Adaptive capacity of Asian populations of Lymantria dispar to non preferred plants during northward expansion

Elena L. Arzhanova
Sirius University of Science and Technology
Sergey V. Pavlushin
Sirius University of Science and Technology
Irina A. Belousova
Sirius University of Science and Technology
Anastasia S. Bastrygina
Novosibirsk State University
Vyacheslav V. Martemyanov
martemyanov79@yahoo.com

Institute of Systematics and Ecology of Animals SB RAS

Article

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Abstract

The spongy moth (*Lymantria dispar*) is considered to be one of the most widespread folivorous insects in the Holarctic, with the capacity to form regular large-scale outbreaks. In the context of global climate change, the geographical distribution of the species is undergoing a shift, which necessitates adaptation to novel host plants, including conifers. The present study analyzed the adaptability of two populations, inhabit the flat region, Novosibirsk population and inhabit the mountain landscape Altai population, to feed on coniferous hosts (pine *Pinus sylvestris* and larch *Larix sibirica*). Silver birch *Betula pendula* were used as the currently preferred host plant. The survival rate of the Novosibirsk population exhibited a decline on pine, while remaining constant on larch. In contrast, the Altai population demonstrated no alteration in survival, neither on larch nor on pine. The findings revealed that both populations exhibited a reduced egg-laying tendency on pine compared to birch and larch, with no discernible differences observed between larch and birch. Furthermore, hatching success was found to be independent of the host plant. Alkaline protease activity increased on larch and pine compared to birch but decreased on pine relative to larch, showing no population-specific dependence. Esterase activity exhibited variation exclusively in males, while malondialdehyde accumulation increased for both populations on larch. The findings demonstrate that the spongy moth exhibits sufficient physiological adaptability to utilize coniferous hosts, thus highlighting its potential to expand into new ecological niches under changing environmental conditions.

Introduction

Climate change has been documented for a considerable period [1,2], with global-average surface temperatures increasing by approximately 0.6°C over the past century [2]. In recent decades, a growing body of research has demonstrated the impact of global warming on insects [3–5]. Bark beetles and moths represent just a partial list of pests that have caused extensive damage to deciduous and coniferous forests across millions of hectares in North America and Fennoscandia over the past 20 years [6–9]. The primary cause of these outbreaks is the positive effect of climate warming on the key stages of insect life cycles, facilitating unprecedented large-scale and hazardous population outbreaks. One of the most severe consequences is the transition of boreal forests to an alternative ecosystem state [10–12]. For instance, there has been a shift to a treeless secondary tundra, as was observed in northern Finland during outbreaks of the autumnal moth in the 1960s [13].

One of the most prevalent phytophagous insects in the Holarctic region is the spongy moth (previously known as the gypsy moth) *Lymantria dispar* [14], which annually forms outbreak areas across hundreds of thousands of hectares [15,16]. Projections derived from CLIMEX modelling suggest the possibility of the spongy moth extending its range by hundreds of kilometers in a scenario of a 2–3°C rise in average annual temperature [17]. Subsequent studies utilizing pheromone monitoring techniques to observe the northern boundary of the Asian *L. dispar* population have corroborated the documented northward shift

in the species' geographical distribution [18,19]. The mean rate of this northward expansion is estimated to be approximately 50 kilometers per year [19].

It is well established that the spongy moth is a broad polyphagous species, with the capacity to damage hundreds of plant species according to various estimates [14]. However, despite its wide polyphagy, populations of the spongy moth exhibit clear preferences for dominant woody plants in different parts of its range. For instance, in Western Siberia, the phytophagous insect primarily damages silver birch (*Betula pendula* Roth.). As the vegetation moves northwards into the light coniferous taiga zone, there is a decrease in the proportion of birch and an increase in the proportion of conifers. Although the boundaries of the taiga will also shift northward over time [20], the rate of these changes in slow-growing trees with multi-year developmental cycles will be significantly lower than that of insects. Additionally, invading populations will face new populations of host plants whose leaf chemistry might depend on the latitude gradient [21]. However, our recent studies demonstrate that most important phytochemical toxicants will not be a serious barrier for northern expansion for *B. pendula* and *Larix sibirica* Ledeb. populations [22]. The adaptation of the spongy moth to feeding on conifers is of particular significance, as coniferous trees generally exhibit reduced tolerance to total defoliation in comparison to deciduous species, resulting in widespread dieback across extensive regions (referred to as "silkworm squares") [23]. Furthermore, the relatively high frequency of spongy moth outbreaks [15,24,25] could potentially result in a catastrophe for boreal forests should this species successfully invade and adapt to new environments.

It has been demonstrated that the spongy moth exhibits significant ecological plasticity, as evidenced by its ability to adapt to North American conifer species [26,27], and certain European conifer species [28], since this species demonstrates significant ecological plasticity. However, recent studies indicate that Asian northern populations respond more favorably to regional thermal conditions compared to North American populations [19]. Specifically, Asian populations exhibit minimal shifts in flight timing, whereas North American populations demonstrate a pronounced delay in flight periods towards autumn as they migrate northwards [19]. These differences may be associated with a lowered temperature threshold for *L. dispar* in Asian populations [29].

The study of enzyme activity involved in food digestion and the detoxification of xenobiotic compounds directly reflects the degree of adaptation to new host plants. Feeding on unsuitable plant species may necessitate the synthesis of higher levels of proteases for efficient protein breakdown [30] or to counteract protease inhibitors [31], albeit at the cost of increased energy expenditure. It has been demonstrated that the protein and carbohydrate content of a diet has a significant impact on the activity of proteases [32]. Furthermore, the intake of alien secondary plant metabolites requires detoxification, which is facilitated by esterases. The equilibrium between antioxidant and pro-oxidant components in the diet has been demonstrated to exert a significant influence on the viability of insects. This, in turn, has been shown to have a direct and indirect impact on their capacity to adapt to a novel host plant.

The objective of the present study was to evaluate the viability indicators of the spongy moth (*Lymantria dispar*) when switching its host plant from birch to coniferous species, specifically siberian larch and scots pine (*Pinus sylvestris* L.), and to assess the persistence of these adaptations in the next generation. Furthermore, given that, certain populations of the spongy moth already include deciduous conifers (e.g., larch) in their diet; two distinct populations were utilized for the investigation. Finally, in order to ascertain the involvement of physiological mechanisms, a comparison was made of the functioning of digestive and detoxification systems in the insect gut when feeding on different plant species.

Materials and methods

Insects

The spongy moth (*Lymantria dispar*) is neither a protected nor a rare species, according to the Red Data Book of Russia and the International Union for Conservation of Nature (IUCN). The European Convention for the Protection of Vertebrate Animals Used for Experimental Purposes (Directive 2010/63/EU) does not regulate the use of insects in research; however, all work was conducted in accordance with internal laboratory standards that aim to minimize animal suffering during experiments. Egg masses were collected in natural conditions during the autumn and stored in the laboratory at 0 to + 4°C. For this study, we selected two populations of the spongy moth. The first population (Novosibirsk “flat” population) was collected in a lowland area near the northern border of the Novosibirsk region (N 55° 40' 52.6584" E 76° 45' 6.0444"), effectively at the northern boundary where the species is capable of forming mass outbreak [15]. If expansion continues, this population will face the necessity of switching its host plant to coniferous species. Its currently preferred host plant is silver birch. The second population (Altai “mountain” population) was collected from mountainous regions of the Altai (N 50°45'00" E 86°09'00"). The Altai population was chosen as a model of a more ecologically plastic population, as outbreaks of the spongy moth in this mountainous area have been observed on both silver birch and siberian larch. Recent population genetic studies have shown that the Altai population forms a distinct genetic cluster [33]. Despite overwintering under different environmental conditions, the temperature regimes during hibernation do not differ significantly. Specifically, Siberian populations overwinter at the base of tree trunks under a layer of snow, while Altai populations overwinter on rocky outcrops [34] and for Altai population adult females obligatorily possess flight ability. In the first case, the population is protected from extreme low temperatures by the insulating snow layer [35], while in the second case, the buffering capacity of rocky outcrops provides protection [34].

Host plants and experimental design

The collection of plant material was conducted in accordance with local legislation pertaining to biodiversity and nature conservation. A survey of the plant species utilized in the study reveals that none of them are classified as endangered or rare according to the Red Data Book of Russian Federation or the IUCN. The species affiliation of plants was determined by Dr. Martemyanov according to

morphological features. Consequently, deciduous forests within the forest-steppe zone are predominantly composed of silver birch, with no overlap observed in the distribution of other species belonging to *Betula* spp. [36]. The species of pine that are characteristic of this zone: *Pinus sibirica* and *Pinus sylvestris*, exhibit striking visual differences in needle length. *Larix sibirica* is the sole larch species in this region. [37]. Silver birch was selected as the control host plant, being the primary host consumed by both the Novosibirsk and Altai populations of the *Lymantria dispar*. Scots pine was used as a model of a potential host plant in the conditions of the West Siberian light-coniferous taiga. Siberian larch was chosen as an "intermediate" option, as it is already known to be damaged by the spongy moth in some regions, including the Altai region. Plant materials were not deposited in a public herbarium, and such deposition was not part of the experimental design

In May, the insects were brought out of diapause. To avoid differences in developmental rates prior to the experiments and to reduce high mortality in the first larval instar, the insects were reared on young birch leaves until they reached the second and third instars. No significant differences were observed in the experimental data between groups, so the groups were combined. In the second instar, the insects were placed into plastic containers (5 liters in volume) with 20 individuals per container. Each population was divided into three feeding groups based on diet: birch, larch, and pine. A total of 340 insects from each population were reared to assess viability until pupation. Parameters such as weight, larval stage duration, survival rate, individual fecundity, and offspring survival were evaluated. Additionally, separate insects from both populations were reared on all tree species to study physiological parameters upon reaching the fourth larval instar.

After pupation, the insects were paired to obtain individual egg clutches. Following summer embryonic maturation, the eggs were overwintered at 0°C and, in the spring of the following year, the second-generation egg masses were brought out of diapause. Individual fecundity (number of eggs per clutch) and hatching success of both generations were recorded. A total of 30 egg clutches from each population were analyzed (10 clutches from each dietary group).

Subsequently, the second generation was reared following the same protocol as the first generation until the third generation was obtained. The viability parameters of the second generation were compared with those of the first generation. Physiological parameters of the second generation were not assessed.

Enzymes activity analysis

To assess physiological parameters, 300 fourth-instar larvae from both populations were selected (100 from each dietary group). As indicators of physiological adaptation to the change in host plant, we measured the activity of digestive enzymes—alkaline proteases, detoxification enzymes – esterases, and lipid peroxidation activity by quantifying malondialdehyde (MDA) accumulation. For the enzyme assays, larval midguts from each group were dissected in ice-cold potassium phosphate buffer (0.2 mol/L, pH 7.8, with 1 mmol/L EDTA) and ground in an ultrasound tissue homogenizer.

Alkaline protease activity was evaluated using a colorimetric method with azocasein as the substrate. The midgut homogenate supernatant was diluted 7-fold with buffer (0.1 M Tris-HCl, 150 mM NaCl, pH 8–9.6), followed by the addition of 140 µl of azocasein solution in NaCl (20 mg/mL). The mixture was incubated for 25 minutes at 28°C, after which the reaction was stopped by adding 40 µl of 30% trichloroacetic acid (TCA). The resulting solution was centrifuged for 10 minutes at 1000g, followed by the addition of 60 µl of 1M NaOH. Absorbance was measured at 440 nm [38].

The esterase activity in the midgut was measured using p-nitrophenyl acetate as a substrate. The reaction mixture (1 ml) consisted of 10 µl of supernatant of midgut homogenate and 200 µl of 0,01 M p-nitrophenyl acetate in 200 mM phosphate buffer (pH 7.2) [39]. The esterase activity was measured as the difference in absorbance at 410 nm after 30 min of incubation with the substrate at 28°C.

Malondialdehyde (MDA) accumulation was also assessed using a colorimetric method with thiobarbituric acid (TBA) as the substrate. To 90 µl of midgut homogenate supernatant, 65 µl of 20% TCA was added, and the mixture was centrifuged for 2 minutes at 20,000g. From the supernatant, 120 µl was collected, and 80 µl of TBA was added. The resulting solution was incubated for 20 minutes at 98°C, and absorbance was measured at 532 nm [40].

Statistical analysis

The data are presented as mean ± standard error (Mean ± SE) for normally distributed samples (number of eggs laid, pupal mass, and alkaline protease activity) and as box plots for samples not described by a normal distribution (larval stage duration, esterase activity, and MDA accumulation), except for survival rate and hatching percentage, which are presented as Mean ± SE. Statistical analysis of the obtained data was performed using Statistica 12.0 (StatSoft, USA) and Past 4.10 [41] software. The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to assess the normality of the distribution of quantitative data. For analyzing non-normally distributed samples, the Kruskal-Wallis test was applied, followed by Dunn's test for post-hoc analysis. For normally distributed samples, Factorial ANOVA was used, with Fisher's test for post-hoc analysis.

Results

Fitness traits of studied populations

Survival and fecundity assessments were conducted on insects from two generations. The survival rate of the first generation of the Altai population on pine was 76.7%, which did not differ from survival on birch and larch (Fig. 1A). However, for the Novosibirsk population, the survival rate of first generation on pine was 60.8%, which was lower than the survival of both populations on birch and larch (Kruskal-Wallis test $H = 12.6$, $p < 0.05$, post-hoc Dunn's test, $p < 0.05$) (Fig. 1A). The survival rate of insects from both populations did not differ when fed on larch compared to birch (Fig. 1A). This trend persisted in the second generation: survival of the Altai population on pine remained unchanged, while survival of the

Novosibirsk population was lower compared to feeding on pine and larch (Kruskal-Wallis test $H = 15.76$, $p < 0.01$, post-hoc Dunn's test, $p < 0.05$) (Fig. 1B).

The number of eggs laid by the first generation decreased on pine compared to birch and larch for both the Altai (Two-way ANOVA host tree effect $F = 48.48$ $p < 0.001$, population effect $F = 42.22$ $p < 0.001$, post-hoc Fisher's LSD test, $p < 0.001$) and Novosibirsk populations (Two-way ANOVA host tree effect $F = 48.48$ $p < 0.001$, population effect $F = 42.22$ $p < 0.001$, post-hoc Fisher's LSD test, $p < 0.001$) (Fig. 1C). However, the number of eggs laid on larch did not differ from that on birch for either population (Fig. 1C). Additionally, the Altai population laid more eggs than the Novosibirsk population across all three host plants (Two-way ANOVA host tree effect $F = 48.48$ $p < 0.001$, population effect $F = 42.22$ $p < 0.001$, post-hoc Fisher's LSD test, $p < 0.01$) (Fig. 1C). In the second generation, the Novosibirsk population laid fewer eggs on pine compared to birch and larch (Two-way ANOVA host tree effect $F = 15.33$ $p < 0.001$, population effect $F = 6.20$ $p < 0.05$, factors interaction $F = 16.20$ $p < 0.001$, post-hoc Fisher's LSD test, $p < 0.01$), but egg counts on larch and birch did not differ (Fig. 1D). For the second-generation Altai population, the number of eggs laid did not differ between pine and birch, but increased in larch compared to birch (Two-way ANOVA host tree effect $F = 15.33$ $p < 0.001$, population effect $F = 6.20$ $p < 0.05$, factors interaction $F = 16.20$ $p < 0.001$, post-hoc Fisher's LSD test, $p < 0.001$) (Fig. 1D).

Hatching success, defined as the percentage of insects hatching from laid eggs, showed no differences between populations or host plants in either the first or the second generation (Fig. 1E, Fig. 1F).

Further studies were conducted on first-generation insects. Feeding on pine by the Altai population resulted in reduced pupal mass for both sexes (Factorial ANOVA host tree effect $F = 415.00$ $p < 0.001$, population effect $F = 508.26$ $p < 0.001$, sex effect $F = 1913.61$ $p < 0.001$, post-hoc Fisher's LSD test, $p < 0.01$) compared to feeding on birch and larch (Fig. 2A) and increased larval stage duration for both sexes (Kruskal-Wallis test $H = 287.81$ $p < 0.001$, post-hoc Dunn's test, $p < 0.01$ for males and Kruskal-Wallis test $H = 287.81$ $p < 0.001$, post-hoc Dunn's test, $p < 0.05$ for females) (Fig. 2B). A similar effect was observed for the Novosibirsk population, feeding on pine reduced pupal mass for both males (Factorial ANOVA host tree effect $F = 415.00$ $p < 0.001$, population effect $F = 508.26$ $p < 0.001$, sex effect $F = 1913.61$ $p < 0.001$, post-hoc Fisher's LSD test, $p < 0.01$) and females (Factorial ANOVA host tree effect $F = 415.00$ $p < 0.001$, population effect $F = 508.26$ $p < 0.001$, sex effect $F = 1913.61$ $p < 0.001$, post-hoc Fisher's LSD test, $p < 0.01$) compared to feeding on birch and larch (Fig. 2A), and increased larval stage duration for both sexes (Kruskal-Wallis test $H = 287.81$ $p < 0.001$, post-hoc Dunn's test, $p < 0.01$) (Fig. 2B).

Feeding on larch by Altai population did not reduce pupal mass for both sexes compared to feeding on birch (Fig. 2A). However, for the Novosibirsk population, female pupal mass decreased when fed on larch compared to birch (Factorial ANOVA host tree effect $F = 415.00$ $p < 0.001$, population effect $F = 508.26$ $p < 0.001$, sex effect $F = 1913.61$ $p < 0.001$, post-hoc Fisher's LSD test, $p < 0.01$), while male pupal mass remained unchanged (Fig. 2A). Additionally, the larval stage duration for both sexes in the Novosibirsk population was longer compared to the Altai population when fed on larch (Kruskal-Wallis test $H = 287.81$ $p < 0.001$, post-hoc Dunn's test, $p < 0.01$ for males and Kruskal-Wallis test $H = 287.81$ $p < 0.001$,

post-hoc Dunn's test, $p < 0.05$ for females) (Fig. 2B). However, the larval stage duration for both sexes in the Altai and Novosibirsk populations did not differ when fed on larch compared to birch (Fig. 2B).

Physiological traits

The activity of alkaline proteases depends exclusively on the host plant rather than population and increases when insects feed on pine compared to birch (Factorial ANOVA host tree effect $F = 44.07$ $p < 0.001$, post-hoc Fisher's LSD test, $p < 0.001$), but decreases compared to feeding on larch (Factorial ANOVA host tree effect $F = 44.07$ $p < 0.001$, post-hoc Fisher's LSD test, $p < 0.01$) (Fig. 3A). When fed on larch, alkaline protease activity increases compared to feeding on birch (Factorial ANOVA host tree effect $F = 44.07$ $p < 0.001$, post-hoc Fisher's LSD test, $p < 0.001$) (Fig. 3A).

Esterase activity in the Altai population does not change when fed on pine compared to birch for both males and females. However, esterase activity increases in males (Kruskal-Wallis test $H = 25.00$ $p < 0.01$, post-hoc Dunn's test, $p < 0.001$) but does not change in females when fed on pine compared to larch (Fig. 3B). Esterase activity in the Altai population does not change when fed on larch compared to birch for both sexes (Fig. 3B). In the Novosibirsk population, esterase activity does not change when fed on pine compared to birch or larch for both sexes, and similarly, no changes are observed when fed on larch compared to birch for both sexes (Fig. 3B). Esterase activity does not differ between populations when fed on birch or larch; however, feeding on pine leads to increased esterase activity in males of the Altai population compared to males of the Novosibirsk population (Kruskal-Wallis test $H = 25.00$ $p < 0.01$, post-hoc Dunn's test, $p < 0.001$), while no differences are observed between females of the two populations (Fig. 3B).

MDA accumulation in the Altai population does not change when fed on pine compared to birch but decreases compared to feeding on larch for both sexes (Kruskal-Wallis test $H = 51.08$ $p < 0.001$, post-hoc Dunn's test, $p < 0.01$ for males and Kruskal-Wallis test $H = 51.08$ $p < 0.001$, post-hoc Dunn's test, $p < 0.05$ for females) (Fig. 3C). Additionally, MDA levels are higher in the Altai population when fed on larch compared to birch for both sexes (Kruskal-Wallis test $H = 51.08$ $p < 0.001$, post-hoc Dunn's test, $p < 0.01$ for males and Kruskal-Wallis test $H = 51.08$ $p < 0.001$, post-hoc Dunn's test, $p < 0.05$ for females) (Fig. 3C). In the Novosibirsk population, MDA accumulation does not change when fed on pine compared to birch but decreases compared to feeding on larch for both sexes (Kruskal-Wallis test $H = 51.08$ $p < 0.001$, post-hoc Dunn's test, $p < 0.001$ for males and Kruskal-Wallis test $H = 51.08$ $p < 0.001$, post-hoc Dunn's test, $p < 0.05$ for females). Additionally, MDA levels are higher when fed on larch compared to birch in males (Kruskal-Wallis test $H = 51.08$ $p < 0.001$, post-hoc Dunn's test, $p < 0.001$) (Fig. 3C). No differences in MDA accumulation are observed between populations on any host plant.

Discussion

The expansion of the spongy moth (*Lymantria dispar*) and the potential shift in host plants have become subjects of extensive research. In particular, the ability of European and North American populations to feed on conifers or other plant species containing specific secondary metabolites has been previously

investigated [26]. It has been demonstrated that certain secondary metabolites belonging to the class of monoterpenes are toxic to lepidopteran larvae. These metabolites have been shown to reduce feeding efficiency and to slow the growth and development of the larvae [42,43]. Moreover, the presence of mono- and diterpenes in larch has been demonstrated to reduce the palatability of food, consequently leading to a decrease in weight gain in spongy moth larvae [44]. When larvae are fed on novel or unsuitable host plants, their digestive enzyme profiles change; depending on the profile of plant secondary metabolites, the activity of both digestive and detoxification enzymes may be altered [45].

According to observations [46], it takes approximately 40 generations (i.e., 40 years, as *L. dispar* is strictly monovoltine) for European subspecies of spongy moth to adapt to a new host plant that was previously outside its natural diet. Larch is regarded as a suboptimal host for the spongy moth, as the synthesis of digestive and detoxification enzymes demands additional energy, which would otherwise be directed towards biomass accumulation [47,48]. The nutritional efficiency of spongy moth larvae feeding on larch is not significantly different from that of larvae feeding on pine, but it is more than two times lower compared to feeding on birch [48]. Nevertheless, no significant differences in fitness have been observed among populations, despite the Altai population utilising larch as frequently as birch.

The survival rates of insects from both populations feeding on larch did not differ significantly from those feeding on birch. Furthermore, no difference in survival was observed among the initially more plastic Altai population on pine, a trend that persisted into the second generation. This finding indicates that feeding on non-preferred host plants does not result in long-term negative consequences for insect survival. The number of eggs laid decreased in both populations when fed on pine, but remained unchanged on larch compared to birch, confirming a higher degree of adaptation to the transitional host plant. Furthermore, the Altai population, which exhibited a greater degree of ecological plasticity, produced a greater number of eggs in the first generation overall. This phenomenon may be interpreted as an adaptive strategy designed to ensure the population's persistence under suboptimal conditions. However, in the second generation, evidence of specialization emerged. The Altai population exhibited a higher fecundity on larch, while the Novosibirsk population demonstrated a higher fecundity on birch. However, variations in egg count across different host plants did not impact hatching success.

Feeding on pine resulted in a reduction in pupal mass and an extension of the larval developmental period in both populations. Feeding on larch was found to have a significant impact on the female pupal mass in the Novosibirsk population, with no such effect observed on the male pupal mass in either of the other populations. Furthermore, the female pupal mass in the Altai population remained unaltered in response to the ingestion of larch. *L. dispar* having aphagous adults has strong positive correlation between female pupae weight and fecundity [49]; however, in our study, the number of eggs laid did not differ between larch and birch.

The present study investigated the physiological adaptations in the digestive system associated with shift of consumed host plant. The study was conducted with a focus on sex differences, given the mounting evidence of sex-specific physiological responses that extend beyond the reproductive system

[50–53]. It is well established that nutrition influences both the overall activity and the specificity of proteases [54]. Plants have evolved a defence mechanism against herbivorous insects in the form of protease inhibitors [55], to which insects can adapt by overproducing proteases [56–58] or synthesizing protease isoforms that are insensitive to these inhibitors [59,60]. Although feeding on pine is not typical for either studied populations, and larch is not common for the Novosibirsk population, both populations were able to survive and develop, indicating sufficient adaptation to the protease inhibitors present in these plants.

Feeding on non-preferred host plants has been demonstrated to result in a reduction of larval weight gain, attributable to diminished digestibility and nutrient availability [61]. Consequently, an augmented production of proteases may act as a compensatory mechanism [30]. In the present study, we observed a decline in mass gain in the Novosibirsk population of females; however, changes in protease activity were evident in both populations and were solely influenced by the host plant. The reduced bioavailability of proteins may be attributable to their conjugation with plant secondary metabolites. However, even feeding on evolutionarily unfamiliar plant species containing such metabolites does not always lead to mortality in *Lymantria dispar* [62], underlining the high degree of polyphagy and adaptability of the species.

The necessity of detoxifying plant secondary metabolites when feeding on novel host plants leads to alterations in esterase activity. Enhanced expression of esterases may contribute to more effective detoxification of secondary metabolites [63]; although, esterase overexpression is not always energetically favorable. However, the presence of larch in the natural diet of the Altai population may explain the higher esterase expression observed during feeding on pine, whereas for the Novosibirsk population, this response appears to be energetically costly. In the present study, increasing of esterase activity were exclusively detected in male subjects. Sex-specific responses to xenobiotics have been documented in various insect species [64,65]. For instance, certain xenobiotics have been observed to increase esterase gene expression in male *Drosophila*, while decreasing it in females [66]. The elevated esterase activity observed in the Altai population may facilitate more efficient detoxification of secondary metabolites present in pine, such as sesquiterpenes, α-pinene, limonene, and others [67], although further investigation is required to confirm this hypothesis.

Oxidative stress, defined as an imbalance favouring oxidants over antioxidants, disrupts redox signalling and is marked by oxidative damage to proteins, lipids, and cellular functions, often assessed through malondialdehyde (MDA), a product of lipid peroxidation [68]. MDA, formed during the oxidation of polyunsaturated fatty acids in cell membranes, is an indicator of uncontrolled lipid peroxidation, which in turn compromises membrane integrity [69] and forming mutagenic adducts with proteins and DNA [70], as evidenced by its accumulation in insects exposed to toxins or adverse conditions [71–74]. The presence of antioxidants in the diet has been demonstrated to reduce lipid peroxidation and MDA accumulation [75]. Compounds like betulin, quercetin, monoterpenes, and polyphenols from plants exhibit protective antioxidant activity [67,76–79]. However, high concentrations of unsaturated fatty acids in larch needles

from Altai and Novosibirsk [22] may serve as substrates for lipid peroxidation, potentially increasing MDA accumulation and intestinal oxidative stress. Furthermore, it has been demonstrated that plant defence compounds have the capacity to disrupt cell membranes and promote inflammation, thereby further exacerbating lipid peroxidation [80].

Conclusions

The findings of the present study demonstrated that, irrespective of population origin, the spongy moth (*Lymantria dispar*) has the capacity to feed and produce viable offspring on non-preferred coniferous species, such as pine. This capacity is saved for at least two generations of herbivores. This finding is consistent with previous studies showing that the adaptability of the spongy moth to consume North American species of coniferous hosts depends more on population-specific traits than on subspecies classification [26]. However, the presence of "transitional" or conditionally favourable coniferous species, such as larch, in the insect's diet enhances the ability of populations to establish themselves on new, previously non-preferred coniferous hosts. This, in combination with higher genetic diversity of Altai population (Martemyanov et al., unpublished data) to compare with flat populations [15], enables the Altai population to make such transitions more successfully.

However, a number of detrimental effects are associated with the adoption of non-preferred host plants, thereby creating uncertainty regarding the capacity of stable populations capable of forming outbreak-level infestations to become established in northern regions. Nevertheless, the development of novel methodologies for the control of spongy moth population dynamics appears to be a promising research direction, given the species' potential to expand into new geographic areas.

Declarations

Additional information

The authors declare that they have no conflict of interest

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Author Contribution

Conceptualization - M.V.V., B.I.A. and P.S.V.; formal analysis - A.E.L. and P.S.V.; investigation - B.A.S., P.S.V. and B.I.A.; writing – original draft - A.E.L. and P.S.V.; writing – review & editing - A.E.L. and M.V.V.

Data Availability

The data used and analyzed during the current study are available from the corresponding author upon request.

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Figures

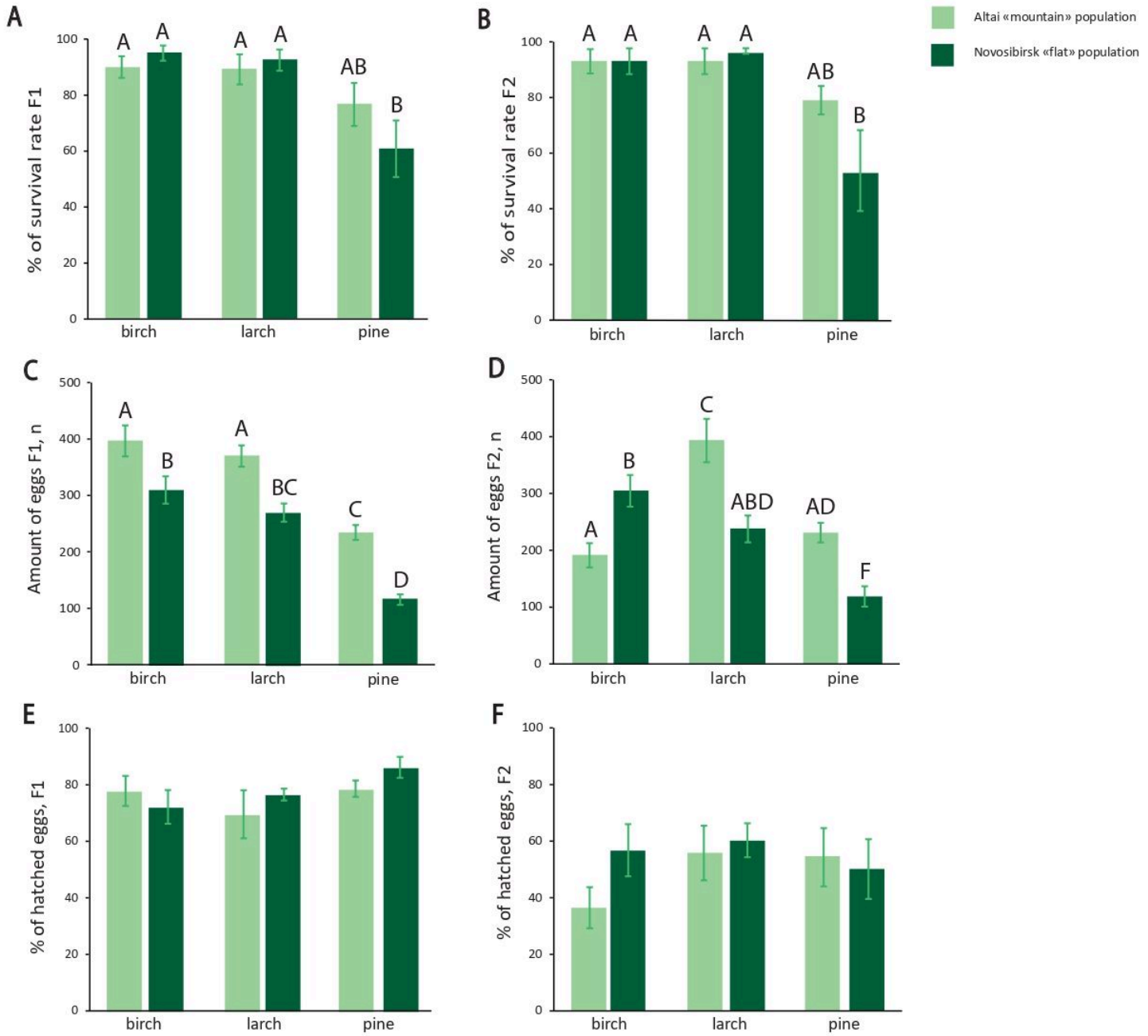

Figure 1

Population associated characteristics of adaptation to host plant changes in two generations of *Lymantria dispar*. **A.** Survival rate of first-generation insects from two populations when fed on three host plants. **B.**Survival rate of second-generation insects from two populations when fed on three host plants. **C.** Number of eggs laid by first-generation insects from two populations when fed on three host plants. **D.** Number of eggs laid by second-generation insects from two populations when fed on three host plants. **E.** Hatching success of insects derived from eggs of the first generation of two populations when fed on three host plants. **F.** Hatching success of insects derived from eggs of the second generation of two populations when fed on three host plants. Different letters indicate statistically significant differences; same letters indicate absence of statistically significant differences between the groups.

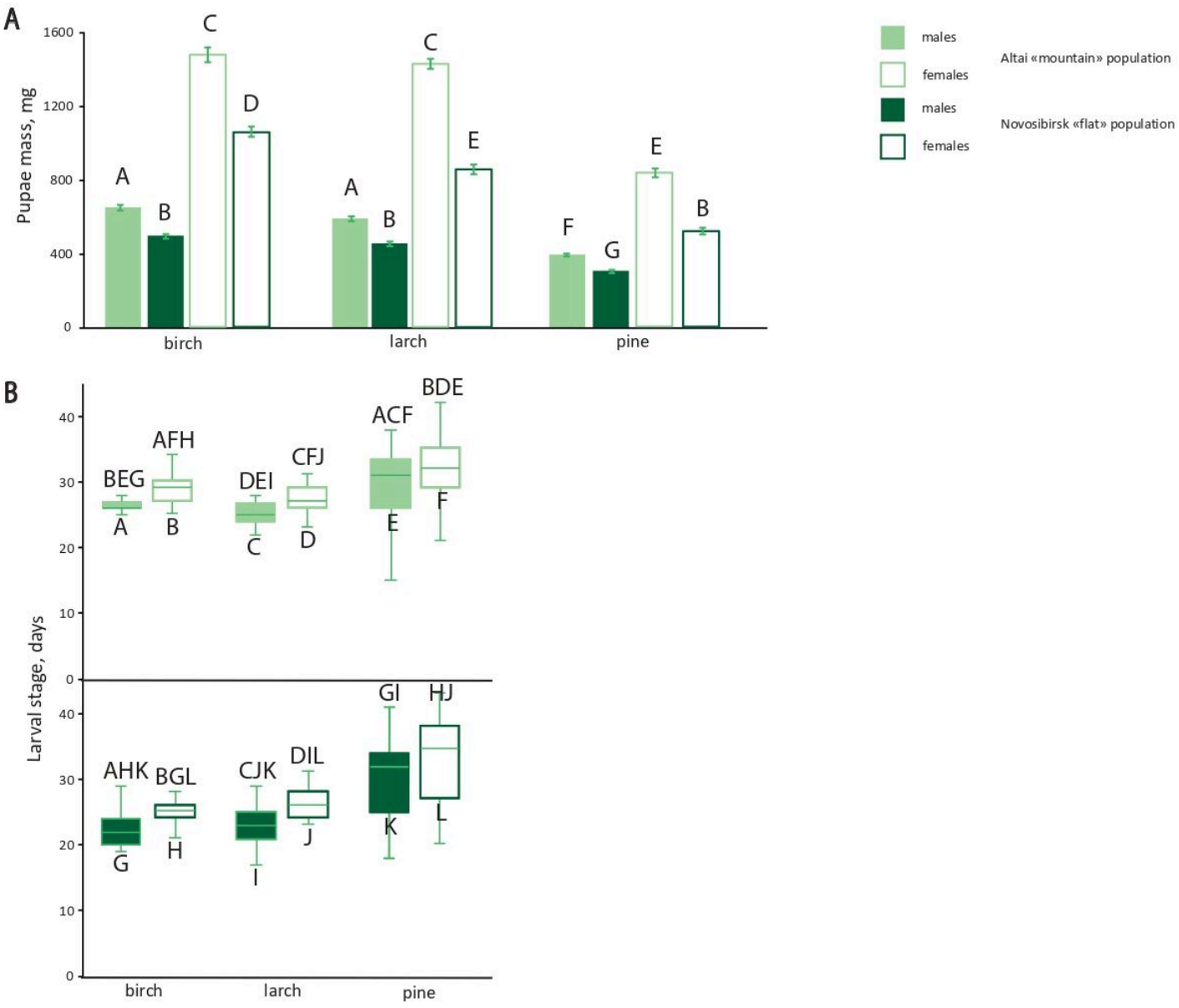

Figure 2

Life history traits of *Lymantria dispar* reared on different host plants. **A.** Pupal weight of insects after feeding on three host plants. Different letters indicate statistically significant differences. **B.**Larval stage duration of insects when fed on three host plants. Letters below the box plot are individual group identifiers, while letters above the box plot indicate groups that are statistically significantly different from this group.

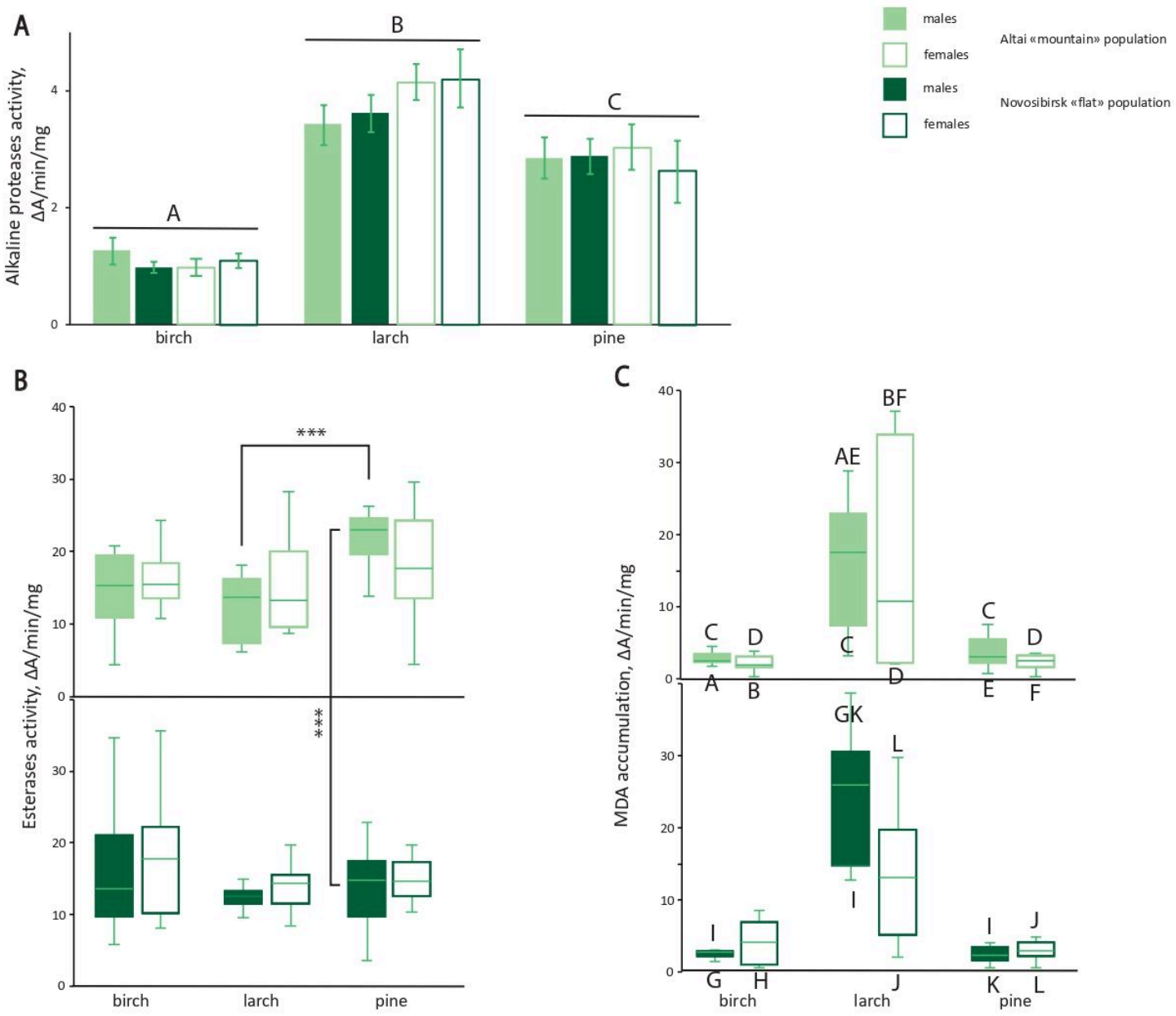

Figure 3

Physiological characteristics of *Lymantria dispar* larvae adaptation to host plant changes. **A.** Alkaline protease activity in the midgut when fed on different host plants. Different letters indicate statistically significant differences. **B.**Esterase activity in the midgut when fed on different host plants. *** $p < 0.001$. **C.** Malondialdehyde (MDA) accumulation in the midgut when fed on different plants. Letters below the box plot serve as individual group identifiers, while letters above the box plot indicate groups that are statistically significantly different from this group.