Physiological approach to explain the ecological success of 'superclones' in aphids: Interplay between detoxification enzymes, metabolism and fitness

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ARTICLE INFO

ABSTRACT

'Superclones' are predominant and time-persistent genotypes, exhibiting constant fitness across different environments. However, causes of this ecological success are still unknown. Therefore, we studied the physiological mechanisms that could explain this success, evaluating the effects of wheat chemical defences on detoxification enzymes [cytochrome P450 monooxygenases (P450), glutathione S-transferases (GST), esterases (EST)], standard metabolic rate (SMR), and fitness-related traits [adult body mass and intrinsic rate of increase (r_m)] of two 'superclones' (Sa1 and Sa2) of the grain aphid, Sitobion avenae. Additionally, we compared 'superclones' with a less-frequent genotype (Sa46). Genotypes were reared on three wheat cultivars with different levels of hydroxamic acids (Hx; wheat chemical defences). Detoxification enzymes and SMR did not differ between wheat hosts. However, GST and EST were different between 'superclones' and Sa46, while Sa1 showed a higher SMR than Sa2 or Sa46 ($p = 0.03$). Differences between genotypes were found for r_m, which was higher for Sa1 than for Sa2 or Sa46. For all cases, genotype–host interactions were non-significant, except for aphid body mass. In conclusion, 'superclones' exhibit a broad host range, flat energetic costs for non-induced detoxification enzymes, and low variation in their reproductive performance on different defended hosts. However, physiological specialization of 'superclones' that could explain their ecological success was not evident in this study.

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1. Introduction

Plant chemistry has been proposed as one of the main selective forces driving the evolution of herbivorous insects (Schultz, 1988; Bernays and Chapman, 1994; Schoonhoven et al., 2005). In fact, plant secondary metabolites have evolved mainly as antiherbivore defences producing antixenosis and antibiosis with significant reduction of insect performance (Bennett and Wallsgrove, 1994; Bernays and Chapman, 1994; Schoonhoven et al., 2005). Simultaneously, insects have developed behavioural, physiological and biochemical strategies to avoid these deleterious effects (Berenbaum, 2001; Karban and Agrawal, 2002; Després et al., 2007). Among biochemical strategies, detoxification enzymes are involved in degradation and neutralization of ingested plant allelochemicals (Schoonhoven et al., 2005), and they have been considered of primary importance in host utilization by insects (Brattsten, 1988).

Insects exposed to host plant chemical defences overproduce detoxification enzymes in order to metabolize and excrete ingested allelochemicals (Feyereisen, 1999; Després et al., 2007; Li et al., 2007). Biosynthesis and maintenance of detoxification enzymes should impose significant demands on insect energy budgets, particularly in insects exposed to a wide range and levels of plant allelochemicals (Krieger et al., 1971; Karban and Agrawal, 2002; Després et al., 2007). As available energy is limited though, more energy allocated to detoxification will leave a smaller proportion of energy to be allocated to other functions, producing energetic trade-offs between detoxification, growth, and reproduction (Appel and Martin, 1992; Karban and Agrawal, 2002). Some studies have associated detoxification costs with reduction in net growth efficiency (the efficiency of conversion of assimilated food into biomass); however, this approach is not a direct measure of metabolic costs because mass reduction could be a consequence of either an increase of metabolic expenditure or a reduced feeding rate (Appel and Martin, 1992). Nevertheless, a straightforward measurement of maintenance and induced costs related to exogenous variables is metabolic rate, which has been useful to evaluate detoxification costs in herbivorous insects

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Table 1
Relative frequency in field collections and allele combinations of the eight multilocus genotypes of *Sitobion avenae*. Studied genotypes are indicated with asterisks, which Sa1 and Sa2 were called 'superclones', whereas Sa46 was considered as a rare, less-frequent genotype. Clones were genotyped by using five microsatellite loci according to methodological procedures described by Figueroa et al. (2005). For microsatellite primer sequences see Simon et al. (1999) and Wilson et al. (2004).

Genotype	Relative frequency	Microsatellite locus				
		S3.R	S5.L	Sm10	Sm11	Sm17
Sa1*	0.38	337 337	225 227	164 166	144 149	178 179
Sa2*	0.36	337 337	223 227	164 166	144 149	178 179
Sa3	0.05	361 361	223 227	164 166	144 149	178 179
Sa45	0.09	345 361	223 227	164 166	144 149	178 179
Sa46*	0.09	345 361	225 227	164 166	144 149	178 179
Sa47	0.01	345 361	225 227	164 166	144 156	178 179
Sa48	0.01	337 337	211 213	164 166	144 156	178 179
Sa49	0.01	345 361	211 213	164 166	144 149	178 179
Total	1.00					

(Appel and Martin, 1992; Cresswell et al., 1992; Castañeda et al., 2009).

Among herbivorous insects, cereal aphids are good study models because they feed on plants of the Poaceae family (i.e. the summer host, both in host alternating (heteroecious) as well as non-host alternating (monoecious) species like the grain aphid – see below), which exhibits a broad concentration range of hydroxamic acids (Hx). Hx are a group of allelochemicals involved in resistance against a wide variety of herbivores, particularly against aphids (Niemeyer, 2009). Hx cause antibiosis, feeding deterrence, and decreased performance and reproduction in aphids (Thackray et al., 1990; Givovich and Niemeyer, 1995). To reduce these deleterious effects, aphids have detoxification systems that are induced when they feed on plants and/or artificial diets containing Hx (Leszczynski et al., 1992; Figueroa et al., 1999; Loayza-Muro et al., 2000). Detoxification systems in aphids, like many other insects, include mainly three main superfamilies of enzymes: cytochrome P450 monooxygenases (P450), glutathione S-transferases (GST) and esterases (EST), all which are involved in allelochemical biotransformation (Leszczynski et al., 1992; Loayza-Muro et al., 2000; Després et al., 2007). Furthermore, Hx have been proposed as modulators of genetic structure in grain aphid, *Sitobion avenae* (Fabricius), populations (Figueroa et al., 2004, 2005). In Chile, *S. avenae* is an introduced pest species thought to reproduce primarily by obligate parthenogenesis, showing high abundance of time-persistent genotypes (the so-called 'superclones', see Vorburger et al., 2003a) with a capacity to exploit a broad range of host plants (Figueroa et al., 2004, 2005).

'Superclones' have been previously reported in several aphid species such as *Myzus persicae* (Sulzer) (Fenton et al., 1998; Vorburger et al., 2003a), *Aphis gossypii* (Glover) (Fuller et al., 1999), and *S. avenae* from French (Haack et al., 2000), British (Llewellyn et al., 2003) and Chilean (Figueroa et al., 2005) populations. 'Superclones' of *S. avenae* have been denominated as generalist genotypes, because they have been found on a wide range of climatic conditions and host plants (Haack et al., 2000; Llewellyn et al., 2004; Figueroa et al., 2005). It has been suggested that these genotypes could be the final product of clonal selection, being able to use a broad range of host plants in environments that vary in temporal and spatial scales (Lynch and Walsh, 1998; Haack et al., 2000; Vorburger et al., 2003a). Therefore, selection could favour physiological strategies (i.e. broad tolerance to allelochemicals) that allow *S. avenae* to exploit and maintain a high fitness across different host plants (Figueroa et al., 2005).

In spite of the increasing effort to understand the high predominance of 'superclones' in the field, the underlying determinants of their ecological success are still elusive (Vorburger et al., 2003a; Vorburger, 2006). In order to contribute in revealing

their apparent success, the main goal of this study was to understand the physiological mechanisms responsible for the broad host range and their consequences on energy budget and fitness of aphid 'superclones'. Hence, in the present study we evaluated allelochemical detoxification (P450, GST and EST activities), energetic costs (standard metabolic rate; SMR, and adult body mass) and fitness-related trait (intrinsic rate of increase) in three multilocus genotypes ('genotypes' for simplicity) of *S. avenae* obtained from populations from Central Chile reared on three wheat cultivars with different Hx levels. Two of these genotypes (Sa1 and Sa2) were considered as 'superclones' because they have a broad host range (i.e. oat, low- and high-Hx wheat, wild grasses) and they have been yearly collected several times since 1996, suggesting persistence in the time with high relative abundance in the field. Specifically, these genotypes corresponded to 68% of a sample of 1749 individuals collected between 1996 and 2000 (Figueroa et al., 2005); whereas in the current sample, they represent a 74% of the total collected aphids (see Table 1). In contrast, the genotype Sa46 is a first-time collected genotype, which exhibits low relative abundances (see Table 1) on a narrow host range in the field such as oat (lacking Hx) and wheats with low Hx levels.

2. Materials and methods

2.1. Insects and sampling site

The grain aphid, *S. avenae*, thrives on cereals and pasture grasses of temperate areas (Blackman and Eastop, 2000). In Chile, it was introduced approximately 35 years ago (Apablaza, 1974) and its present distribution ranges from 32°S to 41°S. During the Austral spring-summer of 2005, 120 living aphids were sampled from three cereal crops near Santiago, Chile (33.3°S): oat, *A. sativa* L. (free of Hx) and the wheats *T. aestivum* L. cultivar Huayún (Hx concentration $\pm$ SE in 6-day old seedlings, $n = 6$: 0.26 ± 0.08 mmol kg^{-1} fresh mass) and *T. turgidum* L. ssp. *durum* cultivar Llareta (Hx concentration $\pm$ SE in 6-day old seedlings, $n = 6$: 12.85 ± 1.46 mmol kg^{-1} fresh mass). To reduce the chance of collecting aphids from the same parthenogenetic colony, a sampling grid in which collecting spots were separated by at least 20 m was used.

2.2. Maintenance and genotyping

Each sampled aphid was individually placed on a 7-day-old wheat seedling (*Triticum aestivum* cv. Huayún) growing in a plastic pot filled with organic compost and caged within plastic cylinders covered with fine mesh textile. All aphid–wheat systems were maintained at 20 ± 1 °C, LD16: 8, to insurance production of clonal

lineages. Clonal lineages were transplanted every 8 days to new 7-day-old wheat seedlings during 5 generations. Afterwards, clonal lineages were genotyped using five microsatellite loci (*Sm10*, *Sm11*, *Sm17*, *S3.R* and *S5.L*) as previously described Figueroa et al. (2005). For microsatellite primer sequences see Simon et al. (1999) and Wilson et al. (2004). Combining allele size of each locus, eight different multilocus genotypes were found (Table 1), and redundant lines were discarded. For the experiments, the two most abundant genotypes (Sa1 and Sa2) and a relatively less-frequent genotype (Sa46) were used (see Table 1 and Section 1 for details).

2.3. Experimental design

To evaluate the effect of plant chemical defences on allelochemical detoxification, energetic costs and fitness-related traits, three wheat (*T. aestivum*) cultivars with different levels of Hx were used (mean ± SE in 6-day old seedlings, $n = 6$): cultivar Huayún (low Hx: 0.26 ± 0.08 mmol kg^{-1} fresh mass); cultivar Ciko (intermediate Hx: 2.09 ± 0.6 mmol kg^{-1} fresh mass); and cultivar Quelén (high Hx: 5.91 ± 1.18 mmol kg^{-1} fresh mass).

To eliminate environmental maternal and grandmaternal effects that could be carried over from the stock culture, genotypes were maintained in the test hosts for two further generations as follow, and traits were assayed in adult aphids of the third generation. Five wingless female aphids (i.e., parental generation) were transferred to a 7 days-old wheat seedling of each one of the experimental hosts. Parental aphids were removed after 3 days of parthenogentic reproduction and the nymphs produced were left on each seedling. After a further 7 days, first generation adult aphids were split into two groups, each one transferred to new seedlings of the same experimental host. Three days later, parental aphids were removed and nymphs were left on the plants. Splitting of each genotype in two groups allowed evaluation and statistical control of variations within experimental hosts (e.g., intra-host effect) (see Castañeda et al., 2009). Finally, after a further 7 days, adult aphids of second generation were transferred to new seedlings of the same experimental host, allowed to produce nymphs and removed after 3 days. Third generation nymphs were maintained in wheat seedlings until adulthood was reached and traits were assayed.

2.4. Standard metabolic rate measurements

From each wheat seedling, 63 wingless adult female aphids of third generation were randomly separated into seven groups of nine individuals with similar body size (each aphid group was considered as an aphid replicate). Hence, 42 aphid replicates per genotype were used (=3 hosts × 2 groups × 7 replicates). However, final sample size was different for some variables, because some replicates were lost during the maintenance or the measurements.

Each aphid replicate was cooled on ice and weighed on a microbalance (±1 μg; Sartorius, Goettingen, Germany) before measuring their standard metabolic rate (SMR). SMR was measured as the volume of CO_2 produced by each aphid replicate using stop-flow system as described by Castañeda et al. (2009). Briefly, each aphid replicate was placed in a glass syringe, which was filled with CO_2-free air and incubated for 3 h until measurements. The volume of CO_2 produced by each aphid replicate was divided by the incubation time and by the number of aphids contained in each aphid replicate (assuming similar metabolic rates among clonal individuals), to give μl CO_2 per hour per aphid.

2.5. Enzymatic determinations

Immediately after metabolic rate measurement, each replicate was separated into three sets of three individuals, using each set to determine the specific activity of one of the three detoxification enzymes. P450, GST and EST enzyme activities were determined by methods previously described by Castañeda et al. (2009). Fluorescence (P450 and GST) and absorbance (EST) were obtained using a Wallac 1420 Victor[3] microplate reader (PerkinElmer, Waltham, MA, USA). All enzymatic activities were expressed as specific activity (U aphid-equiv.$^{-1}$) and were calculated as the ratio between units of net fluorescence or absorbance (U) and aphid-equivalents (Nauen and Stumpf, 2002). Net fluorescence (for P450 and GST) or absorbance (for EST) corresponded to the difference between sample and blank, and aphid-equivalent is an estimation of the number of aphids present in the supernatant [aphid-equivalent = number of homogenized aphids × (analyzed supernatant volume/total homogenizing buffer volume)]. For all enzymatic determinations, solutions without enzymes (i.e. aphid homogenates) were used as blanks. Determinations of GST and EST activities were performed twice and the average of the two records was used as raw data, whereas determination of P450 was performed only once. To determine the reliability of GST and EST determinations, coefficient of variation (CV = SD/mean) between duplicates was estimated. The average CV for duplicates was 10.2% ($n = 115$) for GST activity, and 30.3% ($n = 115$) for EST activity.

2.6. Intrinsic rate of increase

Since aphids used for enzyme determinations cannot be also used for determining intrinsic rates of increase, a parallel experiment was performed following the experimental procedure described in Section 2.3, except for the follow modifications. Second-generation adult aphids were enclosed in clip cages (i.e. cages were made using a plastic Petri disc (35-mm diameter), a foam ring and a metallic clip), and placed on leaves of wheat seedling. After 1 day, adults were removed from plants, and newborn nymphs were left for 6 days on the same plants. Thereafter, all but one randomly selected nymph were removed. In this experiment each caged nymph was considered as a replicate. Therefore, 48 replicates per genotype were used (=3 hosts × 2 groups × 8 replicates). Each caged nymph was checked every 24-h to record when it produced its first nymph, and thus determine the age of first reproduction (i.e. development time; Td). Newborn nymphs produced by each aphid were counted (Md) and removed daily from the clip cage for a period equal to Td (Karley et al., 2002; Hale et al., 2003; Castañeda et al., 2010). Using these measurements, we estimated the intrinsic rate of increase (r_m) for each aphid following this equation: $r_m = 0.738 \times (\log Md) / Td$, where 0.738 is a correction factor extracted from the regression slope between intrinsic rate obtained from life-table and Wyatt and White's methods (1977). This simplified method of estimating r_m has been shown to behave remarkably close to the classical estimator of Birch (1948), which would require a complete life-table and more time-consuming experiments and calculations.

2.7. Statistical analyses

Normality and homoscedasticity assumptions were fulfilled using $\log_{10}$-transformations, except for r_m because it was normally distributed. In a preliminary analysis, we found that body mass was significantly correlated with SMR ($r = 0.51$, $p < 0.0001$; $n = 115$), and with GST activity ($r = -0.50$, $p < 0.0001$; $n = 115$). Therefore, to control for body mass effect on SMR, we extracted residuals from a linear regression between body mass (predictor variable) and SMR (dependent variable). Same analysis was performed between body mass and GST activity. Then, we added 1 to each residual value to get only positive values as dependent variables. Therefore, mass-residuals SMR and mass-residuals GST

activity were used as mass-controlled variables of SMR and GST activity, respectively.

A nested two-way ANOVA was performed to test difference of aphid genotype and effects of wheat host, interaction between wheat host and aphid genotype, and intra-host variation nested in wheat host on P450 and EST activities, mass-residuals GST activity, adult body mass, mass-residual SMR, and intrinsic rate of increase. Bonferroni tests were performed to evaluate *a posteriori* differences among aphid genotypes when this effect was significant. All statistical analyses were performed using Statistica 6.0® (Statsoft, 2004).

3. Results

Descriptive statistics of P450, GST and EST activities, mass-specific SMR, aphid body mass, and intrinsic rate of increase of aphid genotypes reared on wheat host differing in their Hx levels are shown in Table 2.

Detoxification enzymes did not change significantly among wheat hosts for any aphid genotype. The P450 activity (Fig. 1a) did not show any significant effects due to aphid genotype ($F_{2,104} = 2.31$, $p = 0.10$), wheat host ($F_{2,3} = 0.18$, $p = 0.85$), aphid genotype–wheat host interaction ($F_{4,104} = 2.28$, $p = 0.07$) or intra-host variation ($F_{3,104} = 1.69$, $p = 0.17$). On the other hand, mass-residuals GST and EST exhibited significant difference between aphid genotypes. For mass-residuals GST activity (Fig. 1b), significant differences were found between aphid genotypes ($F_{2,104} = 25.99$, $p < 0.0001$): genotype Sa46 exhibited a lower GST activity than Sa1 and Sa2 (Bonferroni test: $p < 0.001$ for both cases), whereas 'superclones' did not significant differ between them (Bonferroni test: $p = 0.93$). Whereas, mass-residuals GST activity did not exhibit significant effects due to wheat hosts ($F_{2,3} = 1.14$, $p = 0.43$), aphid genotype–wheat host interaction ($F_{4,104} = 1.67$, $p = 0.16$) or intra-host variation ($F_{3,104} = 1.31$, $p = 0.28$). For the case of EST activity (Fig. 1c), it showed significant differences between aphid genotypes ($F_{2,104} = 7.41$, $p = 0.001$): Sa46 displayed higher activity than Sa2 (Bonferroni test: $p = 0.001$). But Sa46 was not significant different to Sa1 (Bonferroni test: $p = 0.07$), and no significant difference were found between 'superclones' (Bonferroni test: $p = 0.66$). On the other hand, EST activity did not

exhibit significant effects due to wheat hosts ($F_{2,3} = 0.59$, $p = 0.61$), aphid genotype–wheat host interaction ($F_{4,104} = 1.78$, $p = 0.14$) or intra-host variation ($F_{3,104} = 0.78$, $p = 0.51$).

Mass-residuals SMR displayed (Fig. 2a) significant differences between aphid genotypes ($F_{2,104} = 4.0$, $p = 0.02$): it was higher for Sa1 than for Sa46 (Bonferroni test: $p = 0.008$), but SMR did not differ significantly between 'superclones' (Bonferroni test: $p = 0.10$) and between genotypes Sa2 and Sa46 (Bonferroni test: $p = 0.99$). Non-significant effects of wheat hosts ($F_{2,3} = 3.0$, $p = 0.19$), aphid genotype–wheat host interaction ($F_{4,104} = 1.5$, $p = 0.21$) and intra-host variation ($F_{3,104} = 0.5$, $p = 0.68$) were found for mass-residuals SMR.

Adult body mass (Fig. 2b) exhibited a significant aphid genotype ($F_{2,104} = 16.47$, $p < 0.001$): genotype Sa46 exhibited a higher body mass than Sa1 and Sa2 (Bonferroni test: $p < 0.001$ for both cases), whereas 'superclones' did not significant differ between them (Bonferroni test: $p = 0.99$). Adult body mass showed significant interaction between aphid genotypes and wheat hosts ($F_{4,104} = 3.91$, $p = 0.005$), which is explained because genotype Sa46 tended to decrease its body mass body towards wheat host with high Hx level (Fig. 2b): Sa46 exhibited higher body mass than 'superclones' in the low Hx level wheat host (Bonferroni test: $p = 0.01$ for Sa1 and $p = 0.002$ for Sa2); whereas in the intermediate Hx level wheat host, genotype Sa46 only showed higher body mass than genotype Sa2 (Bonferroni test: $p = 0.12$ for Sa1 and $p = 0.001$ for Sa2); and in the high Hx level wheat host, genotype Sa46 exhibited similar body mass to that 'superclones' (Bonferroni test: $p = 0.99$ for Sa1 and $p = 0.16$ for Sa2). Whereas 'superclones' displayed a constant body mass across wheat hosts differing in Hx levels (Bonferroni test: $p > 0.1$; Fig. 2a). On the other hand, non-significant wheat host effect ($F_{2,3} = 4.0$, $p = 0.14$) nor intra-host variation ($F_{3,104} = 0.34$, $p = 0.80$) were found for adult body mass.

Significant differences in the intrinsic rate of increase (Fig. 2c) were found between aphid genotypes ($F_{2,124} = 7.71$, $p = 0.001$): genotype Sa1 exhibited a lower r_m than Sa2 (Bonferroni test: $p = 0.0001$) and Sa46 genotypes (Bonferroni test: $p = 0.005$); whereas, genotypes Sa2 and Sa46 were not significant different to each other (Bonferroni test: $p = 0.26$). On the other hand, intrinsic rate of increase did not exhibit significant effects due to wheat hosts ($F_{2,3} = 3.06$, $p = 0.19$), intra-host variation

Table 2
Descriptive statistics of cytochrome P450 monooxygenase activity (P450), glutathione S-transferase activity (GST), esterase activity (EST), mass-specific standard metabolic rate (SMR), aphid body mass, and intrinsic rate of increase of aphid genotypes (Sa1 and Sa2 are 'superclones', and Sa46 is less-frequent genotype) of *Sitobion avenae* reared on wheat hosts differing in their levels of hydroxamic acids (Hx; wheat chemical defences). Values are means ± SE (sample sizes are in parentheses).

| Trait	Genotype	Hx levels in wheat hosts		
		Low	Intermediate	High
P450 activity (U aphid-equiv.$^{-1}$)	Sa1	27.57 ± 4.02 (9)	34.14 ± 4.95 (9)	28.48 ± 4.52 (10)
	Sa2	21.67 ± 3.41 (9)	24.45 ± 2.49 (9)	28.32 ± 2.39 (10)
	Sa46	37.25 ± 4.69 (20)	31.48 ± 3.51 (20)	25.37 ± 1.53 (20)
GST activity (U aphid-equiv.$^{-1}$)	Sa1	3647.7 ± 270.6 (9)	4816.9 ± 698.1 (9)	3234.5 ± 335.7 (10)
	Sa2	3229.4 ± 321.0 (9)	4003.8 ± 283.8 (9)	3421.0 ± 418.4 (10)
	Sa46	1674.5 ± 177.6 (20)	1931.3 ± 152.9 (20)	1670.3 ± 196.1 (20)
EST activity (U aphid-equiv.$^{-1}$)	Sa1	0.2224 ± 0.085 (9)	0.3398 ± 0.113 (9)	0.1631 ± 0.094 (10)
	Sa2	0.2578 ± 0.111 (9)	0.1101 ± 0.057 (9)	0.0861 ± 0.034 (10)
	Sa46	0.2794 ± 0.055 (20)	0.4234 ± 0.070 (20)	0.4356 ± 0.065 (20)
Mass-specific SMR ($\mu l\,CO_2\,h^{-1}\,mg^{-1}$)	Sa1	0.6345 ± 0.041 (9)	0.5844 ± 0.061 (8)	0.5898 ± 0.049 (10)
	Sa2	0.5221 ± 0.028 (9)	0.5775 ± 0.063 (10)	0.5035 ± 0.041 (10)
	Sa46	0.4927 ± 0.013 (20)	0.4767 ± 0.017 (20)	0.4529 ± 0.017 (20)
Aphid body mass (μg)	Sa1	399.88 ± 18.02 (9)	381.63 ± 32.89 (8)	394.83 ± 23.56 (10)
	Sa2	385.31 ± 14.80 (9)	351.96 ± 13.69 (10)	447.88 ± 24.42 (10)
	Sa46	506.81 ± 16.81 (20)	473.14 ± 19.04 (20)	434.13 ± 15.40 (20)
Intrinsic rate of increase (day^{-1})	Sa1	0.2278 ± 0.0094 (10)	0.2488 ± 0.0113 (6)	0.2332 ± 0.0129 (10)
	Sa2	0.2729 ± 0.0058 (15)	0.2948 ± 0.0115 (15)	0.2509 ± 0.0087 (12)
	Sa46	0.2486 ± 0.0058 (21)	0.2699 ± 0.0067 (28)	0.2647 ± 0.0106 (19)
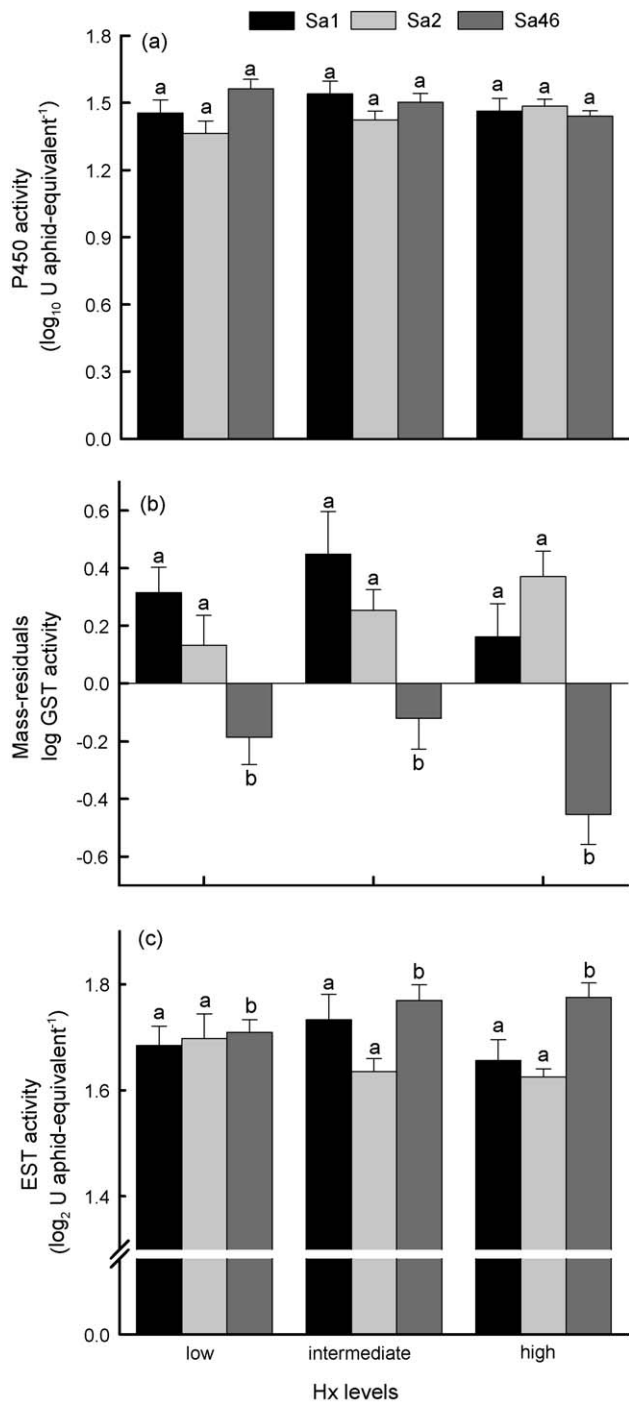
Fig. 1. Mean (±SE) of specific activities of (a) $\log_{10}$ cytochrome P450 monooxygenase (P450), (b) mass-residuals of natural logarithm glutathione S-transferase (GST; it was expressed as U aphid-equiv.$^{-1}$) and (c) $\log_2$ esterases (EST) of aphid genotypes (Sa1 and Sa2 are 'superclones', and Sa46 is less-frequent genotype) of *Sitobion avenae* reared on wheat hosts differing in their levels of hydroxamic acids (Hx; wheat chemical defences). Letters show statistical difference between aphid genotypes at $p < 0.05$ level.

($F_{3,124} = 1.21$, $p = 0.31$) or aphid genotype–wheat host interaction ($F_{4,104} = 1.43$, $p = 0.23$).

4. Discussion

4.1. Hx effects on detoxification systems

Detoxification systems of 'superclones' exhibited non-significant responses to chemical defences present in the tested wheat
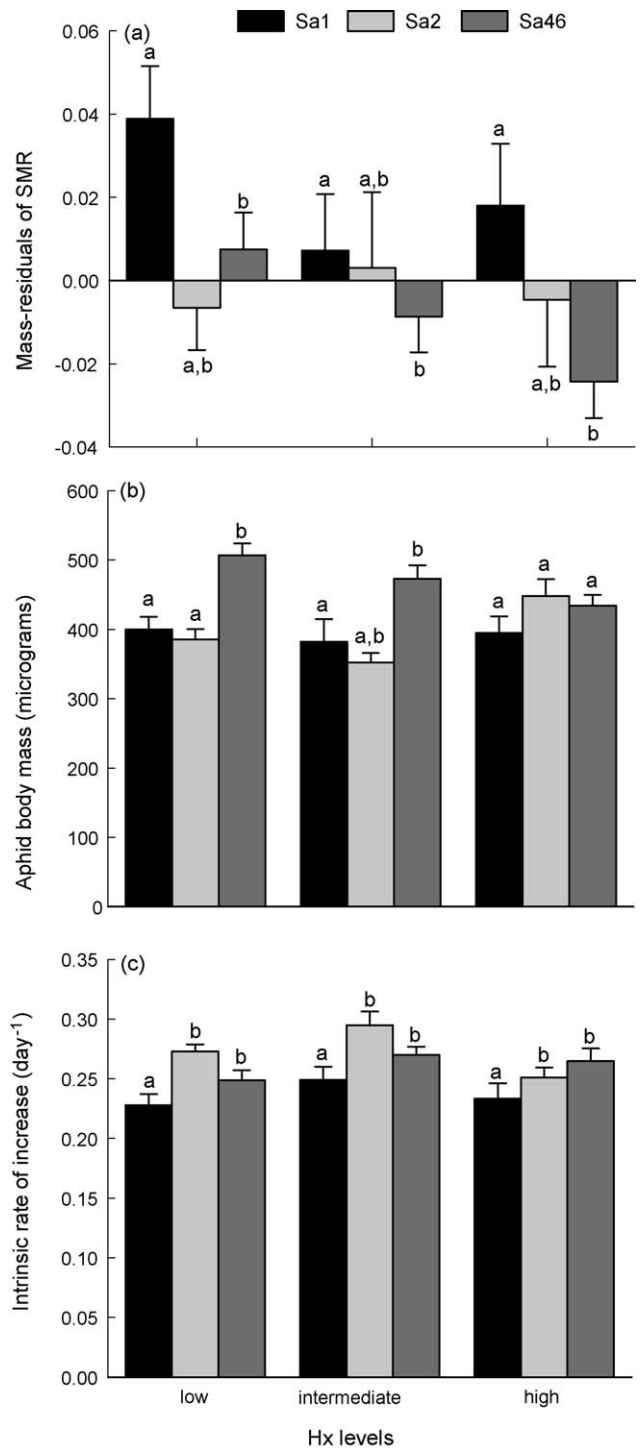

Fig. 2. Mean (±SE) of (a) aphid body mass, (b) mass-residuals of standard metabolic rate [SMR; it was expressed as μl CO_2 h^{-1} aphid^{-1}] and (c) intrinsic rates of increase of aphid genotypes (Sa1 and Sa2 are 'superclones', and Sa46 is less-frequent genotype) of *Sitobion avenae* reared on wheat hosts differing in their levels of hydroxamic acids (Hx; wheat chemical defences). For (a) and (c), letters show statistical difference between aphid genotypes at $p < 0.05$ level. Whereas for (b), letters show statistical difference between aphid genotypes within wheat hosts at $p < 0.05$ level.

hosts, similar to that the rare, less-frequent genotype Sa46. Several studies have reported changes in enzyme activities due to Hx in plant hosts (Leszczynski et al., 1992; Yan et al., 1995; Mukanga-nyama et al., 2003). Nevertheless, our results showed than aphid genotypes exhibited phenotypic constancy in terms of their enzymatic activities with respect to Hx levels in wheat hosts.

The responses of detoxification systems exhibited by 'superclones' may be associated to their efficient capacity to exploit host plants with a broad range of chemical defences levels (Mukanganyama et al., 2003). If so, it could explain why 'superclones' can use hosts lacking Hx (e.g. oat) as well as wheat hosts with Hx levels more than twofold the maximum concentration used in our experimental design (e.g. wheat var. Llareta). For the case of genotype Sa46, a non-induction of detoxification enzymes when it is feeding on defended wheat plants could reduce its detoxifying capacity. Hence, the rigid response exhibited by genotype Sa46 could explain why this genotype was found in the field only in plant without or low Hx levels.

4.2. Costs of 'superclones' detoxification

Metabolic rates have been widely used as a proxy to evaluate energetic costs in insects (Chown and Nicolson, 2004). In fact, metabolic rate is a universal estimation of whole-organism energy expenditure for a wide range of species (Nespolo et al., 2008). In the case of allelochemical detoxification, increase of metabolic rates related to induction of detoxification systems occur when insects are exposed to high levels of allelochemicals (Cresswell et al., 1992; but see Appel and Martin, 1992; Castañeda et al., 2009). In the present study, we found that all aphid genotypes exhibited similar SMR across wheat hosts with different Hx levels. This result indicates that metabolic costs are constant even if aphids are exploiting highly defended wheat plants, which is consistent with constitutive (i.e. non-induced) detoxification capacity across chemically defended wheat plants. A similar result was reported in a multiclonal population of S. avenae, which found low correlations between enzyme activities (P450, GST and EST) and SMR (Castañeda et al., 2009). Furthermore, the absence of an association between metabolic rate and allelochemical levels in host plants possibly excludes the existence of energetic costs in other defensive mechanisms that were not evaluated, such as rapid excretion or sequestration of plant allelochemicals (Schoonhoven et al., 2005; Després et al., 2007).

In the case of adult body mass, we found significant genotype–host interaction, showing that 'superclones' exhibited similar body mass across wheat plants with different Hx levels. Contrastingly, genotype Sa46 tended to decrease its body mass towards highly defended wheat hosts. Although that this response was not significant, it could explain the significant genotype–host interaction. Changes in body mass as well as other growth parameters such as growth rate and conversion efficiency have been used as a proximal effect of plant chemical defences in insects (Appel and Martin, 1992; Berenbaum and Zangerl, 1994; Schoonhoven et al., 2005). Some critics have arisen about the use of gravimetric methods because causes of changes in growth parameters are difficult separate (Van Loon et al., 2005). For instance, reduction of body mass could be linked to different causes such as increase of costs of detoxification systems (i.e. energy trade-off between detoxification and growth) and/or feeding deterrence (i.e. reduced ingested food). However, because we did not find induced metabolic cost of detoxification enzymes, changes in body mass of genotype Sa46 towards highly defended wheat plants may well be due to feeding deterrence effects or reducing efficiency of food utilization mediated by Hx (Niemeyer et al., 1989). Therefore, changes in body mass could be related to the sensibility to toxic and deterrent effects mediated by plant defences, where 'superclones' seem to be more tolerant to Hx than the rare, less-frequent genotype Sa46 (Figueroa et al., 2004).

Our results for intrinsic rate of increase, a proxy of fitness, showed non-plastic responses for 'superclones' in relation to Hx levels in wheat hosts. A similar result was reported in previous study that evaluated the effect of host chemistry on r_m of S. avenae, revealed that genotypes Sa1 and Sa2 exhibited similar r_m values on three hosts with different Hx levels (i.e., lacking-, low- and high-Hx hosts) (Figueroa et al., 2004). Nevertheless, comparisons between cyclical and obligate parthenogens (including aphid 'superclones') of the peach-potato aphid, M. persicae, showed plastic fitness across different hosts (Vorburger et al., 2003b) and temperature-dependent performance for both reproductive modes (Vorburger, 2004). In the current study, the low variance in fitness of 'superclones' under laboratory conditions may allow them to use a wide range of host plants, including defended plants at low energy costs. At the same time, genotype 46 also exhibits non-significant effects on fitness related to host plant chemistry, even when it was reared on the wheat plant with the highest Hx level. However, under field conditions, Sa46 was found only on oat plants (a lack-Hx host), being absent on Hx-defended wheats. This discrepancy between laboratory and field responses may perhaps be due to interaction with other stressful factors such as predators, parasitoids, thermal variability and/or insecticide applications that could constrain the use of highly defended host plants found under field conditions. For instance, strong effects of parasitoids have been reported when aphids are feeding on plants with high Hx levels (Fuentes-Contreras and Niemeyer, 2000; Brewer and Elliott, 2004). This plausible trade-off between detoxification systems and resistance to parasitoids could explain the limited host used observed by Sa46 in the field. An additional explanation for this contrasting result could be related to our collection procedure. In order to collect a larger number of genotypes in the field, aphids were sampled early in the season, when genotypes with low fitness have not yet been culled by selection. This could be the case of genotype Sa46, which under favourable conditions (i.e. the laboratory) would have displayed a higher fitness. In fact, less abundant S. avenae genotypes sampled early in the season are absent in subsequent samplings performed during mid and late seasons (Figueroa et al., 2002).

5. Conclusion

Because of 'superclones' exhibit high abundance and time-persistence in the field, it may be concluded that they represent successful 'solutions' for the invasion and host range expansion of the grain aphid on cultivated and wild Poaceae, especially in regions of intense cultivation (Loxdale et al., 2010). These results partly reveal the origin of the ecological success of 'superclones': broad range of chemically defended host plants, non-induced energetic costs of detoxification systems and low variation in their reproductive performance on hosts with different allelochemical levels. Unfortunately, we did not find clear-cut differences between 'superclone' and the 'non-superclone' genotypes, which would have helped to elucidate the physiological mechanisms behind of 'superclones' success. Among the factors that could have affected the results and hence biased our conclusions, was the use of experimental plants, which could differ in features other than Hx levels. An alternative experimental setting would be the use of artificial diets (which can be made to vary only in Hx concentrations), but cereal aphids exhibit high mortality under these conditions (usually they do not reproduce nor survive) (Caillaud and Rahbe, 1999).

Lastly, the integrative approach (i.e. biochemical, physiological and fitness-related traits) here used hopefully constitutes a starting point for future studies addressing 'superclone' biology. In addition, further complementary studies that include other environmental factors (i.e. temperature, parasitoids, predators, etc.) and more genotypes are needed to discern the nature of the advantage of 'superclones' in nature and indeed determine, as we believe, that 'superclones' are a true biological reality.

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