies were maintained under an LD 16 : 8 h photocycle at 20 ± 1 °C in discrete generations to ensure parthenogenetic reproduction, by transferring five wingless adults to new 7-day-old pepper seedlings every 10 days. Aphid colonies were maintained on pepper seedlings for at least ten generations before being analyzed.

Microsatellite genotyping, allelic discrimination and enzymatic determination

Each aphid colony was genotyped using six previously described microsatellite loci (*M2*, *M3*, *M25*, *M35*, *M37*, *M40*) (Sloane *et al*., 2001; Wilson *et al*., 2002; Vorburger *et al*., 2003, Fuentes-Contreras *et al*., 2004; Malloch *et al*., 2006; Vorburger, 2005, 2006; Blackman *et al*., 2007; Margaritopoulos *et al*., 2007a; Kasprowicz *et al*., 2008). Multilocus genotypes (MLG) were obtained after combining alleles from each amplified locus in the whole sample, which resulted in fifty different MLG (information regarding microsatellite profiles for each genotype and the number of copies for each MLG is provided in the Supporting information, Table S2). Out of 94 genotypes, 44 were redundant (i.e. colonies from the same genotype repeated more than once) and were discarded. Most redundant genotypes were sampled from closed collection points and only two aphid genotypes presented a widespread distribution according to the sampling transect (Table S2). Thirty of the 50 genotypes that remained were screened for the presence of IRMs using allele discrimination based on quantitative polymerase chain reaction assays developed by Anstead *et al*. (2004) for *kdr* (L1014F) and super-*kdr* (M918T) mutations, and Anstead *et al*. (2008) for MACE mutations. Total esterase activity was evaluated using the microplate bioassay, as described previously by Devonshire *et al*. (1992), and with five independent biological replicates and three technical replicates per measurement.

Breeding design and reproductive fitness measurements

Ten individual, wingless (parental) aphids of each genotype were transferred to ten adult sweet pepper plants, with one nymph per plant being collected after 24–48 h. Each of these nymphs (F_1) was transferred to a Blackman box containing a 2-month-old pepper seedling, and maintained until maturity. Parental aphids were removed from seedlings after 3 days of parthenogenetic reproduction and all but one nymph (F_2) were discarded 2 days later. This process was repeated twice; hence, focal individuals were from the F_4 generation. All aphids were maintained under an LD 16 : 8 h photocycle at 20 ± 1 °C. This protocol was repeated (i.e. ten lines per genotype) for all genotypes because of mortality in some of the replicates. Thus, 20 individual lineages were developed from each genotype.

The intrinsic rate of natural increase (r_m) was estimated as described in Wyatt & White (1977). Essentially, the age from birth to first reproduction (T_d) and the number of offspring produced in an equivalent time (M_d) are determined. If an aphid has its first offspring 5 days after being born (i.e. T_d) the progeny are counted for 5 days (i.e. M_d). Then, the intrinsic rate of natural increase is calculated as $r_m = 0.74 \cdot (\log_e M_d)/T_d$.

Standard metabolic rate

The standard metabolic rate (SMR) was determined after the estimation of r_m. It was measured as the volume of CO_2 produced in a given period by an individual aphid using a closed-system metabolic chamber previously described by Castañeda *et al*. (2009). Each single wingless aphid was placed in a sealed 2-mL syringe filled with CO_2 free air to a volume of 1.5 mL and placed in a dark climate-controlled chamber at 20 ± 1 °C. After 2 h in the climate-controlled chamber, 1 mL of air from each syringe was injected into a Bev-A-line® tube (Thermoplastic Processes, Stirling, New Jersey) connected to a CO_2 analyzer with a resolution of 1 p.p.m. of CO_2 gas in air (Li-6262, LI-COR Bioscience, Lincoln, New England). Flow rates of CO_2-free air (ambient air was scrubbed of CO_2 by passing it through a soda lime column) were maintained at 100 mL min^{-1} (±1%) with a mass flow controller (Sierra Instruments, Monterey, California). The output from the CO_2 analyzer was recorded with Expedata software (Sable Systems International, Las Vegas, Nevada). Rates of CO_2 production (in L CO_2 h^{-1}) were calculated from the complete record and by transforming the concentration from p.p.m. to CO_2 fraction, and then multiplying by the flow rate. From each peak of CO_2 observed in the record (i.e. corresponding to each injection of air), the area under the curve (e.g. the integral of mL CO_2 min^{-1} versus min) was calculated (Lighton, 2008).

This area is equal to the volume of CO_2 in the syringe produced by each aphid. The volume is divided by the total period of measurement (2 h), and multiplied by 1000 to give L CO_2 h^{-1}, which corresponds to the metabolic rate per aphid per hour. Fifteen empty syringes were sampled as described above to be used as blanks for calculations of CO_2 concentration. Aphids were cooled on ice for a few seconds and weighed to the nearest microgram on a microbalance (MXA5; Radwag, Czech Republic) before SMR measurements.

Statistical analysis

The effect of IRM on total esterase activity was evaluated by one-way analysis of variance using a mean value of activity per genotype. A linear mixed modelling approach was used to evaluate the effect of IRM and total esterase activity on metabolic rate, and of the overall effect of all these on r_m, at the same time as taking into account the nested structure of the experimental design (i.e. the presence of mutations that were genotype-specific) and some unbalance. Hypothesis testing for fixed effects was based on marginal F tests and, for testing for random effects, was based on likelihood ratio test of nested models (Pinheiro & Bates, 2000). Body mass, SMR and r_m were $\log_{10}$-transformed to meet normality assumptions. Statistical analysis was conducted using the NLME package (Pinheiro *et al*., 2011) for R software, version 3 (R Development Core Team, 2009).

Results

Genotypes and allelic discrimination

Of a total of 30 genotypes that were characterized using microsatellites, 27 were found to be homozygous susceptible for MACE (i.e. *ss*) and 23 homozygous susceptible for *kdr*, whereas all were homozygous susceptible for super-*kdr*. Three genotypes were heterozygotic (i.e. *sr*) for MACE and seven for *kdr*. No genotype was found to be homozygous resistant (i.e. *rr*) for either MACE or *kdr*. Because all 30 genotypes were susceptible for super-*kdr*, this information was not included in any statistical analysis. Because of the high unbalance of the dataset (i.e. *kdr* was always associated with MACE in all genotypes analyzed), it was decided to test just the overall effect of having at least one IRM.

Fitness measurements and standard metabolic rate

Descriptive statistics of intrinsic rate of increase (r_m), body mass, SMR and esterase activity for aphid genotypes with or without IRMs are presented in Table 1.

Enhanced esterase production in resistant genotypes

Significant differences in esterase activity were found between genotypes with and without an IRM ($F_{1,28} = 6.705$, $P = 0.015$). Aphid genotypes carrying an IRM exhibited 80% higher esterase activity compared with 'susceptible' genotypes (with IRM: 0.300 ± 0.050 SE, $n = 7$; without IRM: 0.167 ± 0.025 SE, $n = 23$).

Lack of energy costs associated with insecticide resistance

Esterase activity was not found to be associated with SMR ($F_{1,27} = 1.343$, $P = 0.257$) or IRM ($F_{1,27} = 0.129$, $P = 0.723$), indicating that high esterase activity and IRM do not necessarily involve an increase of energy expenditure in *M. persicae*. Metabolic rate was only affected by body size (on a log-log scale $b = 0.503 \pm 0.110$ SE, $n = 163$; $F_{1,162} = 20.307$, $P < 0.0001$). Thus, residuals of metabolic rate against body size were used in subsequent analysis. Because no statistical effect of IRM on metabolic rate was found (i.e. no nesting needed), the variation among aphid genotypes in SMR regardless of IRM was evaluated. It was found that there was not much variation in metabolic rate as a result of genotypes ($\chi^2 = 1.612$, d.f. $= 1$, $P = 0.204$).

No reproductive fitness cost is associated with insecticide resistance or energy expenditure

No evidence was found for a reproductive fitness cost associated with total esterase activity ($F_{1,27} = 0.020$, $P = 0.889$), IRM ($F_{1,27} = 0.036$, $P = 0.851$) or metabolic rate (residuals) ($F_{1,162} = 0.011$, $P = 0.917$). Because no statistical effect of IRM on reproductive fitness was found (i.e. no nesting needed), the possibility of variation in r_m among aphid genotypes regardless of IRM was evaluated. The results show an important variation among genotypes in reproductive fitness ($\chi^2 = 10.015$, d.f. $= 1$, $P = 0.002$). However, the effect appeared to be confined to just one genotype (North 36-1) that had reduced values of reproductive fitness (Table 1). When this genotype was removed from the analysis, the effect of genotype also disappeared ($\chi^2 = 3.088$, d.f. $= 1$, $P = 0.079$).

Discussion

The allocation principle, a conceptual foundation in physiological ecology, contends that functions that compete for finite inputs of energy cannot be maximized at the same time (Sibly & Calow, 1986; Brown *et al*., 1993). The main prediction of this principle is that natural selection should maximize the residual available energy, and therefore higher maintenance costs will be associated with lower fitness if no compensation in other functions is available. In a pest species, resistance to insecticides provides important benefits for the individuals in agricultural crops that are sprayed with insecticides. However, it may be associated with fitness costs in environments that are free of insecticides if the mechanisms that confer the resistance cannot be turned off (Roush & McKenzie, 1987; Carrière *et al*., 1994; Foster *et al*., 1999, 2000, 2003; Berticat

Table 1. Characteristics of aphid clones sampled from crops and weeds along a north-south 1830 km transect in Chile, showing the occurrence of at least one insecticide resistance mutation (IRM), together with sample size of each genotype n and descriptive statistics for intrinsic rate of increase (r_m), body mass, standard metabolic rate (SMR) and constitutive esterase activity (EST).

Genotype	IRM	n	r_m (day^{-1})	Body mass (g)	SMR (L CO_2 h^{-1})	EST activity (U aphid-equivalent^{-1})
N 36-1	No	6	0.258 ± 0.013	0.379 ± 0.064	0.498 ± 0.072	0.133 ± 0.022
N 21B-1	No	7	0.292 ± 0.011	0.406 ± 0.044	0.383 ± 0.098	0.132 ± 0.006
N 44-1	No	2	0.335 ± 0.008	0.258 ± 0.029	0.352 ± 0.107	0.219 ± 0.015
N 30A-1	Yes	7	0.309 ± 0.008	0.350 ± 0.040	0.293 ± 0.078	0.390 ± 0.026
N 50-1	No	9	0.333 ± 0.014	0.322 ± 0.043	0.270 ± 0.047	0.538 ± 0.019
N 30B-1	No	8	0.313 ± 0.011	0.241 ± 0.030	0.363 ± 0.196	0.099 ± 0.005
N 49-1	No	1	0.374	0.287	0.211	0.373 ± 0.016
N 46-1	No	7	0.345 ± 0.005	0.385 ± 0.036	0.323 ± 0.044	0.069 ± 0.011
N 47-2	No	6	0.324 ± 0.016	0.255 ± 0.051	0.200 ± 0.075	0.092 ± 0.010
N 41-1	No	6	0.318 ± 0.024	0.218 ± 0.029	0.252 ± 0.065	0.246 ± 0.044
N 42-2	Yes	14	0.305 ± 0.008	0.303 ± 0.031	0.261 ± 0.032	0.334 ± 0.028
N 34-3	No	9	0.336 ± 0.016	0.358 ± 0.047	0.368 ± 0.060	0.228 ± 0.019
N 39-1	Yes	6	0.333 ± 0.016	0.207 ± 0.093	0.269 ± 0.035	0.487 ± 0.042
N 29-1	No	8	0.306 ± 0.008	0.223 ± 0.063	0.238 ± 0.047	0.070 ± 0.006
N 48-1	No	8	0.325 ± 0.018	0.176 ± 0.023	0.176 ± 0.040	0.047 ± 0.008
Teno7B	No	3	0.308 ± 0.018	0.328 ± 0.115	0.291 ± 0.033	0.092 ± 0.006
Talca3-1BA	No	4	0.282 ± 0.015	0.319 ± 0.018	0.148 ± 0.015	0.183 ± 0.004
Sfdo2C	No	5	0.331 ± 0.009	0.270 ± 0.042	0.222 ± 0.041	0.087 ± 0.002
Durazno Talca 2B	Yes	5	0.347 ± 0.012	0.311 ± 0.034	0.154 ± 0.028	0.160 ± 0.013
Curi3C	No	2	0.341 ± 0.033	0.357 ± 0.240	0.136 ± 0.099	0.201 ± 0.015
Sfdo1A	No	9	0.339 ± 0.016	0.300 ± 0.022	0.535 ± 0.157	0.120 ± 0.010
26A	Yes	8	0.324 ± 0.011	0.150 ± 0.023	0.274 ± 0.054	0.384 ± 0.034
Hijuelas 3-1	No	9	0.313 ± 0.011	0.141 ± 0.010	0.278 ± 0.057	0.331 ± 0.011
Talca1A	No	8	0.336 ± 0.019	0.218 ± 0.045	0.366 ± 0.114	0.078 ± 0.007
Paine7C	No	7	0.334 ± 0.012	0.390 ± 0.062	0.203 ± 0.071	0.117 ± 0.016
Talca4A	No	8	0.302 ± 0.007	0.197 ± 0.021	0.173 ± 0.041	0.070 ± 0.004
Sur 25A-3	No	5	0.302 ± 0.008	0.281 ± 0.044	0.154 ± 0.017	0.295 ± 0.008
Sur 74-1	Yes	6	0.313 ± 0.015	0.403 ± 0.073	0.254 ± 0.056	0.142 ± 0.011
Sur 46-3	Yes	5	0.301 ± 0.007	0.315 ± 0.065	0.248 ± 0.052	0.205 ± 0.015
Varas3-1	No	5	0.378 ± 0.022	0.333 ± 0.072	0.190 ± 0.049	0.062 ± 0.005

Sample size for esterase determinations was $n = 5$, with the following exceptions: N 44-1 ($n = 3$), N 30B-1 ($n = 2$), N 46-1 ($n = 3$), N 47-2 ($n = 4$), N 41-1 ($n = 4$), N 48-1 ($n = 4$) and Sfdo2C ($n = 4$). Values are the mean ± SE.

et al., 2008). Although fitness costs have been reported extensively, the mechanistic or physiological bases for such costs are generally still lacking; but see also Foster *et al*. (2007b). Nevertheless, fitness costs are suggested to be a consequence of trade-offs in the allocation of energy between traits that underlie insecticide resistance and fitness (Roush & McKenzie, 1987; Ghadamyari *et al*., 2008; Fenton *et al*., 2010). In the present study, the physiological costs in the form of standard metabolic rate, a widely used proxy of energy costs in insects (Chown & Nicolson, 2004), are incorporated explicitly. In particular, it is found that genotypes having an IRM show higher constitutive levels of esterase activity than genotypes without an IRM, that there is no evidence of an energy cost associated with total esterase activity or IRM and no evidence for a reproductive fitness cost associated with total esterase activity, IRM or SMR.

The results suggest that heterozygotic target-site mutations do not impose an energy or reproductive fitness cost on the aphids carrying the mutation. To some extent, this result is not unexpected. For example, *kdr* is a point mutation in the gene encoding for the sodium channel protein and thus it is unlikely this would impose any energy cost because this mutation produces only a structural change (Foster *et al*., 2007a). However, it is reported that resistant aphids having *kdr* mutations show behavioural alterations with important fitness costs. For example, *kdr* mutations in *M. persicae* are correlated with a reduced response to alarm pheromone, which affects their response to external stimuli, such as the presence of parasitoids, and has a direct effect on their survival (Foster *et al*., 1999, 2005, 2007b). More recently, Fenton *et al*. (2010) correlate homozygotic *kdr* resistance with reproductive fitness costs in *M. persicae*. Although homozygotic mutations for *kdr* are not found in the present study, a more intensive (i.e. increased number of samples collected) and extensive (i.e. more sampling locations) survey during 2010 shows that homozygous *kdr* mutations are at an extremely low frequency (i.e. 16% for *M. persicae* in Rosaceae; 1% in Brassicaceae; and 4% in Solanaceae; C. Figueroa, A. Silva & E. Fuentes-Contreras, unpublished observations) and are restricted to a small geographic zone in Chile. Thus, future studies are needed to evaluate whether these genotypes show reproductive fitness costs. By contrast, the relationship between MACE mutations

and fitness costs in *M. persicae* is not so clear from these other studies as the relationships involving *kdr* mutations or overproduction of detoxification enzymes (Fenton *et al*., 2010). In the present study, aphid genotypes carrying MACE only are not found (i.e. MACE is associated with *kdr* in all genotypes analyzed); hence, it is difficult to disentangle the contribution of MACE to fitness or energy costs in *M. persicae*. However, previous work by Foster *et al*. (2003) finds that aphids carrying the MACE mutation exhibit a lower intrinsic rate of increase compared with aphids without the mutation, irrespective of the production of carboxylesterases (i.e. a metabolic resistance mechanism by gene duplication). Furthermore, MACE-resistant mosquitoes show fitness costs in the form of longer development time (*Culex pipiens*; Bourguet *et al*., 2004) and reduced numbers of emerging females (*Culex quinquefasciatus*; Berticat *et al*., 2008).

It is well known that metabolic resistance to insecticides (mainly to organophosphates) is achieved through the overproduction of specific carboxylesterases (E4 and FE4) that degrade the insecticide esters before they reach the insect nervous system. Thus, it is more likely that an increase in the production of detoxification enzymes will have important effects on the energy budget of the aphid compared with IRM (Roush & MacKenzie, 1987; Foster *et al*., 2007a). However, the total esterase activity measured in the Chilean genotypes ranges between the S (susceptible) and R1 (resistant 1) levels according to the S/R1/R2/R3 classification described by Devonshire *et al*. (1992). If the present study had found resistant genotypes at level R2 or R3, the detection of a significant increase in energy costs may have been more likely. At least within the S and R1 levels of resistance, the present results suggest that total esterase activity does not correlate with any energy costs (measured as SMR), probably because the aphids are tested under benign conditions and resistance mechanisms may be switched off to avoid any related costs (Hoffmann & Merilä, 1999; Fry, 2001). In agreement with this suggestion, a recent study that evaluates the energy costs of plant allelochemical detoxification in the grain aphid (*Sitobion avenae*), finds a weak relationship between detoxifying enzymes (i.e. cytochrome P450 monooxygenase) and SMR (Appel & Martin, 1992; Castañeda *et al*., 2009). In the same context, and given that esterase activity is not related to energy costs, it is also not unexpected to find that esterase activity is not associated with reproductive fitness cost. Nevertheless, a reduced ability to survive winter conditions (frost, rain and wind) associated with reduced mobility at low temperatures, is reported as a potential fitness cost in *M. persicae* carrying higher levels of esterase activity (Foster *et al*., 1996, 1997).

With some exceptions, genotypes without an IRM generally have lower levels of esterase activity compared with those having at least one IRM. Such linkage disequilibrium is probably related to clonal selection of asexual lineages as a result of insecticide usage rather than to a tight chromosomal linkage of the resistance genes (Devonshire *et al*., 1998; Foster *et al*., 2000). This nonrandom association between insecticide resistance mechanisms, although not universal, is reported in several studies with *M. persicae* in different crops and countries. For example, an association between resistance mechanisms is described for large-scale surveys in the U.K. (Field & Foster, 2002; Foster *et al*., 2002), in Greece (Margaritopoulos *et al*., 2007b), Italy (Criniti *et al*., 2008) and New Zealand (van Toor *et al*., 2008). Finally, metabolic costs *per se* do not show an impact on reproductive fitness, suggesting that they either do not represent an important energetic demand, or that metabolic expenditure does not reach its physiological limits or that energy inputs are not restricted (Bacigalupe & Bozinovic, 2002).

In summary, no evidence is found for energy or reproductive fitness costs associated with total esterase activity or IRMs (*kdr* and MACE) in *M. persicae* from Chile. Furthermore, the genotypes examined do not differ in metabolic rate and reproductive fitness energy, thus offering little additional information to explain the worldwide ecological success of *M. persicae*. It is hoped that these findings will encourage future research on other relevant traits, such as feeding behaviour and dispersal ability. Studies of fitness costs in this context have an important eco-evolutionary and applied impact because they provide informative tools to help understand the role of constraints on insecticide resistance evolution and the spread of resistance mechanisms in pest populations.

Acknowledgements

This study was funded by FONDECYT grant 1080085 to L. D. Bacigalupe. Aphid genotyping and esterase determination were funded by FONDECYT grant 1090378 to C. C. Figueroa. L. E. Castañeda was supported by FONDECYT grant 3090056. A. X. Silva and P. A. Cortés acknowledge CONICYT Doctoral scholarships. Other than the first and last authors, the order of authorship is alphabetical. We would like to thank Dr Carola Otth (UACh) for allowing use of her laboratory for esterase determination. Comments from two anonymous reviewers and Dr Robert Weaver helped us to clarify the intended message.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: DOI: 10.1111/j.1365-3032.2011.00793.x

Table S1. Clonal lineages, sampling sites and hosts from where aphids were collected. Aphids collected in *Brassica rapa* were found either far from or close to agricultural fields.

Table S2. Allele combinations at six microsatellite loci of *Myzus persicae* genotypes used in the present study. # Clone copy = number of copies of the same genotype; Region = part of the country where the different copies were found.

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Accepted 4 May 2011
First published online 22 July 2011