Chemical defence in a sawfly: genetic components of variation in relevant life-history traits

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Larvae of several tenthredinid sawfly species readily release droplets of haemolymph through their integument when attacked by predators. This defence mechanism via 'bleeding' is characterised by a low integument resistance and a high haemolymph deterrence. Both traits are variable, and negatively correlated among species. We sought to determine if such differences in the propensity to bleed also occur intraspecifically by studying the heritability of traits potentially associated with the bleeding phenomenon in the turnip sawfly *Athalia rosae ruficornis* Jakovlev (Hymenoptera: Tenthredinidae, Allantinae). For three European populations, heritabilities were estimated in the laboratory in a parent–offspring and a full-sib design for *haemolymph deterrence* (measured as concentration of sequestered glucosinolate), *integument resistance*, *body mass* of eonymph and adult, and *developmental time*. Within *A. rosae*, no significant

negative phenotypic correlation was found between the two traits directly related to the defence mechanism: *integument resistance* and *haemolymph deterrence*. However, the significant heritabilities found for these traits in the full-sib analysis (0.39 and 0.35, respectively, for males in the Swiss population) show that the variation has a genetic component. While full-sib analysis revealed highly significant heritabilities for most traits in all the three populations, parent–offspring regression revealed little or no evidence of heritable variation. Effects of common environment for siblings and variation in the host-plant quality between insect generations are likely to be the main factors explaining these differences. A consequence of such host-plant variation in the wild might be that genetic variation of such chemical defensive traits is largely invisible to natural selection.

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Introduction

Although traits related to chemical defence mechanisms of insect herbivores are directly subjected to natural selection, their genetic architecture is poorly understood. A few studies have measured genetic and phenotypic variation of chemical defences, for example, in species of beetles (eg Eggenberger and Rowell-Rahier, 1992; Holloway *et al*, 1993) or butterflies (Camara, 1997). Two arguments might explain the lack of more investigations of this type. Firstly, as the chemistry of the insect is often very closely linked to that of the host plant, chemical variation in the latter might overshadow genetic variation in the former. Secondly, the effort required to measure chemical variation *qua* time and technique is usually higher than for measurements of morphological or developmental traits, thus tending to lead to more limited sample sizes. Nevertheless, the intriguingly high diversity of chemical defence mechanisms utilised in sawflies through self-synthesised or sequestered toxins (Codella and Raffa, 1993) offers an interesting field for investigations. Several species of the tenthredinid sawflies exhibit a so-called 'easy bleeding' defence (Boevé and Schaffner, 2003); when the larvae are touched by, for

example, pointed mandibles of a predator, their integument will disrupt readily at this spot and a droplet of haemolymph is released. This droplet has been shown to have deterrent effects on predators such as birds and ants (Ohara *et al*, 1993; Schaffner *et al*, 1994; Müller *et al*, 2002). The deterrent component is often derived from bioactive host-plant metabolites that are sequestered in the haemolymph of the sawflies (Schaffner *et al*, 1994; Müller *et al*, 2001; Boevé and Schaffner, 2003). Two quantitative traits characterise this bleeding phenomenon, namely the resistance of the integument and the haemolymph deterrence. These traits vary in different tenthredinid species and have been shown to be negatively correlated across 22 species (Boevé and Schaffner, 2003). A less-resistant integument results in a more rapid contact between the exuding haemolymph of the prey and an attacking predator, whereby this haemolymph apparently needs to be more toxic to deter a predator efficiently.

As tenthredinid species differ in their propensity to 'bleed', we sought to determine whether variation in traits associated with this defence mechanism occurs intraspecifically and whether it is heritable. Such quantitative genetic variation could then be the basis for macroevolutionary patterns (Schluter, 1996; Merilä and Björklund, 1999). The Turnip sawfly, *Athalia rosae ruficornis* Jakovlev (Hymenoptera: Tenthredinidae, Allantinae), was chosen for our study. *A. rosae* sequesters glucosinolates of its cruciferous host plants into the haemolymph (Müller *et al*, 2001). A deterrent effect on the ant species *Myrmica rubra* (Hymenoptera:

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Formicidae) is at least partly caused by this plant secondary metabolite (Müller *et al*, 2002). *A. rosae* is a haplodiploid insect and thus, unfertilised eggs develop into haploid males while fertilised eggs produce diploid females. An advantage of haplodiploids for studies of heritability is that there is no effect of dominance in the production of male offspring, and thus only additive genetic variance is estimated in sib analysis of brothers (Liu and Smith, 2000). Parent–offspring and sib analyses were carried out in the laboratory for potential traits related to the defence mechanism, and the phenotypic and genetic correlations between all the traits were examined. Sawflies from three different populations were studied to attempt to obtain a wider range of variation.

Material and methods

Plants

Plants of *Sinapis alba* L. (cultivar: Salvo, seeds from Advanta Seeds B.V., The Netherlands) were grown in a greenhouse (20°C, 70% relative humidity, L16:D8). Preflowering plants at 4–5 weeks were used.

Insects

A. rosae were collected as fertilised adult females while nectaring on Dipsacaceae at Autreppe, France (F). Larvae were collected from mustard fields both at Delémont, Switzerland (CH) and Mühlheim, Germany (D). Adults were provided with honeywater-soaked cottonwool. Larvae were reared on shoots or leaves of *S. alba*. Eonymphs (the final, nonfeeding larval instar) were offered a mixture of sand and soil for pupation.

Offspring of individual females from France were reared as separate families. In the next generation, crosses of females were made to males of unrelated families of the same population to minimise inbreeding. The resulting adults and those that emerged from the larvae collected at Switzerland and Germany were used as the grandparent (GP) generation for the heritability studies in which all the three populations were analysed separately. Insects were reared at 22°C, 70% relative humidity, and L16:D8 in the Leiden laboratory. Plant material for all families of one generation was of the same age, and shoots or leaves were picked at random from plants.

Heritability studies

In the GP generation, one-half of the females was not mated to produce male offspring. The other half were mated individually to males, by placing couples together in Petri dishes for 1 day. Virgin and mated females were allowed to oviposit for 24 h on individual shoots of *S. alba* at 3–4 days after emergence from pupa when the number of mature eggs reaches a maximum in females (Sawa and Oishi, 1989). After 14 days of common development on the shoot, larval offspring (parent-generation *P*) were counted and five to 10 larvae of each family placed individually in Petri dishes lined with moist filter paper. Larvae were provided daily with freshly picked *S. alba* leaves.

Integument resistance was measured (see below) on the second day of the final feeding larval instar (males 4th, females 5th instar). For larvae of the Swiss *P* and male *F1*-generation, haemolymph samples of about 2 µl

were collected from the resulting wound, weighed in Eppendorf caps (1.5 ml) and frozen at –80°C for later analysis of glucosinolate concentration (see below).

When larvae reached their eonymph stage, they were weighed and placed in plastic cups (diameter: 5 cm, height: 6.5 cm) filled to 2 cm height with a mixture of sieved sand and soil in which they formed cocoons to pupate. After 1 week, cocoons were carefully removed and placed in Petri dishes lined with moistened filter paper. Adult mass was measured on the day of emergence. Developmental times both from egg to eonymph and from eonymph to adult were recorded.

P-adults were set up in a half-sib design. Males from a range of families were each mated to two to three individual females from different families. Matings were made assortatively based on high (>3 g) and low (<3 g) integument resistance values to increase the precision of the heritability estimates (Falconer and Mackay, 1996). Mated females, as well as individual virgin females, were allowed to oviposit on individual *S. alba* shoots for 1 day, 3–4 days after emergence. After 14 days, seven to 10 *F1* larvae per family were taken randomly and placed individually into Petri dishes. Quantitative traits were measured sequentially as in the parent generation. As the number of female offspring in the *F1* was very low, an additional generation of mated females from the French and Swiss populations was set up for oviposition, and the same traits measured in the *F2*.

Measurement of integument resistance

An advanced force gauge (Mecmism, AFG 2.5 N) with an iron needle (diameter: 0.2 mm; tip cut straight, but with a fine rounded border) was attached to a micromanipulator. A larva was placed laterally in a curled position under a gauze frame (3.5 cm diameter, mesh width: 1.6 mm). The force gauge was moved perpendicular at 0.1 mm/s towards the larva, until the needle disrupted the integument. The maximum force applied to the larva was recorded from a digital display.

To obtain information about the reliability of the data, four groups of 50 larvae from our stock were measured repeatedly. The first measurement was taken in all the larvae when they had reached the second day of the final feeding instar. A second measurement was taken at the opposite lateral body side of the same individuals either after 0, 3, 6, or 18 h. The maximum force was always measured left to the centre of the larva (in the first measurement the larva's head was placed on the left side, in the second measurement on the right side, or the other way round in half of the larvae measured per treatment).

Analysis of glucosinolate concentration

Haemolymph samples were lyophilised. Glucosinolates were extracted in 70% boiling methanol after adding 20 µl of benzylglucosinolate as internal standard. Glucosinolate analysis was elaborated by conversion to desulfoglucosinolates as described elsewhere (Agerbirk *et al*, 2001). For analysis of desulfoglucosinolates, a high-performance liquid chromatograph coupled to a photodiode array UV detector (Waters 990) was equipped with an ODS 2 column (Alltech: Allsphere, length 150 mm, ID 4.6 mm). Methanol (15%) in water was used as solvent, at a flow rate of 1 ml/min. Peaks were quantified by the

peak area at 226 nm (bandwidth 4 nm) relative to the area of the internal standard peak. The relative response factors reported by McGregor (1985), as cited by Buchner (1987), were used for sinalbin (*p*-hydroxybenzylglucosinolate) (0.50), and benzylglucosinolate (0.86). Identification of glucosinolates in haemolymph samples of *A. rosae* had been confirmed previously (Müller *et al*, 2001). In several samples, trace amounts of two unidentified peaks were found, one possibly 1-methylpropylglucosinolate (Müller *et al*, 2001) and the other with a close retention time and similar spectrum to sinalbin. For both, a response factor of 1 was assumed. Glucosinolate concentration was expressed as total glucosinolate concentration in $\mu\text{mol/g}$ fresh weight, summing the concentrations of sinalbin and the two unknown glucosinolates (concentration of the latter always $<1 \mu\text{mol/g}$ fresh weight).

Allozyme analysis

To compare the populations, male and female adults of the *P*-generation were frozen and analysed for allozyme polymorphism using electrophoresis on cellulose–acetate gels. Staining and buffer recipes were taken from Richardson *et al* (1986) and Hebert and Beaton (1989). Allozyme data were analysed using GDA, version D12 (Lewis and Zaykin, 1999).

Statistical analysis

Repeatability of integument resistance measurements was calculated using one-way analysis of variance (ANOVA) (Lessels and Boag, 1987). Heritabilities (h^2) were estimated using offspring-on-parent regression analysis (Falconer and Mackay, 1996) adapted to haplo-diploid insects by assuming gene dosage compensation in the haploid males (Liu and Smith, 2000). Sexes were analysed in different ways: $h^2 = b \times 2$ (b : regression coefficient) for mother–son regression and $h^2 = b$ for mid-parent–daughter regression. Linear regressions of parents to mean values of offspring were weighted for family size.

Sib analysis was carried out by ANOVA with restricted maximum likelihood estimation and with ‘family’ as a random factor. Heritabilities were estimated for male sibs as $h^2 = 2 \times \text{Var}(\text{family}) / [\text{Var}(\text{family}) + \text{Var}(\text{error})]$ and for female sibs as $h^2 = 4/3 \times \text{Var}(\text{family}) / [\text{Var}(\text{family}) + \text{Var}(\text{error})]$. Several *P* females had probably not mated successfully as they only produced sons. Thus, data did not allow the partition of variance into components due to sire and dam nested within sires for female offspring. Heritability estimates of the trait *integument resistance* were corrected for assortative mating by the formula $h^2 = [-1 + \sqrt{(1 + 4rh_0^2)}] / 2r$ (where r is the correlation between the father and mother value and h_0^2 is the uncorrected h^2 estimate) (Falconer and Mackay, 1996). ANOVA was used to test for effects (phenotypic differences) of population and generation. Data were only pooled when there were no significant effects in the latter analysis.

Phenotypic correlations between different traits were estimated by Pearson product–moment correlation of individual measurements. For genetic correlations, means of offspring per family were weighted and analysed by Pearson product–moment correlation.

Results

Repeatability of integument measurement data

The repeatability of measurements of the integument resistance was 89% after 0 h ($P < 0.001$, $n = 48$), 62% after 3 h ($P = 0.01$, $n = 49$), and 96% after 6 h ($P < 0.001$, $n = 50$). These values indicate that most of the variance was among individuals rather than within individuals, and that, therefore, both measurement accuracy (Becker, 1992) and potential heritability was high. When the second measurement was made on the following day after 18 h, data were not repeatable ($r = -0.11$, $P = 0.12$, $n = 50$) indicating the necessity of careful standardisation of timing of integument measurements with respect to developmental stage.

Heritability estimates – male offspring

Mother–son regressions revealed no statistically significant heritabilities for the traits *glucosinolate concentration*, *integument resistance*, *eonymph mass*, and *adult mass* in the three populations (glucosinolate concentration only tested for the Swiss population) (Table 1). The lack of significance was partly due to high standard errors (eg in two traits of the French population) and partly because the estimates of h^2 were close to zero (eg all traits in the Swiss population, Table 1). The developmental time from egg to eonymph and eonymph to adult was significantly heritable only in the German population. The sole trait with no significant differences in phenotype between populations was *integument resistance*. For all other traits, there were significant effects of populations ($P < 0.01$, ANOVA): for eonymph mass, differences in phenotype were significant between the French and Swiss, and Swiss and German populations ($P < 0.001$, Scheffé-test); for adult mass, differences were significant between the French and Swiss ($P < 0.001$), and French and German populations ($P = 0.04$); for developmental times, differences were significant between the Swiss and German populations ($P = 0.03$), and for time from eonymph to adult additionally between the French and German populations ($P < 0.001$).

In contrast to mother–son regressions, full-sib analysis of sons revealed highly significant heritabilities in all traits and populations, except *body masses* of eonymphs and adults which were only significantly heritable in the Swiss population. Mean heritabilities differed between populations but the ranges were overlapping (Table 1).

Heritability estimates – female offspring

Parent–offspring regressions for female offspring were examined over two generations due to a lack of a sufficient number of female offsprings in one generation. There was a significant effect of generation for the phenotypic values of all traits measured ($P < 0.05$, ANOVA; there were significant differences between *F1* and *F2* for all traits). Furthermore, only three females of the German population produced female offspring and thus, data from this population for this sex could not be analysed.

Mid-parent–daughter regression revealed a statistically significant heritability only for one trait, *integument resistance*, in one generation (*F2*) of the French population (Table 2). On the other hand, once again, full-sib analysis of daughters revealed significant heritabilities for all

Table 1 Narrow-sense heritability estimates for male *A. rosae* of three populations from France (F), Switzerland (CH), and Germany (D), calculated by parent–offspring regression (linear regression) of quantitative traits between mothers and mean value of sons, weighted for number offspring per family, and by full-sib analysis (ANOVA)

Trait	Pop.	Mean $\pm$ SD mothers	Mean $\pm$ SD sons	Ind. (n)	Fam. (n)	$h^2 \pm SE$ parent-offspring	$h^2 \pm SE$ full-sib
Gls. Conc.	CH	19.23 $\pm$ 8.51	13.71 $\pm$ 7.22	186	41	−0.001 $\pm$ 0.16 n.s.	0.35 $\pm$ 0.19***
Integument resistance (g)	F	3.09 $\pm$ 0.77	2.58 $\pm$ 0.45	90	19	0.34 $\pm$ 0.27 n.s.	0.44 $\pm$ 0.29**
	CH	2.92 $\pm$ 0.53	2.61 $\pm$ 0.44	260	41	0.09 $\pm$ 0.26 n.s.	0.39 $\pm$ 0.17***
	D	2.80 $\pm$ 0.88	2.84 $\pm$ 0.50	72	9	0.47 $\pm$ 0.39 n.s.	0.50 $\pm$ 0.52**
	F+CH+D	2.93 $\pm$ 0.66	2.64 $\pm$ 0.46	422	69	0.24 $\pm$ 0.17 n.s.	0.40 $\pm$ 0.14***
Mass eonymph (mg)	F	49.92 $\pm$ 3.70	30.93 $\pm$ 1.50	89	19	−0.08 $\pm$ 0.20 n.s.	0.13 $\pm$ 0.19 n.s.
	CH	49.34 $\pm$ 5.82	28.53 $\pm$ 2.62	256	41	0.03 $\pm$ 0.14 n.s.	0.75 $\pm$ 0.24***
	D	46.61 $\pm$ 3.21	31.71 $\pm$ 1.00	70	9	0.01 $\pm$ 0.24 n.s.	0.11 $\pm$ 0.18 n.s.
Mass adult (mg)	F	22.13 $\pm$ 2.58	11.14 $\pm$ 0.75	88	19	−0.005 $\pm$ 0.14 n.s.	0.22 $\pm$ 0.22 n.s.
	CH	21.80 $\pm$ 2.36	10.18 $\pm$ 1.01	253	41	0.003 $\pm$ 0.14 n.s.	0.69 $\pm$ 0.22***
	D	21.08 $\pm$ 0.85	10.37 $\pm$ 0.85	58	9	−0.14 $\pm$ 0.43 n.s.	0.97 $\pm$ 0.88 n.s.
Dev. egg–eon. (d)	F	18.75 $\pm$ 0.68	17.95 $\pm$ 0.80	89	19	0.34 $\pm$ 0.56 n.s.	1.33 $\pm$ 0.62***
	CH	19.47 $\pm$ 0.79	18.11 $\pm$ 1.53	256	41	0.07 $\pm$ 0.62 n.s.	1.60 $\pm$ 0.40***
	D	18.70 $\pm$ 0.42	17.44 $\pm$ 0.86	70	9	3.97 $\pm$ 0.41***	1.70 $\pm$ 0.96***
Dev. eon.–adult (d)	F	14.15 $\pm$ 0.52	16.35 $\pm$ 0.81	88	19	1.25 $\pm$ 0.70 n.s.	1.20 $\pm$ 0.61***
	CH	15.42 $\pm$ 0.72	15.80 $\pm$ 0.53	246	41	−0.24 $\pm$ 0.23 n.s.	0.66 $\pm$ 0.21***
	D	14.14 $\pm$ 0.35	15.24 $\pm$ 1.68	58	9	7.27 $\pm$ 2.39*	0.90 $\pm$ 0.57***

Significant differences from zero: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s. not significant. Statistically significant heritability estimates are highlighted in bold. ind.=individuals; fam. = families; gls=glucosinolate, conc. = concentration ($\mu\text{mol/g}$ fresh weight); eon. = eonymph; dev. = developmental time. Differences in phenotype between populations were examined by ANOVA. The only trait without a significant population effect is the integument resistance ($P = 0.058$). For all other traits $P < 0.01$.

Table 2 Broad-sense heritability estimates for female *A. rosae* of two populations from France (F), and Switzerland (CH) for two generations (F1, F2), calculated by mid-parent–offspring regression (linear regression) of quantitative traits between mean value of parents and mean value of daughters, weighted for number offspring per family, and by full-sib analysis (analysis of variance)

Trait	Pop./gen.	Mean $\pm$ SD mid-parents	Mean $\pm$ SD daughters	Ind. (n)	Fam. (n)	$h^2 \pm SE$ parent-offspring	$h^2 \pm SE$ full-sib
Integument resistance (g)	F F1	2.66 $\pm$ 0.76	2.63 $\pm$ 0.82	59	12	0.17 $\pm$ 0.27 n.s.	0.43 $\pm$ 0.28**
	F F2	2.70 $\pm$ 1.16	3.12 $\pm$ 0.61	61	12	0.33 $\pm$ 0.07***	0.48 $\pm$ 0.31***
	CH F1	2.81 $\pm$ 0.53	2.44 $\pm$ 0.64	49	10	−0.04 $\pm$ 0.30 n.s.	0.43 $\pm$ 0.41**
	CH F2	2.40 $\pm$ 0.90	3.03 $\pm$ 0.64	81	13	−0.006 $\pm$ 0.07 n.s.	0 n.s.
Mass eonymph (mg)	F F1	40.99 $\pm$ 2.59	51.995 $\pm$ 3.996	59	12	0.47 $\pm$ 0.32 n.s.	0.36 $\pm$ 0.26**
	F F2	40.22 $\pm$ 2.35	51.79 $\pm$ 5.12	61	12	0.07 $\pm$ 0.37 n.s.	0.17 $\pm$ 0.17 n.s.
	CH F1	38.51 $\pm$ 2.34	45.95 $\pm$ 4.72	48	10	0.21 $\pm$ 0.40 n.s.	0.24 $\pm$ 0.30 n.s.
	CH F2	37.43 $\pm$ 3.25	49.67 $\pm$ 6.79	81	13	0.02 $\pm$ 0.26 n.s.	0.04 $\pm$ 0.10 n.s.
Mass adult (mg)	F F1	17.16 $\pm$ 2.17	21.55 $\pm$ 1.58	55	12	0.05 $\pm$ 0.16 n.s.	0.32 $\pm$ 0.27*
	F F2	15.98 $\pm$ 1.25	22.18 $\pm$ 2.35	60	12	0.37 $\pm$ 0.34 n.s.	0.22 $\pm$ 0.20*
	CH F1	16.60 $\pm$ 1.80	19.85 $\pm$ 1.67	45	10	−0.02 $\pm$ 0.16 n.s.	0 n.s.
	CH F2	14.97 $\pm$ 1.02	21.50 $\pm$ 3.03	81	13	0.55 $\pm$ 0.33 n.s.	0.07 $\pm$ 0.12 n.s.
Dev. egg–eon. (d)	F F1	17.75 $\pm$ 0.45	19.05 $\pm$ 1.65	59	12	−0.74 $\pm$ 0.12 n.s.	1.13 $\pm$ 0.55***
	F F2	18.33 $\pm$ 0.91	16.62 $\pm$ 0.93	61	12	−0.14 $\pm$ 0.26 n.s.	0.83 $\pm$ 0.44***
	CH F1	18.65 $\pm$ 0.97	19.25 $\pm$ 1.18	48	10	−0.12 $\pm$ 0.34 n.s.	0.95 $\pm$ 0.57***
	CH F2	18.23 $\pm$ 1.09	16.33 $\pm$ 1.26	81	13	0.11 $\pm$ 0.30 n.s.	1.06 $\pm$ 0.49***
Dev. eon.–adult (d)	F F1	14.00 $\pm$ 0.83	15.25 $\pm$ 0.97	55	12	0.32 $\pm$ 0.31 n.s.	0.89 $\pm$ 0.48***
	F F2	15.58 $\pm$ 0.70	14.77 $\pm$ 1.00	60	12	0.30 $\pm$ 0.37 n.s.	0.66 $\pm$ 0.39***
	CH F1	15.85 $\pm$ 0.47	14.28 $\pm$ 1.03	46	10	−0.28 $\pm$ 0.66 n.s.	0.78 $\pm$ 0.50***
	CH F2	15.19 $\pm$ 0.56	14.58 $\pm$ 0.72	81	13	0.09 $\pm$ 0.27 n.s.	0.56 $\pm$ 0.30***

Significant differences from zero: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s. not significant. Statistically significant heritability estimates are highlighted in bold. ind. = individuals; fam. = families; eon. = eonymph; dev. = developmental time.

Table 3 Phenotypic (P) and genetic (G) correlations (Pearson product-moment correlation) between quantitative traits, measured in males (F2) of the Swiss population of *A. rosae*

Trait	Integument resistance		Glucosinolate conc. (Gc)		Mass eonymph (Me)		Mass adult (Ma)		Dev. Egg-eon. (De)	
	P	G	P	G	P	G	P	G	P	G
Gc	-0.13N.S.	0.02N.S.								
Me	-0.03N.S.	0.01N.S.	0.15*	0.37***						
Ma	-0.04N.S.	-0.09N.S.	0.12N.S.	0.25***	0.71***	0.77***				
De	0.16*	0.37***	0.13N.S.	0.14N.S.	0.01N.S.	0.04N.S.	-0.21**	-0.29***		
Da	-0.06N.S.	-0.13N.S.	-0.16*	-0.31***	-0.04N.S.	-0.17N.S.	-0.20N.S.	-0.07N.S.	0.02N.S.	0.12N.S.

Significant differences from zero: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s. not significant. Statistically significant correlations are highlighted in bold. conc. = concentration; dev. = developmental time; eon. = eonymph; Da: dev. eon.-adult.

traits measured in the French population in both generations except the *eonymph mass* of the F2. In the Swiss population, *integument resistance* was heritable in one generation (F1) and *developmental times* in both generations (Table 2).

Correlations among different traits

Significantly positive phenotypic correlations between the mass of the eonymphs and of the adults were found in all the generations and populations ($r > 0.5$, $P < 0.01$). A detailed overview of the correlations is given for the male offspring of the Swiss population (Table 3 and Figure 1) since this population is also representative of the other two. Glucosinolate concentration showed a negative phenotypic relation with integument resistance although the correlation did not reach significance. Furthermore, this chemical trait was positively correlated with the eonymph mass, but negatively with developmental time from eonymph to adult (Figure 1), although again these correlations were rather low and levels of significance weak. The genetic correlations were of the same sign but stronger than the phenotypic correlations in several cases, for example, those between glucosinolate concentration and either body mass or developmental time (Table 3). However, there was no indication of any genetic correlation between the two defensive traits.

Allozyme analysis

The F_{ST} values for males and females of the investigated populations are low and indicate 'little' genetic differentiation (according to the classification of Hartl and Clark, 1997) (Table 4).

Discussion

This study is the first to examine the quantitative genetics of an insect chemical defence mechanism that involves several traits, namely the concentration of a deterrent compound and an integument adaptation. Although because of the fact that some females produced no or only male offspring, sample sizes are lower than we had planned, some clear results were obtained. Most heritability estimates for both defence and life-history traits in the sawfly *A. rosae* were significant when

analysing full-sib data but not when regressing offspring on parents. Full-sib analysis often overestimates heritability compared to parent-offspring regression, because of both dominance variance and common environments for sibs. However, as highly significant values were also obtained for male full-sibs, where no dominance variance is present, the common environment issue is more important. Such common environment conditions result in a small within-family variation and thus heritabilities for the full-sib-analysis are inflated as within-family variation forms the denominator in the estimates. Detailed explanations for possible causes of the differences between heritability estimates in *A. rosae* are given in the following discussion of the individual traits.

The chemical trait of *glucosinolate concentration* in the haemolymph of *A. rosae* showed significant genetic variation ($h^2 = 0.35 \pm 0.19$) when analysing full-sib data (Table 1). The glucosinolate concentration serves as an indicator for the deterrence of the haemolymph towards predators such as ants (Müller *et al*, 2002). In the leaf beetle *Oreina gloriosa* (Coleoptera: Chrysomelidae), the concentration of defensive compounds secreted by adults was significantly heritable in estimates of full-sib analysis ($h^2 = 0.57$), and of mother-offspring regression ($h^2 = 0.51$) (Eggenberger and Rowell-Rahier, 1992). The defensive secretion of *O. gloriosa* contains a complex mixture of cardenolides and ethanalamines that are not sequestered but synthesised *de novo* (Rowell-Rahier *et al*, 1995) and thus are unlikely to be dependent on host-plant quality. In contrast, for *A. rosae* the host-plant quality is crucial for the concentration of the defence compound in the haemolymph. Analysis of experiments involving switching larvae to host plants with other glucosinolate profiles during the last feeding of larval instar revealed that the glucosinolate composition of the larval haemolymph corresponds to that of the new leaf in a very short time: after 20 h it closely resembles the new host (Müller, unpublished). In the full-sib experiment, we minimised the effects of common environment for families by placing larvae individually at least 2 days before sampling of haemolymph on randomised leaves in Petri dishes. After this time, the glucosinolate concentration of the haemolymph will be more influenced by the quality of that particular leaf than the previous food supply. Furthermore, it might be affected by the ability of the larva to concentrate glucosinolates

Figure 1 Regression of different traits on the glucosinolate concentration (as measurement for deterrence) in males of the Swiss population of *A. rosae*. For *r*- and *P*-values refer to the phenotypic correlations in Table 3.

up to a certain threshold level. Although our *S. alba* plants were all grown from the same seed source and under the same environmental conditions, their glucosinolate content and overall quality may have differed between batches used for subsequent generations, as it does in many Brassicaceae due to seasonal variation and induction events (Rosa, 1997; Agrawal *et al*, 1999; Agerbirk *et al*, 2001). This could explain the observed lack of correlation between haemolymph glucosinolate concentrations for parents and their offspring in *A. rosae*.

For *integument resistance*, the full-sib analysis revealed for male and female siblings in all the three populations

Table 4 F_{ST} -values for *A. rosae* populations from Switzerland (CH), France (F) and Germany (D) obtained by allozyme electrophoresis

Sex	overall	CH/F	CH/D	F/D	n Total
Male	0.03	0.07	0.01	0.05	49
Female	—	0.05	N.D.	N.D.	94

Analysis of nine different loci from the enzymes (malate dehydrogenase, MDH; 1.1.1.37), (malic enzyme, ME; 1.1.1.40), (isocitrate dehydrogenase, IDH; 1.1.1.42), (aspartate aminotransferase, AAT; 2.6.1.1), (β -esterase, β -est.; 3.1.1.1), (glucose-6-phosphate isomerase, GPI; 5.3.1.9), and (phosphoglucosmutase, PGM; 5.4.2.2) (nomenclature following Murphy *et al*, 1996, EC numbers given). Presence of private alleles: two in Swiss, and two in French females. No linkage disequilibrium was detected. N.D., not detected.

moderate heritability estimates of about 0.4 (0.38–0.50). The similarity between female and male heritability estimates suggests that dominance variance is of minor influence on this trait (brother–brother analysis estimates narrow-sense heritability only). This may in turn indicate that selection on this particular trait is low and may be over-ridden by environmental variance (Crnokrak and Roff, 1995). The integument resistance measurements on living larvae are a sum of the integument strength and the hydraulic pressure of the haemolymph. Hydration has a marked effect on the integument properties (Hepburne and Joffe, 1976) which is dependent on environmental conditions such as humidity and host-plant quality. Variation in these factors during the experiment might again account for the absence of significant correlations between measurements from different generations. For the integument resistance, the timing of measurement is critical and closely linked to the moulting cycle of the larvae. Our measurement in the mid-phase of the last feeding larval instar is highly repeatable, while measurements closer to the end of the phase are no longer comparable probably because the new integument of the eonymph is already formed under the larval cuticula. To our knowledge, heritabilities of integument resistance have not been estimated for other insects.

The life-history traits of *body mass* of eonymphs and of adults were only significantly heritable in some populations/generations. Where significant, heritabilities varied between 0.22 ± 0.20 (Table 2) and 0.75 ± 0.24 (Table 1). In other haplodiploid sawflies, heritabilities for body mass estimated by half-sib analysis were comparable [eg 0.38, SE not given, Sequeira and Mackauer, 1992; 0.55 ± 0.15 , Kauser *et al*, 2001]. For *developmental time*, very high values for heritabilities were found for full-sib analysis of all *A. rosae* populations and generations. However, some caution is needed in interpreting our estimates because they have rather high and overlapping standard errors. Furthermore, those for developmental time are likely to be even more sensitive to common environment effects, especially in early development where larvae shared environments. A high variation of estimated heritabilities for morphological and life-history traits is typical for many studies including other sawflies (eg Chenot and Raffa, 1998). Furthermore, for several tree feeding sawfly species, it has been shown that plant chemistry can have a strong effect, particularly on developmental time (Geri *et al*, 1993).

Differences in heritabilities were found between the *A. rosae* populations, most strikingly in the trait *body mass*. Such differences could indicate different histories of selection, for example, due to regional climates and habitat diversity. A high variation in heritability estimates for body size of wild populations along a latitudinal gradient has been demonstrated for *Drosophila* (van 't Land *et al*, 1999). Differences between populations could also be because of different founder effects or bottleneck events resulting in loss of genetic variation or inbreeding depression (eg Saccheri *et al*, 1996). Molecular markers are a practical tool for investigating such effects (eg Brown *et al*, 1997; de Jong *et al*, 2001). Allozyme analyses of *A. rosae* revealed little genetic variation between the three populations (Table 4). Thus, the differences found in heritability estimates might be partly explained by differences in genetic history. However, molecular variability has only a limited ability to predict quantitative genetic variability (Reed and Frankham, 2001), making a different history of selection the most likely reason for the differences between populations.

A high phenotypic and genetic correlation between the body mass of eonymph and adult was consistently found in all the three *A. rosae* populations. However, all the other phenotypic correlations between the quantitative traits were weak (Table 3). Body mass and developmental time of larvae have been shown to be clearly positively correlated in a comparative study on six birch-feeding sawfly species when food was of stable high quality and constraints on long development were relaxed (Kause and Morin, 2001; Kause *et al*, 2001). Seasonal variation of host-plant quality has a major influence on the phenotypic and genotypic architecture of this insect guild (Kause *et al*, 2001). The influence of host-plant quality on the performance of *A. rosae* is the subject of further studies.

Within *A. rosae*, a high concentration of glucosinolates in the haemolymph seems to be linked to a high eonymph weight and a short developmental time from eonymph to the adult stage, as indicated by the respective signs for the phenotypic and genetic correlations between these traits, Table 3, Figure 1). Thus, there is no obvious trade-off involved in sequestration of the chemical defence compound. There was no significant phenotypic correlation between the two defence traits integument resistance and haemolymph deterrence; however, the sign of this correlation was negative (Figure 1). A clearly significant negative correlation between these traits has been demonstrated among tenthredinid species where haemolymph deterrence was measured in bioassays with *M. rubra* (Boevé and Schaffner, 2003). The link between these traits is thought to be functionally, rather than physiologically based, and to have evolved under the selection pressure of predation (Boevé and Schaffner, 2003). The correlation might represent an emergent property, becoming more obvious only when a wide range of differences in the traits are considered, as has been reported recently for egg size in relation to adult body size between and within butterfly species (Fischer *et al*, 2002).

Heritabilities in *A. rosae* both for life-history traits and defence traits of a chemical nature appeared to depend on the level of relatedness used to estimate them, probably because of their high sensitivity to environ-

mental effects. These are likely to arise from the tight coupling of the insect herbivore traits to host-plant quality and chemistry. In the field, sawfly larvae are confronted with an even wider range of plant nutrients and plant secondary compounds than in our laboratory conditions. Our findings imply that genetic variation is unlikely to be visible in the field as it will be readily overshadowed by environmental variation. However, there is clearly some potential for adaptive evolution in *A. rosae*, for example, in response to progressive change in environmental conditions. Such genetic variation within species must have been the basis for evolution of phenotypic differences found across species. The apparent profound effects of a variable host plant on the chemical defence trait may have also led to maintenance of genetic variation in the sawfly populations for traits that are potentially closely related to fitness.

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