

Drivers of host plant shifts in the leaf beetle *Chrysomela lapponica*: natural enemies or competition?

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Abstract. 1. The leaf beetle, *Chrysomela lapponica*, originally uses the salicyl glucosides (SGs) of its host plants to sequester salicylaldehyde, which serves as a defence against generalist enemies but attracts specialist enemies. However, some populations of *C. lapponica* have shifted to SG-poor hosts, and their secretions do not contain salicylaldehyde.

2. It was suggested that beetles shift to SG-poor hosts to escape from specialist enemies. To test this hypothesis, we compared field mortality between two populations of *C. lapponica* that were associated with SG-rich willow, *Salix myrsinifolia* (Kola Peninsula and Finland) and two populations that fed on SG-poor willows, *S. glauca* (Ural) and *S. caprea* (Belarus).

3. Mortality from generalist enemies was significantly higher in Belarus than in three other populations, whereas mortality from specialists did not differ among populations. A specialist predator (syrphid fly larvae, *Parasyrphus nigritarsis*) and specialist parasitoids (phorid flies, *Megaselia* spp.) were attracted to the secretions of larvae reared on both SG-rich and SG-poor hosts.

4. Feeding on leaves of *S. caprea* and *S. myrsinifolia* both previously damaged by leaf puncturing and by the larvae of potentially competing species *Chrysomela vigintipunctata*, decreased the weight and prolonged the development of *C. lapponica*.

5. Thus, populations of *C. lapponica* that have shifted to SG-poor willow species did not obtain enemy-free space because specialist enemies have developed adaptations to herbivores that switched to a novel host plant. We suggest that in some populations host plant shift was favoured by interspecific competition with the early season SG-using specialist, *C. vigintipunctata*.

Key words. Defensive secretions, enemy free space, generalist, host plant specialisation, parasitoid, predator, salicylaldehyde, sequestration, specialist, willows.

Introduction

One of the ecological mechanisms causing the diversification of phytophagous insects is the differentiation of herbivore lineages that attack different hosts (Berlocher & Feder, 2002). This host-associated differentiation starts with the addition of a new species to a host range and may occur with or without abandonment of the original host (Heard *et al.*, 2006). Host plant shifts are quite common in herbivorous insects, although herbivore individuals that shifted to a novel host plant may

have lower fitness compared with individuals feeding on the original host (Berenbaum & Zangerl, 1991; Feder, 1995) as a result of differences in host plant chemistry. Therefore, the shift can occur only when herbivores obtain some benefits from the novel host, such as escape from natural enemies (Price *et al.*, 1980; Jeffries & Lawton, 1984) or competitors (Feder *et al.*, 1995; Despres & Cherif, 2004), which counterbalances the decrease in performance.

Escape from natural enemies is one of the major evolutionary forces leading to specialisation in herbivorous insects (Bernays & Graham, 1988; Jaenike, 1990; Dicke, 2000). Some herbivores obtain protection from natural enemies by sequestration of toxins from their host plants. In particular, a number of leaf beetle species specialise on host plants with a high

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content of phenolic glycosides, and these species sequester the major component of their defensive secretion (salicylaldehyde) from the salicyl glycosides (SGs; i.e. salicin and salicortin) of their host plants (Pasteels *et al.*, 1990). Advantages of this specialisation are clear: escape from competition with generalist herbivores that are not adapted to use SG-rich plants, as well as escape from generalist natural enemies due to low-cost defence by sequestering plant components (Smiley *et al.*, 1985; Rowell-Rahier & Pasteels, 1986; Kearsley & Whitham, 1992).

The ancestral host plants of the leaf beetle *Chrysomela lapponica* L. are Salicaceae rich in SGs (Termonia *et al.*, 2001). Several populations exhibit fidelity to SG-rich willow *Salix myrsinifolia* Salisb. (Zvereva *et al.*, 1995; Gross *et al.*, 2004a). However, some populations have shifted to SG-poor Salicaceae, such as *S. caprea* L. and *S. glauca* L. (Zvereva *et al.*, 2010) or to Betulaceae (Hilker & Schulz, 1994; Gross & Hilker, 1995; Mikhailov, 2001). For example, the Belarus population of *C. lapponica* feeds exclusively on the SG-poor willow species *S. caprea* (Kruglova & Zvereva, 2006), although ancestral SG-rich species *S. myrsinifolia* is abundant in the same locality (Zvereva *et al.*, 2010). However, beetles from this population, as well as from the Ural population (feeding on several SG-poor willow species), perform equally well on SG-rich *S. myrsinifolia* and on novel hosts (SG-poor *S. caprea* or *S. glauca*) when reared in the laboratory (Zvereva *et al.*, 2010). These results allow us to reject the hypothesis that these shifts occurred as a result of better nutritional quality of novel host plants (Berdegue *et al.*, 1996).

The compositions of larval defensive secretions of *C. lapponica* depend on concentrations of SGs in their host plants: SG-specialised populations release mostly host plant-derived salicylaldehyde, whereas populations from hosts totally lacking SGs, or containing only small amounts of them, release mostly autogenous secretion with esters of carboxylic acids acting as the main defensive compounds (Hilker & Schulz, 1994; Schulz *et al.*, 1997; E. L. Zvereva, unpublished). Because some specialist natural enemies use defensive secretions to locate their prey, it has been repeatedly hypothesised that shifting to SG-poor hosts could provide an escape from these specialists (Rank & Smiley, 1994; Köpf *et al.*, 1997; Termonia & Pasteels, 1999; Zvereva & Rank, 2004). However, data supporting this hypothesis are still scarce (but see Gross *et al.*, 2004a).

Escape from competitors can also facilitate host plant shifts in phytophagous insects (Taper, 1990; Feder *et al.*, 1995), among which interspecific competition is frequent and widespread (Denno *et al.*, 1995; Morris *et al.*, 2004). In the Belarus population, *C. lapponica* shows high fidelity to *S. caprea* in the field, although ancestral *S. myrsinifolia* is abundant in the same habitats. As *S. myrsinifolia* in this locality is exploited by several abundant, sometimes eruptive (e.g. *Chrysomela vigintipunctata* Scop.) herbivores (Kruglova & Zvereva, 2006), we hypothesised that specialisation of *C. lapponica* on *S. caprea* evolved because of a selection pressure imposed by interspecific competition.

The aim of the present study was to explore two factors that could drive shifts of *C. lapponica* from ancestral SG-rich to novel SG-poor host plants: escape from specialist natural enemies and escape from competition with other herbivores. To

test the enemy-free space hypothesis (Berdegue *et al.*, 1996), i.e. populations of *C. lapponica* have shifted to novel SG-poor host plants to obtain enemy free space, we compared fitness (in terms of mortality) in the presence of natural enemies (e.g. in the field) in populations of *C. lapponica* that fed on ancestral SG-rich and novel SG-poor hosts. We (i) assessed field mortality of *C. lapponica* caused by different groups of generalist and specialist enemies and (ii) conducted experiments to determine whether natural enemies developed adaptations to host herbivores that have shifted to SG-poor plants. To test the second hypothesis, that the shift to SG-poor host plants could be driven by interspecific competition, we (i) compared the abundances of potential competitors of *C. lapponica*, and foliar damage imposed by these competitors, between SG-rich and SG-poor hosts and (ii) conducted experiments to determine whether host damage by early season herbivores imposed adverse effects on the development of *C. lapponica*.

Materials and methods

Study objects, populations, and host plants

The leaf beetle *C. lapponica* is widely distributed in the Palaearctic region (Koch, 1992; Warchalowski, 1994). Adults hibernate in soil and start feeding and copulating on host plants soon after leaf flush. Females lay batches of 35–40 eggs on host plant leaves. Larvae feed for about 1 month and pupate on the host plants. When disturbed, larvae release droplets of a defensive secretion, the composition of which depends on the host plant chemistry (Hilker & Schulz, 1994; Gross & Hilker, 1995).

The studied populations of *C. lapponica* differ in their primary host plants. In the Kola Peninsula, Russia (near Nikel, 69°24'N, 30°13'E), and in Finnish Lapland (near Nuorgam, 70°05'N, 27°51'E), *C. lapponica* feeds almost exclusively on *Salix myrsinifolia* subsp. *borealis* (Fr.) Hyl., a willow species with high concentrations of SGs. In Polar Ural, Russia (near Vorkuta, 67°03'N, 63°34'E), this beetle is recorded on two willow species, *S. dasyclados* Wimm. and *S. glauca*, which have low concentrations of SGs. Although no willow species with higher SG concentrations grow in the surveyed habitats around Vorkuta, larvae of this population performed equally well on SG-rich and SG-poor host plants in experiments (Zvereva *et al.*, 2010). In Belarus (Berezinsky Reserve, near the village of Domzheritsy, 54°30'N, 28°45'E), *C. lapponica* feeds exclusively on *S. caprea*, a species with no or very low concentrations of SGs, although SG-rich *S. myrsinifolia* was also frequent in this locality. Information on host-plant association in different populations of *C. lapponica* and on SG content in both host plants and co-occurring willow species is summarised in Table 1. For additional information on host-plant association and host chemistry, consult Zvereva *et al.* (2010) and Julkunen-Tiitto (1989), respectively.

Host plant preference tests

Laboratory preference trials with the leaves of five plant individuals of each of two willow species contrasting in SG

Table 1. Willows (*Salix* spp.) that are abundant in four study areas, and their use by *Chrysomela lapponica*.

Locality	Willow species and salicylic glycoside (SG) content in their foliage*	
	Used by leaf beetle in nature	Not used by leaf beetle in nature
Kola	<i>S. myrsinifolia</i> (SG-rich)	<i>S. caprea</i> (SG-poor) <i>S. glauca</i> (SG-poor) <i>S. phylicifolia</i> (SG-poor)
Finland	<i>S. myrsinifolia</i> (SG-rich)	<i>S. caprea</i> (SG-poor) <i>S. glauca</i> (SG-poor) <i>S. phylicifolia</i> (SG-poor)
Belarus	<i>S. caprea</i> (SG-poor)	<i>S. myrsinifolia</i> (SG-rich) <i>S. fragilis</i> (SG-poor) <i>S. aurita</i> (SG-poor) <i>S. cinerea</i> (SG-poor)
Ural	<i>S. dasyclados</i> (SG-poor) <i>S. glauca</i> (SG-poor) <i>S. phylicifolia</i> (SG-poor) <i>S. lanata</i> (SG-poor)	—

*SG-poor: salicortin concentrations are below 10 mg/g dry weight; SG-rich: salicortin concentrations are over 40 mg/g dry weight (chemical data from Julkunen-Tiitto, 1989).

content (*S. myrsinifolia* vs. *S. caprea*) were performed at room temperature (*c.* 22 °C) and natural illumination. The experimental unit consisted of a plastic Petri dish (90 mm in diameter) with moistened filter paper on the bottom. Leaf disks (10 mm in diameter) were taken with a cork borer from the third or fourth leaf of the annual shoot. Leaf disks were symmetrically arranged in a Petri dish so that two disks of each host species (taken from two different individuals) were offered (four disks per dish altogether). One adult beetle was placed in each Petri dish for 24 h. The proportion of the disk area that was consumed by the beetle was visually estimated to the nearest 5% (Zvereva *et al.*, 1995; Zvereva & Kozlov, 1996). Ten naive (newly hatched) beetles were tested from each population. To account for individual learning, we also tested 10 beetles from both the Kola and Belarus populations collected in the field from their preferred host plants (*S. myrsinifolia* in Kola and *S. caprea* in Belarus). The relative (i.e. adjusted for feeding activity) consumptions of two willow species were compared using the Kruskal–Wallis test.

Mortality from natural enemies

Species composition of natural enemies attacking *C. lapponica* is documented for several populations (Zvereva & Kozlov, 2000; Gross *et al.*, 2004a). Mortality caused by different natural enemies was recorded separately for eggs, larvae, and pupae of *C. lapponica* in two sites (3–20 km apart) within each locality to account for within-locality variation. The mortality of adult beetles was not considered because their defensive secretion is always synthesised *de novo* and, therefore, is not influenced by host-plant species (Pasteels *et al.*, 1990).

To assess egg mortality from predators, at the time of mass oviposition in each locality we collected 25–50 unhatched egg batches of *C. lapponica* from the primary host plant and

investigated them under a dissecting microscope. Empty eggs in a batch were attributed to the activity of generalist predatory bugs (Zvereva & Kozlov, 2000). From 2005 to 2007, egg batches of *C. lapponica* were also examined for the presence of eggs and young larvae of a specialist predator, *Parasyrphus nigrirarsis* (Zett.) (Diptera, Syrphidae). Infested batches were presumed dead because one predatory syrphid larva usually completely eliminated all eggs or newly hatched larvae in a batch (Zvereva & Kozlov, 2000).

Larval mortality was assessed by conducting enemy exclusion experiments (as described by Zvereva & Kozlov (2000)). In each site, five individuals of locally used host plants of *C. lapponica* were selected at least 2 m apart from the nearest conspecific neighbour, and all adults and egg batches of *C. lapponica* were removed from these plants. At the time of egg hatching (dates vary with locality), two branches of each plant were each infested with 7 (in Ural) or 15 (other localities) first instar larvae hatched from field-collected batches. One of the infested branches was covered with a mesh bag ('sleeve cage') to exclude vertebrate and invertebrate predators; another (uncaged) branch served as a control. We regularly checked that syrphid larvae did not appear near the experimental larvae, and after 20–25 days (i.e. shortly after pupation), survivors were collected. The difference in survival of caged and uncaged larvae was attributed to the activity of generalist predators, such as bugs, ants, and spiders (for a list, consult Zvereva & Kozlov, 2000).

To assess parasitism rates, in 2006, we collected all prepupae and pupae from 5 to 10 randomly chosen individual plants of natural host species at each of eight study sites (two per locality) at the time of mass pupation, which yielded approximately 80 prepupae plus pupae per locality. Material was kept in the laboratory until adult beetles hatched, and then was sorted to the following five categories: alive (beetle hatched); killed by the tachinid fly *Cleonice nitidiuscula* (Zett.); killed by the phorid fly *Megaselia humeralis* (Zett.) in the Belarus population and *M. opacicornis* Schmitz in three other populations; killed by the calcidoid wasp *Schizonotus sieboldi* (Ratz.); and dead for unknown reasons. The calcidoid wasp *S. sieboldi* is a generalist feeder with respect to SG-specialisation of the host, as it infests a number of leaf beetles, including both species that do and do not sequester SGs (Dowden, 1939; Urban, 1997, 2005). *Cleonice nitidiuscula* was reared from only two SG-using leaf beetle species (Richter & Zvereva, 1996), and therefore, we assumed that it is a specialist parasitoid. Phorid flies *M. opacicornis* and *M. humeralis* are specialist parasitoids of some leaf beetles (Disney *et al.*, 2001; Disney & Zvereva, 2008); they attack prepupae and use the defensive secretion of their hosts as a search cue to locate their prey (Zvereva & Rank, 2004). Parasitism rates by each of the three parasitoid species were calculated for each plant individual, and among-population variation was assessed by one-way ANOVA.

Bioassays with natural enemies

To investigate how responses of natural enemies to secretions of *C. lapponica* differ between SG-specialised (Kola)

and non-SG-specialised (Belarus) populations of *C. lapponica*, a number of tests with a specialist predator (syrphid fly *P. nigritarsis*), generalist predator (wood ants *Formica polyctena* Förster), and specialist parasitoids (phorid flies *Megaselia* spp.) were conducted.

Larvae of syrphid flies from SG-specialised populations use a salicylaldehyde-containing larval defensive secretion to locate their prey; they preferred faecal traces from larvae fed SG-rich host plants when searching for prey in experiments (Rank & Smiley, 1994; Gross *et al.*, 2004a). We conducted experiments with *P. nigritarsis* found on egg batches of *C. lapponica* on *S. caprea* in the Belarus population. Only three larvae were collected in the year of the experiment (2007), and therefore, each larva was used in several tests. Between tests, larvae were allowed to feed on their natural prey (larvae of *C. lapponica* reared on *S. caprea*) for at least 24 h. We followed the methods of Gross *et al.* (2004a) who studied SG-specialised populations (Finland) so that our data could be compared with his results. Trails of faecal suspensions of *C. lapponica* larvae from the Belarus population that were fed either *S. myrsinifolia* or *S. caprea* were painted on filter paper in a Y-shape form. The larvae were placed at the base of the Y, and the trail that was chosen was recorded.

To compare the attractiveness of different defensive secretions to syrphid fly larvae, we used secretions from the Belarus population of *C. lapponica* reared either on *S. myrsinifolia* or on *S. caprea*. Small pieces of filter paper (50 mm²) were used to disturb a last instar larva so that all released secretions were absorbed by these pieces. Two such pieces with secretions of a larva from different host plant species were placed on the round arena (Petri dish 90 mm in diameter), and the syrphid larva was released onto the arena. The behaviour of the larva was observed for 5 min, and the number of visits to each of the pieces of paper accompanied by investigation (i.e. repeated touching by mouthparts) was recorded. The attractiveness of faecal traces, as well as of the defensive secretion of larvae fed on *S. myrsinifolia* and *S. caprea*, were compared using Wilcoxon's signed-ranks test (Sokal & Rohlf, 1995).

Phorid parasitoids also use larval defensive secretions of *C. lapponica* to locate their prey (Zvereva & Rank, 2004). We compared the attractiveness of secretions of larvae reared either on *S. myrsinifolia* or on *S. caprea* for local phorid parasitoids, using sticky traps with cotton balls soaked with either water plus secretions taken from two to three larvae of *C. lapponica* or with water alone (Zvereva & Rank, 2004). Seven bushes of *S. myrsinifolia* in Kola and eight bushes of *S. caprea* in Belarus were selected, and three traps (one control and two with larval secretions of larvae from *S. myrsinifolia* or *S. caprea*) were placed in the crown of each bush about 20 cm apart. Attracted phorids were counted after 3 h of exposure (when most of the secretion had already evaporated). Effects of host species within each of two localities were analysed using one-way ANOVA.

Field experiments with wood ants were conducted in the Kola Peninsula with the Kola population and in Turku (Finland) with the Belarus population. Two nests of *F. polyctena* were chosen in each locality. Larvae from each population were reared on both *S. myrsinifolia* and *S. caprea*. In each

test, one fourth instar larva (about 20–25 mg fresh weight) was placed on the ground at a distance of 3 m from the margin of the nest; 8–12 larvae (half from *S. myrsinifolia* and half from *S. caprea*) were tested near each nest, and each subsequent larva was placed 50 cm from the previous one to avoid the potential influences of chemical signalling from foraging ants that had discovered the previous prey. As a measure of defence efficiency, we used the number of ants that discovered and contacted the larva but did not attack it, i.e. the number of ants that were repelled prior to prey removal. The effect of the prey's host plant on the number of repelled ants was analysed using one-way ANOVA.

Field herbivory on *S. myrsinifolia* and *S. caprea*

The species richness of herbivores damaging *S. myrsinifolia* and *S. caprea* was explored in 2006 and 2007 by carefully surveying 20 equally sized plants of each willow species in each two sites in the Kola and Belarus localities. Total foliar damage was assessed in 10 plants of *S. myrsinifolia* and 10 plants of *S. caprea* as the proportion of damaged leaves in a random sample of 100 leaves per plant individual (Alliende, 1989). Surveys were conducted at the beginning of larval development of *C. lapponica*: early June in Belarus and early July in Kola.

Effect of herbivore damage on plant quality for *C. lapponica*

The experiment examining the effects of early season leaf damage on the performance of *C. lapponica* was initiated on 7 June 2007, in Belarus. At this time, *C. vigintipunctata* (the presumed primary competitor of *C. lapponica*) had almost completed its feeding on *S. myrsinifolia*, whereas larvae of *C. lapponica* just started to hatch from eggs. Five multi-stem clones of both *S. myrsinifolia* and *S. caprea* were chosen; all herbivores naturally occurring on these plants were removed, and stems of the same individual were attributed to different treatments. On *S. myrsinifolia*, both natural damage by *C. vigintipunctata* and artificial damage were applied; on *S. caprea*, only artificial damage was applied because *C. vigintipunctata* did not feed on this species.

To impose natural damage, 10–15 last instar larvae of *C. vigintipunctata* were introduced to the test stem of *S. myrsinifolia* and were enclosed with a mesh bag for 24 h to prevent larvae from dispersing as a result of disturbance from manipulations. The control stem was also covered with a mesh bag for the same period to account for possible changes in leaf quality because of enclosure. On the third stem of *S. myrsinifolia*, all leaves were artificially damaged by puncturing 10 holes (diameter 2.5 mm); the fourth stem was used as a control to artificial damage. On *S. caprea*, only two stems were used: artificial damage and control; 20 holes were applied to each leaf because leaves of *S. caprea* were bigger than leaves of *S. myrsinifolia*. On average, 5–10% of the leaf area was removed in artificial damage treatments; in natural damage treatments, all leaves bearing signs of damage by *C. vigintipunctata* (from 5 to 50% of leaf area removed) were used for the experiment.

The performance test with *C. lapponica* started the day after the first treatment. Seven newly hatched larvae were placed in 50-ml vials with foliage from either damaged or control stems; one vial corresponded to each plant \times treatment combination. Altogether, 20 vials for *S. myrsinifolia* (4 treatments \times 5 plants) and 10 vials of *S. caprea* (2 treatments \times 5 plants) were used. At the end of larval development, the survival (proportion of hatched beetles), duration of development, and fresh weight of newly emerged beetles were estimated. The effects of artificial damage and host plant species on the duration of development and weight of *C. lapponica* were analysed (mixed model ANOVA with plant individual within species as a random variable). The difference between artificial and natural damage of *S. myrsinifolia* was tested in a separate analysis (conducted in a similar way). Beetle survival was analysed using logistic regression and the events/trials syntax (procedure LOGISTIC; SAS Institute, 2009). In this case, trial was the number of larvae placed in each experimental unit and event was the number of emerged adults.

Disks made from damaged leaves (either artificially or by *C. vigintipunctata*) were also offered to naive beetles of *C. lapponica* in laboratory preference trials (for the description of methods, see above).

Results

Host plant preference

Naive beetles from Belarus populations strongly preferred *S. caprea*, whereas naive beetles from three other populations did not show any preference for either willow species (Fig. 1). Field-collected beetles preferred host plant species on which they had preferentially fed in nature: *S. myrsinifolia* in the Kola population and *S. caprea* in the Belarus population (Fig. 1).

Mortality from natural enemies

Egg mortality caused by the generalist sucking predators and total larval mortality in the enemy exclusion experiment were highest in Belarus and similar in the three other populations; mortality from the specialist syrphid predator did not differ among populations (Fig. 2). Parasitism by tachinid flies was higher in the two populations feeding on *S. myrsinifolia* (Kola and Finland) than in the two populations feeding on SG-poor hosts (Belarus and Ural), whereas parasitism by phorid flies was highest in Ural and parasitism by chalcidoid wasps was considerable only in Belarus (Fig. 2).

Behaviour of natural enemies

In dual choice tests, the larvae of syrphid flies from Belarus preferred trails with faeces of *C. lapponica* (fed on their natural host plant, *S. caprea*) to water trails in all nine tests. However, syrphid larvae showed no preference between faeces of *C. lapponica* larvae fed on *S. myrsinifolia* or on *S. caprea*

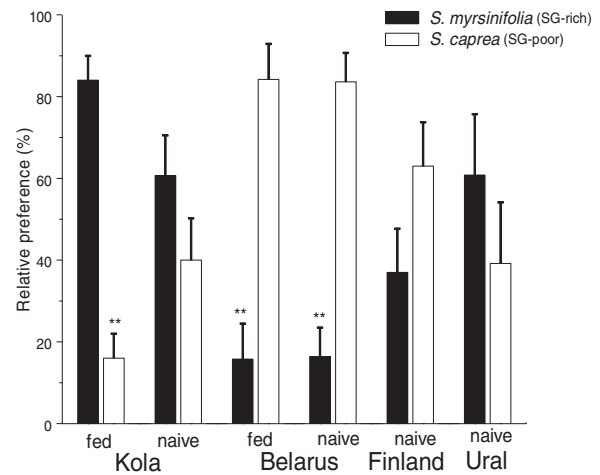


Fig. 1. Relative preference (proportion of leaf area consumed) of *Salix myrsinifolia* and *S. caprea* by adult beetles of *Chrysomela lapponica* from four populations in choice test. Naive – beetles emerged in laboratory, not fed after hatching; fed – beetles collected in the field on their natural host plants (*S. myrsinifolia* for Kola and *S. caprea* for Belarus). Means (\pm SE) are based on 10 beetles; asterisks indicate significant differences between willow species: ** $P < 0.01$ (Kruskal–Wallis test).

(Wilcoxon's signed-ranks test, $W = 3$, $n_{s/r} = 5$, $P > 0.05$) or between pieces of filter paper with defensive secretions of *C. lapponica* larvae reared either on *S. myrsinifolia* or on *S. caprea* ($W = 1$, $n_{s/r} = 5$, $P > 0.05$).

Defensive secretions of larvae fed on *S. myrsinifolia* in the Kola locality were more attractive to phorid parasitoids than secretions of larvae fed on *S. caprea*, whereas in Belarus, the attractiveness of these secretions was similar (Fig. 3a).

Wood ants were significantly more repelled by defensive secretions of larvae fed on *S. myrsinifolia* than on *S. caprea* in tests with both the Kola and Belarus populations (Fig. 3b).

Potential competitors and host plant damage

Species richness of willow feeding herbivores was higher in Belarus than in Kola. In Belarus, *S. caprea* was damaged by a higher number of abundant generalist species [*Gonioctena viminalis* (L.), *Plagioderma versicolora* (Laich.), *Crepidodera aurata* Marsh., *Anisoplia austriaca* Hrbst., *Melolontha melolontha* (L.)] than *S. myrsinifolia* (*G. viminalis* and *C. aurata*). In this locality *C. lapponica* was feeding on *S. caprea* only, whereas three specialists (*Chrysomela populi* L., *C. vigintipunctata*, and *Phratora vitellinae* L.) fed on *S. myrsinifolia*. In Kola, only one species was found on *S. caprea* (generalist leaf beetle *Gonioctena arctica* Mannerheim) and two specialist beetles (*C. lapponica* and *P. vitellinae*) fed on *S. myrsinifolia*.

Proportion of damaged leaves was higher in Belarus than in Kola, and distribution of damage between two willow species differed between populations: the proportion of damaged leaves was higher in *S. caprea* than in *S. myrsinifolia* in Belarus and lower in Kola (Fig. 4).

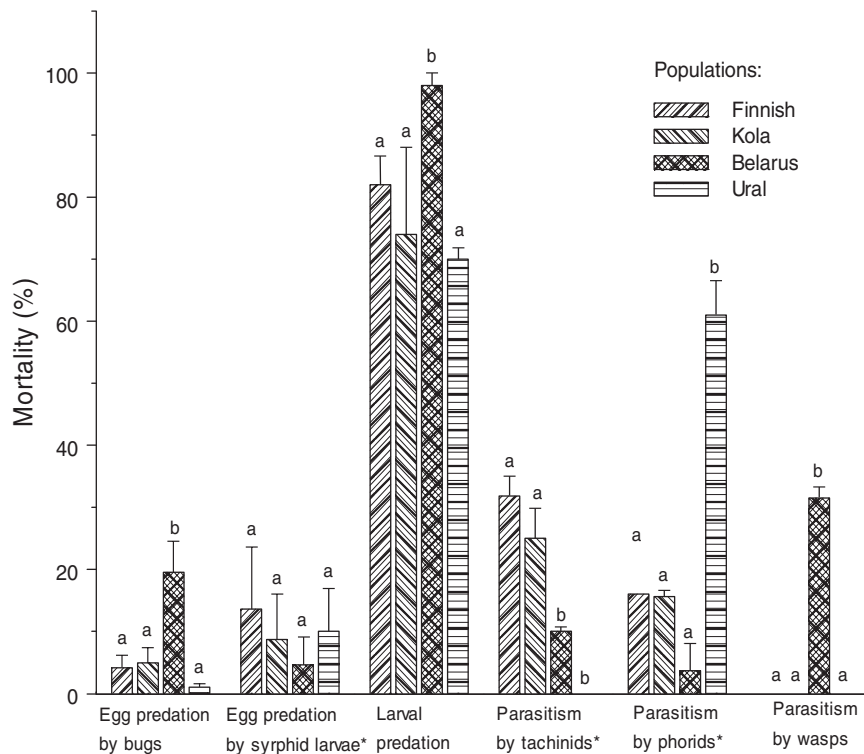


Fig. 2. Mortality of pre-adult stages of *Chrysomela lapponica* caused by different natural enemies in four populations. Means (\pm SE) are based on two sites within each population. Values marked with different letters indicate significant differences between populations (Duncan's test, $P < 0.05$). An asterisk denotes specialist enemies.

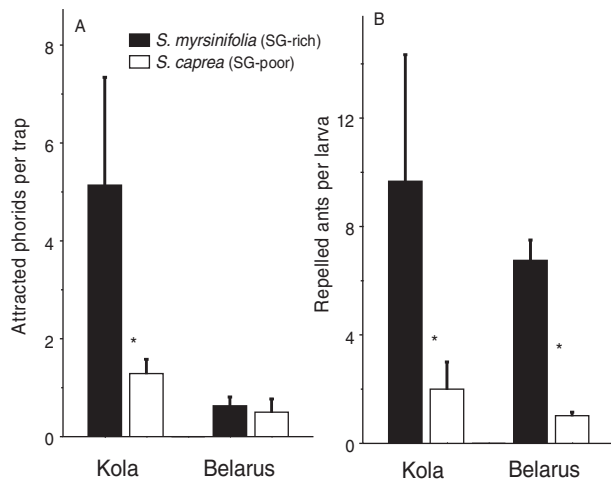


Fig. 3. Behaviour of specialist (phorid fly parasitoids *Megaselia opacicornis* in Kola and *M. humeralis* in Belarus) (a), and generalist (wood ant *Formica polyctena*) (b) enemies in relation to composition of larval defensive secretion (reared either on *Salix myrsinifolia* or on *S. caprea*) in two populations of *Chrysomela lapponica*. Means (\pm SE) are based on seven to eight traps for the number of attracted phorids and on two ant nests (eight to six larvae from each host plant was tested around each nest) for the number of repelled ants. Asterisks indicate significant differences between host plant species (Duncan's test, $P < 0.05$).

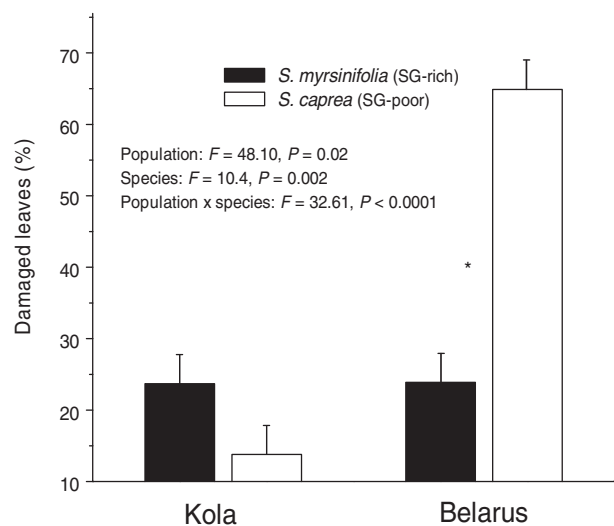


Fig. 4. Effects of willow species (*Salix myrsinifolia* and *S. caprea*) and locality (Kola and Belarus) on the cumulative foliar damage (the proportion of damaged leaves in 100-leaf sample) imposed by chewing herbivores. Least square means (\pm SE) are based on 10 plants. Asterisk indicates significant ($P < 0.0001$) differences between willow species (mixed model ANOVA, Tukey–Kramer test).

Table 2. Effect of artificial damage of two host plant species, *Salix caprea* and *S. myrsinifolia*, on performance of *Chrysomela lapponica* from Belarus.

Character	Variables	d.f.	F/WCH*	P
Duration of development [†]	Damage	1	23.5	<0.0001
	Species	1	32.3	<0.0001
	Damage × species	1	0.3	0.58
Weight of newly emerged beetles	Damage	1	24.3	<0.0001
	Species	1	1.22	0.27
	Damage × species	1	0.33	0.57
Survival [‡]	Damage	1	0.86	0.35
	Species	1	14.84	0.0001
	Damage × species	1	2.95	0.09

*F, Fisher's test, refers to ANOVAs used to analyse beetle weight and duration of development; WCH, Wald Chi-Square, refers to logistic regression model used to analyse survival data.

[†]Duration of development from hatching of larvae to emergence of adults (mixed model ANOVA, with plant individual within species as random variable).

[‡]Survival from hatching of larvae to emergence of adults.

Effect of foliar damage on plant quality for *C. lapponica*

In preference tests, *C. lapponica* beetles avoided artificially damaged leaves of *S. myrsinifolia*, but preferred leaves of this species damaged by *C. vigintipunctata*; on *S. caprea*, the effect of damage on beetle preference was not significant ($P = 0.09$, Fig. 5d).

Leaf puncturing caused a significant decrease in performance of *C. lapponica* larvae on both host plants (Table 2, Fig. 5) in terms of prolonged development and beetle weight at hatching (Fig. 5a,b) but did not affect survival (Fig. 5c). In *S. myrsinifolia*, artificial damage by leaf puncturing and natural damage by *C. vigintipunctata* resulted in similar changes in performance (Tukey–Kramer test: $P = 0.91$ for duration of development and $P = 0.21$ for beetle weight).

Comparisons between performance indices of beetles fed with damaged *S. myrsinifolia* and undamaged *S. caprea* showed inconsistent results. Beetle weight was higher on undamaged *S. caprea* than on damaged *S. myrsinifolia* (Tukey–Kramer test: $P = 0.05$), survival was higher on damaged *S. myrsinifolia* than on undamaged *S. caprea* (Tukey–Kramer test: $P = 0.005$), while duration of development showed no differences (Tukey–Kramer test: $P = 0.88$).

Discussion

Host plant preferences in different populations

Kola and Finland populations of *C. lapponica* were shown to possess local adaptations to their primary SG-rich host, *S. myrsinifolia* (Zvereva *et al.*, 2010), and field-collected beetles from Kola populations preferred this host plant in experiments (Fig. 1). However, naive beetles from Kola and Finland populations, as well as from the Ural population,

did not prefer SG-rich to SG-poor host plants, suggesting within-population variation in host plant use. In contrast, in Belarus not only experienced beetles (both in the field and in the experiment), but also naive beetles clearly preferred the novel host plant, SG-poor *S. caprea*, indicating that host plant selection is genetically fixed. The reduced variation in behavioural traits related to host plant use likely indicates that the Belarus population has been, and still is subjected to strong directional selection for preference of an SG-poor host. This selection maintains a high preference for a novel host in spite of better performance on the ancestral host (Fig. 5a,c) and the ability of larvae to sequester their secretion from SG-rich host plants when forced to feed on them (E. L. Zvereva, unpublished). Absence of correlation between adult preference and larval performance on a novel host plant has been demonstrated in a number of studies (Valladares & Lawton, 1991; Gassman *et al.*, 2006), and our result is consistent with the hypothesis that host shifts are often initiated by evolution of behaviour (Dethier, 1970; Futuyma *et al.*, 1993).

The Ural population of *C. lapponica* did not demonstrate local adaptations to their SG-poor host plants, *S. glauca* and *S. dasyclados* (Zvereva *et al.*, 2010). Although these willows have 5–10 times lower concentrations of SGs than *S. myrsinifolia*, larvae of *C. lapponica* still sequester salicylaldehyde from them (unpublished data). Therefore, feeding on *S. glauca* and *S. dasyclados* is not accompanied by dramatic changes in host-plant mediated interactions of *C. lapponica* with their natural enemies. In this population, as in some other situations (Gassman *et al.*, 2006), feeding on a novel plant seems to be favoured simply by its availability because SG-rich hosts are locally rare.

Thus, only one (Belarus) of two populations of *C. lapponica* feeding on SG-poor host plants was subjected to natural selection that favoured a shift in host plant and the maintenance of specialisation on *S. caprea*. As SG-poor host plants are of lower quality (Zvereva *et al.*, 2010; Fig. 5a,c), factors other than bottom-up effects, e.g. top-down (predation) or horizontal (competition) forces, are likely to favour shifts of *C. lapponica* to SG-poor hosts.

Selection forces: escape from enemies?

In the Belarus population that shifted to SG-poor *S. caprea*, mortality from specialist enemies (either the syrphid predator or phorid parasitoid) was at about the same level as in populations feeding on ancestral SG-rich hosts. This result differs from the results of Gross *et al.* (2004a) who found lower mortality from specialist enemies in a Czech population feeding on birch. Larval defensive secretions in both Belarus and Czech populations lack salicylaldehyde (Hilker & Schulz, 1994; Gross & Hilker, 1995; E. L. Zvereva, unpublished). Therefore, the low pressure of specialist enemies in the Czech population (Gross *et al.*, 2004a) is likely to result from factors other than the composition of secretions. This is confirmed by similarly low (0.6%) rates of parasitism by phorid flies of *C. vigintipunctata* (which, like *C. lapponica*, sequesters salicylaldehyde from SG-rich food plants) in this region (Urban, 1998).

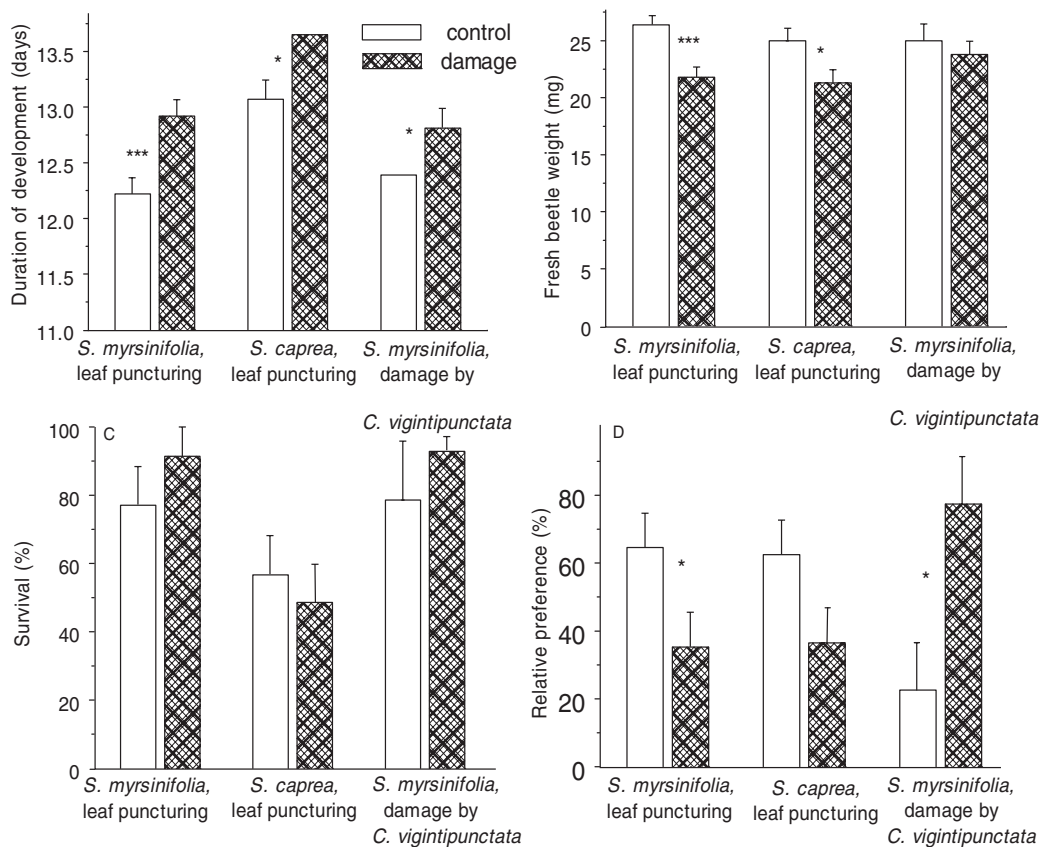


Fig. 5. Effects of artificial damage (leaf puncturing) of *Salix myrsinifolia* and *S. caprea* and natural damage of *S. myrsinifolia* by *Chrysomela vigintipunctata* on duration of development from hatching of larvae to emergence of adults (a), fresh weight of newly emerged beetles (b), survival from hatching of larvae to emergence of adults (c), and relative preference of damaged versus undamaged leaves (d) in *C. lapponica*. Means (\pm SE) are based on five replicates (larval groups) in a, b and c, and on 8–13 beetles in d. Asterisks indicate significant (***)- $P < 0.0001$; *)- $P = 0.05$) differences between treatments (mixed model ANOVA, Tukey–Kramer test for a, b and c; Kruskal–Wallis test for d). For more statistics, consult Table 1.

Our study of the search behaviour of syrphid larvae and phorid flies further supports the view that changes in the chemistry of defensive secretions cannot provide an escape from specialist enemies in populations that have shifted to SG-poor hosts. Syrphid larvae collected from an SG-using Finland population showed a clear preference for faecal trails and defensive secretions of larvae fed with SG-rich willows (Gross *et al.*, 2004a), whereas syrphid larvae collected from the Belarus population feeding on *S. caprea* did not show this preference. Similarly, the phorid fly *M. opacicornis* preferred secretions of *C. lapponica* larvae that fed on *S. myrsinifolia* over secretions of larvae that fed on *S. caprea* in Kola, whereas *M. humeralis* seemed to use both kinds of secretions to locate their prey in Belarus (Fig. 3a). Although results of trials with syrphid larvae should be interpreted cautiously as a result of low number of replicates, still two preference experiments consistently indicate that both predator and parasitoids tested in our study have developed adaptations to locate their prey on a new host.

Previously, it was demonstrated that within the Kola population, *C. lapponica* obtained enemy-free space from specialist

parasitoids when larvae were transferred to SG-poor *S. caprea* in the same locality (Zvereva & Rank, 2003). This may indicate that herbivores can escape from natural enemies on a novel host plant only in the initial stages of host shifting, where the enemy-free space may be a transitional phase as a result of a lag in the evolution of parasitoid searching behaviour (Grosman *et al.*, 2005). Although the reactions of specialist enemies to specific chemical cues may be genetically fixed (Steidle *et al.*, 2003), adaptive changes in search behaviour have been demonstrated to evolve over three generations in a specialist parasitoid, *Diadegma semiclausum* (Rossbach *et al.*, 2006), which developed the ability to locate its host on a novel food plant. These adaptations may be favoured by olfactory learning of chemical cues (Pasteels & Gregoire, 1984; Vet & Groenewold, 1990), as well as by pre-adult learning, e.g. preference for the host herbivore that feeds on the same plant species as the host on which the parasitoids were reared (Barron, 2001; Gandolfi *et al.*, 2003). Importantly, feeding of SG-specialised leaf beetles on SG-poor hosts does not reduce the performance of their specialist natural enemies (Rank & Smiley, 1994; Zvereva & Rank, 2003). Thus, although escape

from specialist enemies could favour the initial stages of the shift of *C. lapponica* to SG-poor hosts, fidelity to novel hosts (reported by Gross *et al.*, 2004b; Zvereva *et al.*, 2010) in populations that have already specialised on SG-poor host plants should be maintained by other factors.

The highest mortality from generalist enemies is found in the Belarus population specialised on an SG-poor host plant (Fig. 2); this may result from both the highest variety and abundance of insects (predators and parasitoids in particular) in southern localities (Hawkins *et al.*, 1997; Schemske *et al.*, 2009) and from a lower efficiency of chemical defence from generalist enemies. The latter suggestion is supported by our experiments with wood ants, which were more repelled by secretions of larvae fed on *S. myrsinifolia* than by secretions from larvae fed on *S. caprea* (Fig. 3b).

To conclude, in spite of high mortality from generalist enemies and the lack of enemy-free space from specialists, the Belarus population of *C. lapponica* exhibited high fidelity to SG-low *S. caprea*, although in the experiments it demonstrated high fitness on the SG-rich ancestral host, *S. myrsinifolia*, growing in the same locality (Fig. 5a,c). This indicates that factors other than natural enemy pressure maintain this specialisation.

Selection forces: escape from competition?

While competition theory historically emphasised direct interactions (i.e. exploitative competition, when one species reduces the quantity of resources available for competitors: Begon *et al.*, 2005), over two-thirds of observations included in a meta-analysis of interspecific competition revealed indirect interactions mediated by plants and/or natural enemies (Kaplan & Denno, 2007). In host-plant mediated competition, even a small level of damage can induce important ecological effects (Kaplan & Denno, 2007). We found negative effects of both artificial damage and damage by *C. vigintipunctata* larvae on the performance of *C. lapponica*. These adverse effects may have negative consequences for populations of *C. lapponica*: prolonged development may result in increased mortality from natural enemies (Haggstrom & Larsson, 1995), while lower weight may be associated with lower fecundity (Crowe, 1995). However, these results do not support the hypothesis that the host plant shift was driven by host-plant mediated competition, because the quality of the undamaged novel host (*S. caprea*) was still not superior to the quality of the damaged ancestral host (*S. myrsinifolia*) (Fig. 5). On the other hand, direct competition could be an evolutionary force behind the observed shift of *C. lapponica* to the novel host, *S. caprea*.

Damage of both *S. myrsinifolia* and *S. caprea* in Northern Europe is generally rather low (Fig. 4, and E. L. Zvereva, pers. obs.), partly explaining why Gross *et al.* (2007) did not detect exploitative competition for SG-rich *S. myrsinifolia* between *C. lapponica* and *P. vitellinae*. In contrast, in Belarus willow-feeding herbivores are more diverse and more abundant, and therefore interspecific competition in this locality may be more important than in Northern Europe. Although *S. caprea* in Belarus is highly damaged by generalist herbivores already at

the beginning of the larval development of *C. lapponica*, this leaf beetle keeps high fidelity to its novel host plant. We suggest that this pattern could have had evolved as an escape from competition with *C. vigintipunctata*, a specialist SG-using leaf beetle (Soetens *et al.*, 1998) which is common in Belarus, and feeds there almost exclusively on *S. myrsinifolia* (Kruglova & Zvereva, 2006). Because interspecific competition is especially strong among closely related species and among specialists (Denno *et al.*, 1995), competition with *C. vigintipunctata* for the ancestral *S. myrsinifolia* can be more important for *C. lapponica* than competition with generalists for *S. caprea*.

Interestingly, in preference tests, *C. lapponica* beetles avoided artificially damaged leaves but preferred naturally damaged leaves to undamaged leaves of *S. myrsinifolia*. This discrepancy is in line with the results of Kendrick and Raffa (2006), who found that leaf wounding by *Chrysomela scripta* Fab. (SG-specialised leaf beetle), but not artificial wounding, enhanced the attractiveness of foliage for this beetle. This attractiveness is governed by volatiles released by both damaged plants, beetles, and beetle frass (Kendrick & Raffa, 2006). Other leaf beetle species may also be attracted to wound-induced foliage (Loughrin *et al.*, 1995; Kalberer *et al.*, 2001). Damage-induced volatiles, both alone and in combination with compounds produced by beetles, may serve as aggregation pheromones that promote the attraction of beetles to plants suitable for feeding and larval development or used in searching for a mate (Kalberer *et al.*, 2001; Tansey *et al.*, 2005). The attraction of *C. lapponica* beetles to leaves damaged by *C. vigintipunctata* may be explained by the close similarity in the relationships of these leaf beetles with host plants, which may result in emissions of similar plant volatiles in response to damage. The attraction of ovipositing females of *C. lapponica* to plants damaged by *C. vigintipunctata*, in combination with the adverse effects of these plants on *C. lapponica* progeny, could enhance the negative effects of competition.

Although in study years we have not observed outbreaks of *C. vigintipunctata*, this modern situation does not necessarily reflect the situation in the past when the shift occurred. Severe outbreaks of *C. vigintipunctata* were recorded in Central Europe, for example, in Germany (1985–1988: Topp *et al.*, 1989), in Czechia (1994–1995: Urban, 1997), and in Slovakia (1994–1995: Kodr k, 1995 and 2002–2004: Kodr k *et al.*, 2006). The densities of *C. vigintipunctata* during these outbreaks were so high that the preferred willows (mostly *S. fragilis*) were already completely defoliated in May for 2 or 3 consecutive years (Topp *et al.*, 1989; Urban, 1997). In this situation, co-occurring herbivores that start development later in the season and have the same feeding preference (*C. lapponica* in particular) are completely lacking their normal host plants. Because *C. lapponica* possess within-population variation in host plant use, and some individuals prefer low-salicylate species and perform well on them (Zvereva *et al.*, 2010), these genotypes are likely to gain selective advantages during outbreaks of *C. vigintipunctata*. SG-poor willows are available for *C. lapponica* even when an outbreak of *C. vigintipunctata* is extremely severe, because SG-poor plants (*S. caprea*, in particular) are damaged by

C. vigintipunctata to a much lesser extent than SG-rich willows (Topp *et al.*, 1989; Urban, 1997). Under strong selection pressure, a shift in feeding preference in the population may happen over a few generations (Messina *et al.*, 2009).

An analysis of the geographical distribution of *C. vigintipunctata* and *C. lapponica* provides indirect support for the hypothesis of strong exploitative competition between these two species. Their ranges overlap in Central Europe, but *C. lapponica* is distributed much further to the north than *C. vigintipunctata* (Koch, 1992; Warchalowski, 1994). In the areas where *C. vigintipunctata* is absent, such as Norway, the Kola Peninsula, Finland, and the Queyras Mountains (France), *C. lapponica* feeds on the ancestral SG-rich hosts (Zvereva *et al.*, 1995; Gross *et al.*, 2004a; Machkour-M'Rabet *et al.*, 2008). On the other hand, *C. lapponica* populations that have shifted to SG-poor hosts are known from Bavaria, Czechia, Poland, Belarus, the Baikal area, and Massive Central in France (Hilker & Schulz, 1994; Warchalowski, 1994; Gross & Hilker, 1995; Machkour-M'Rabet *et al.*, 2008; Zvereva *et al.*, 2010), where distributions of these leaf beetle species overlap.

Thus, competition with *C. vigintipunctata*, which is especially strong during the outbreaks, could have forced *C. lapponica* to use novel SG-poor host plants. On the other hand, escape to a lower quality host may be indicative of the bet hedging strategy – an evolutionary adaptation that facilitates persistence in the face of fluctuating (unpredictable) environmental conditions (Beaumont *et al.*, 2009) caused by periodical defoliation of ancestral host plant during outbreaks of the early season competitor.

Conclusion

Populations of *C. lapponica* that have shifted to an SG-low host plant, *S. caprea*, have a number of disadvantages compared with populations feeding on the ancestral host, *S. myrsinifolia*. These disadvantages include lower larval performance (Fig. 5a,c), high sensitivity to diseases (Gross *et al.*, 2002), high competition with abundant generalist herbivores, and high mortality from generalist enemies (Fig. 2) as a result of low efficiency of defensive secretions (Fig. 3a). Importantly, increased mortality from generalist enemies is not counterbalanced by the expected decrease in mortality from specialists, because specialists rapidly develop adaptations to locate their prey on a novel host plant, in spite of substantial changes in the chemistry of compounds used as search cues. Still, *C. lapponica* has demonstrated shifts to SG-poor hosts which may be partly explained by avoidance of competition with co-occurring congeneric species, SG-using specialist *C. vigintipunctata*, for ancestral SG-rich host plant.

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