

Predator protection versus rapid growth in a montane leaf beetle

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Summary. Adults and larvae of *Chrysomela aenicollis* (Coleoptera: Chrysomelidae) feed on foliage of *Salix* species (Salicaceae) between 2,400–3,400 m above sea level in the east-central Sierra Nevada mountains of California. We predicted that (1) cold climatic conditions would be a more frequent source of mortality at higher elevations, (2) mild-weather agents of mortality such as predation should be more severe at lower elevations, and (3) populations of *C. aenicollis* would be adapted to the local selective regime at each elevation. We tested these predictions in 1984 and 1985 by transferring over 6,000 eggs and larvae within and between two sites at 2,810 and 3,240 m elevation above sea level. During mild summer weather at both sites, survivorship on *Salix* branches isolated by a barrier of sticky resin was similar to that on control branches, and we concluded that aerial predators were the primary cause of mortality. At least one major predator, a solitary wasp (*Symmorphus* sp., Hymenoptera: Eumenidae), was specifically associated with *C. aenicollis* at the lower site, where beetle mortality was highest. At both sites in 1984 and 1985, larvae originating from the lower site remained in aggregations and survived more frequently than larvae from the upper site, suggesting that they are better defended against predators. During a storm with cold weather late in the 1984 season, larvae and pupae died more frequently at the upper site, and there was a marginally significant trend ($P < 0.1$) for the lower site individuals to die more frequently than upper site larvae during the cold storm. Upper site larvae grew approximately 10% faster than lower site larvae at the lower site and under controlled conditions in the laboratory. These findings indicate that upper and lower site populations were adapted to the local selective regime, which suggest how populations of montane phytophagous insects may adapt to changing elevations.

Key words: *Chrysomela* – *Salix* – Elevation difference – Predator protection – Growth rate

Most species of herbivorous insects suffer severe mortality while feeding on their host plants, owing to food shortage, predators, and adverse weather conditions (Price 1984). Insects adapt to these sources of mortality in different ways. For example, many insects rely on rapid growth to exploit unpredictable environments (e.g., Rockwood 1974), while others rely on concealment, chemical defenses or a thick

exoskeleton to repel predators (Strong et al. 1984). The effectiveness of such adaptations determines the range and abundance of herbivorous insect species, as well as affecting the insect's choice of host plant (Price et al. 1981). Herbivorous insects growing along elevation gradients may be subject to opposing selection pressures at different elevations. At high elevations the short growing season and low temperatures potentially cause frequent mortality, while at lower elevations the insects may be subject to increased mortality from predators or desiccation. We predict that populations of locally adapted insects should possess adaptations to increase growth rate or resist cold at higher elevation sites, whereas they should increase predator defense or desiccation resistance at lower elevation sites. Investigation of such adaptations and the possible tradeoffs involved should improve our knowledge of how the physical and biotic environment affects evolution of associations between plants and herbivorous insects (e.g., Watt 1977, Kingsolver and Watt 1984).

Chrysomela aenicollis (Shaeffer) feeds on *Salix* sp. from 2,400 to 3,500 m above sea level in the Eastern Sierra Nevada, California. At the lower end of its distribution the snow melts in May–June while at the upper elevations (5–10 km away) snow melt is in July–August. Furthermore, sub-freezing temperatures and foliage-yellowing occur at upper elevations after early September, and during late summer storms. At those elevations, the fitness of these univoltine beetles is likely to be reduced by the short, cool growing season and the likelihood of cold weather during storms. At lower elevations, willow foliage retains its water content during dry weather (Smiley, personal observation), and desiccation is probably not a factor. Predation and parasitism are likely to be severe, as with most foliage-feeding insects. *C. aenicollis* larvae aggregate to reduce predation (M. Wade, K. Miller, personal communication). The larvae also possess eversible glands which contain salicylaldehyde, an odorous, irritating compound derived from salicin in the host plant (Pasteels, et al. 1983a, Rowell-Rahier 1984) which repels ants and other predators (Wallace and Blum 1969, Matsuda and Sugawara 1980, and Pasteels et al. 1983b).

We investigated agents of mortality at high and low elevations by comparing survival rates of beetles at two sites. We predicted that during warm summer weather in July and early August, daily survival rates would be higher at the high elevation site, but that during storms or cold weather survival would be superior at the low elevation site. Since beetles exhibited little movement between plants

in the field we also predicted the populations to be locally adapted to each site, such that beetles from low elevation populations should have superior survivorship rates during warm summer weather, while beetles from high elevation populations should survive better during storms and cold weather. To test our predictions we reciprocally transferred beetles from one site to the other and compared survival against appropriate controls. We also tested the prediction that beetles from higher elevations should grow faster by measurements of growth rate under field and controlled conditions.

Materials and methods

We studied *C. aenicollis* populations in June–August, 1984 and 1985. Beetles were feeding on *Salix* and *Populus* species growing along Big Pine Creek, west of the town of Big Pine, Inyo County, California (37°7'N/118°29'W). Temperatures in the Big Pine Creek drainage were mild in the summer of 1984 and 1985, with abundant rainfall in July, 1984. However, on August 16–18, 1984, a cold storm caused near-freezing temperatures as well as dehiscence and yellowing of some *Salix* leaves at the upper site while not visibly affecting the lower site. This unusually early event enabled us to examine the effects of late-season cold weather on beetle survivorship.

C. aenicollis is univoltine and overwinters in the adult stage (Brown 1956), crawling to the base of the host trees and hiding in cracks among the debris. In about mid-June or after snow melt, whichever is later, the adults feed on new growth of the host plant, mate, and lay eggs in clutches of about 30 on *Salix* foliage. The eggs hatch and the larvae remain together in groups until the second or third instar, when larvae tend to feed solitarily or in groups of 2–10. When larval feeding is completed, individuals aggregate at the tops of the host plants to pupate. Pupae retain the larval skin around their abdominal segments, and salicylaldehyde glands in the larval skin continue to function under control of the pupa (Wallace and Blum 1969). After eclosure, adults feed on the host tree until the onset of cold weather. Eclosure of adults is easily counted since the empty pupal/larval skin remains attached to the plant.

In the Big Pine Creek drainage, *C. aenicollis* feeds primarily on the shrubs, *S. orestera* C.K. Schneid. and *S. lasiolepis* Benth. var. *lasiolepis*. These plants were assumed to grow in clones, since plants within a clump were highly uniform in morphology and chemistry as compared with variability among clumps. The amount of *C. aenicollis* infestation is strongly dependent on the amount of salicin production in the clone, but not on the species of *Salix* (Smiley et al. 1985).

Field sites for between-elevation transfers were chosen at the highest and lowest sites where *C. aenicollis* was abundant in the Big Pine Creek drainage. Both sites were on southeast-facing slopes. The lower site (L) was at 2,810 m above sea level, and the beetles there were feeding on a clone of *S. lasiolepis* which on average contained 5% salicin in its leaves. The upper site (U) was at 3,240 m, where the beetles fed upon a clone of *S. orestera* which also contained 5% salicin.

Summer 1984 transfer experiments. At each site we isolated 12 willow plants by clipping foliage and clearing leaf litter

until each plant was surrounded by a 25 cm bare zone. Each plant was assigned to one of three categories: unmanipulated control (LC and UC), within-site transfer (LTL and UTU), and between site transfer (LTU and UTL). Transfer plants were very evenly matched in terms of size (approximately 1.0 m³), but the unmanipulated controls tended to be small individuals. Eggs and newly hatched larvae were counted on the unmanipulated controls, but were removed (still attached to a leaf) from the other treatments and placed in cups. After one day, approximately half of these were placed on the within-site transfer plants and the other half transported to the other site where they were placed on the between-site transfer plants. Transfers were done on June 20–21, 1984, henceforth referred to as day 0.

Leaves bearing eggs and first instar larvae were attached to recipient plants by carefully taping them to another leaf using Scotch Magic tape. Newly hatched larvae were capable of walking across the tape to the new host, and in most cases, transferred individuals successfully completed this maneuver. Eggs and newly hatched larvae were counted after 10 days to estimate early stage mortality. Egg clutches laid after day 0 were removed from the plant at this time. Surviving individuals were also censused after 20, 51, and 64 days to estimate developmental stage and survivorship for each treatment. Empty pupal skins were removed after counting on day 51 to prevent counting them again on day 64. Five plants (UTL # 902, UTL # 906, LTU # 916, LC # 922, and LC # 925) had a large, undetermined number of late ovipositions or physical contact with neighboring plants and were removed from the experimental design. Other treatments showed no evidence of larval immigration or emigration after transfer up until day 51. After day 51, three plants (LTU # 914, UTU # 909, and UC # 917) at the upper site were accidentally brought into contact with other *Salix* during a trailclearing operation, and subsequent beetle survival could not be measured.

Eggs and larvae at the lower site began the 1984 growing season approximately one week in advance of those at the upper site. For this reason, the transferred individuals from lower site plants consisted of approximately 80% eggs and 20% larvae, while those from upper site plants were 99% eggs. Since mortality rates of eggs and larvae differ, this creates a bias in estimating egg-adult survivorship. To partially correct for this, we measured the survivorship of individual egg clutches for a 10 day period. We calculated a mean egg survivorship (p) for each treatment, and, by multiplying $1/p$ times the number of larvae, back-calculated the number of eggs which would have been required to produce the observed number of larvae. This procedure overestimates mortality because it assumes beetles to be in "double jeopardy", once in the estimated 10-days of egg mortality and again in the actual first 10 days. However, since we apply this correction procedure almost entirely to lower site larvae, which we predict to have reduced mortality (during the mild part of the growing season), the actual difference in mortality between upper and lower site larvae will be greater than that estimated. The procedure is therefore conservative when used to estimate the predicted difference, and we employed it in our analyses of survival (Tables 2 and 3).

Percent survival during a time interval was calculated as $100 \times N_f/N_b$, where N_f and N_b were the number of beetles alive at the finish and beginning of the time interval,

respectively (Table 2). We calculated egg survival and survival from day 51 to day 64 (cold storm survival) from uncorrected field data, whereas survival from day 0 to day 51 (warm weather survival), and survival from day 0 to day 64 (overall survival) were calculated using the correction procedure explained above. For each time interval, we tested for significant differences among treatments using arcsin-transformed percentages (Sokal and Rohlf 1981). A two-way analysis of variance was calculated for each time interval using CRISP (CRunch Interactive Statistical Package, Stegner and Bostrom 1983) on a Corona PC microcomputer. Table 3 shows the statistical significance of differences in percent survival at the two sites ("site" effect). Also shown are statistical differences in survival between larvae originating from each site ("source" effect), and interaction effects between site and source of larvae. The site effect tests our prediction that selective forces of mortality change with elevation, and the source and interaction effects test for adaptation in local populations.

Summer 1985 transfer experiments. On July 10–12, 1985 one of us (J.S.) isolated 50 branches of *S. lasiolepis* and 20 branches of *S. orestera* at the lower site, and 50 branches of *S. orestera* at the upper site, by clipping the surrounding foliage and applying a ring of sticky resin (Tree Tanglefoot, Tanglefoot Company) to the base of the branch. Branches were numbered sequentially, and grouped in units of five. Every fifth branch, designated as a control, had a lodgepole pine twig (*Pinus murrayana*) attached which bridged the barrier to the surrounding foliage, allowing access by walking predators such as ants. One cluster of small-medium first-instar larvae was attached to each branch using techniques described above. Control branches at each site received larvae from that site (LC and UC, respectively), and all other branches alternately received larvae from the lower and upper site respectively. Surviving larvae were counted after 10 and 26 days, and on day 26 all survivors were collected to determine parasitism rates. Differences between treatments were tested using the Wilcoxon signed ranks test (Sokal and Rohlf 1981), calculated for adjacent branches. Testing adjacent pairs factored out the considerable variance in survivorship at different spatial locations within the field site.

At the upper site, sticky traps consisting of 30 cm square plastic sheets covered with sticky resin were placed 30 cm below five experimental branches. These were subsequently examined to determine if *C. aenicollis* larvae had fallen from the branches.

Table 1. Number of surviving *C. aenicollis* immediately before cold storm (day 51, 1984), after cold storm (day 64, 1984), and during mild summer weather (day 26, 1985). Small, medium, and large individuals are distinguished for 1985 third instar larvae only. Upper site individuals (UTL) pupated as soon or sooner than lower site individuals (LTL) at the lower site during both years. At the upper site the relative development rates could not be distinguished

Treatment	Number alive on day:									
	51 (1984)			64 (1984)			26 (1985)			
	larvae	pupae	adults	larvae	pupae	adults	small	med	large	pupae
LTL	16	62	144	5	18	208	12	10	7	0
UTL	1	14	80	0	0	104	3	4	12	6
LTU	29	64	34	10	3	68	20	71	18	0
UTU	53	28	2	36	14	19	8	55	6	0

Measurements of growth rate. Growth rates of larvae were measured by collecting them at the field sites and transporting them in cups to the White Mountain Research Station in Bishop, CA. At the start of the measurements, body weights from the upper and lower sites averaged 0.0121 ± 0.041 and 0.0120 ± 0.041 g, respectively. Larvae were starved for 24 hours at 20° C, weighed, and placed in a plastic cup with a transparent lid with foliage of *S. orestera* containing 2.5% salicin collected near Lake Sabrina, 14 km from Big Pine Creek at 2,896 m elevation (37°13'N/118°37'W). Cups were randomly placed in water baths in 1 cm of water, with the temperatures maintained at 5, 10, 17, 22, 27, and 35° C. Humidity in the cups was high, with some condensation on the lid, but larvae were not in physical contact with water droplets. Larvae were weighed after 48 hours and their relative growth rate (r/day) calculated as $(\ln W_f/W_s)/2$, where \ln equals natural logarithm, W_f equals final weight, W_s equals starting weight, and 2 equals the number of days from start to finish.

Curves of growth rate (r) as a function of temperature (T) were fit to a normal curve, using BMDP non-linear regression (Dixon 1983), based on the methods of Ralston and Jennrich (1978). Fitting to a normal curve facilitates comparison to tables in Taylor (1981), as well as estimating parameters R_m , T_m , and T_s (maximum growth rate, temperature at maximum growth rate, and temperature tolerance, respectively) which have a simple biological interpretation. We also tested for effects of body weight (W_s) on growth rate by incorporating a linear body size effect B into the non-linear regression model:

$$r = R_m(e^{-((T - T_m)/T_s)^2}) + BW_s$$

Results

Summer 1984: Most *C. aenicollis* larvae completed their development during the 64 days of observation. At the lower site, where temperatures ranged from 5.6° C on the coldest night to 28° C on the warmest day, 65% of LTL beetles and 84% of UTL beetles had eclosed from the pupa by day 51 prior to the cold storm (Table 1). Since LTL adult beetles were about the same length (8.4 ± 0.71 mm, $N=60$) as UTL beetles (8.6 ± 0.76 mm, $N=38$), and since the upper site larvae began development approximately one week later than the lower site larvae, UTL beetles must have grown at least 10% (6/64 days) more rapidly than LTL beetles. At the upper site, where temperatures ranged from 1.0 to 24.4° C, 77% of LTU beetles and 36% of UTU

Table 2. Number of *C. aenicollis* eggs and larvae, and their subsequent survival, on *Salix* plants. Transfer treatments have three-letter codes, i.e. LTL=larvae transferred from lower site to other plants in lower site. Unmanipulated controls have two-letter codes. L=lower site and U=upper site. The number of larvae was adjusted for having passed through egg to larval mortality, and added to the number of eggs to give the estimated "corrected" number of eggs present on day zero. This procedure underestimates actual survival rates (see text)

Treatment	Day 0			Percent survival			
	# eggs	# larvae	"corrected" # eggs	day 0 to 10 egg	day 0 to 51 egg-pupae	day 51 to 64 pupae	day 0 to 64 total
LTL	1305	293	2114	38	11	92	10
UTL	1393	53	1597	25	7	97	7
LTU	1271	248	1851	43	19	22	3
UTU	1128	0	1128	36	9	67	7
LC	399	177	753	50	11	82	9
UC	785	0	785	52	17	44	11

Table 3. Analysis of variance in survival probability, designed to test for differences between sites and between larvae from the two sites (source), after variation among plants (within treatments) has been eliminated. Survival probability was transformed using arcsin $p^{1/2}$ (Sokal and Rohlf 1981). * $P < 0.1$, ** $P < 0.05$, **** $P < 0.001$. Variation among plants was so great that few significant differences ($P < 0.05$) were seen, except (1) that lower site larvae prior to the storm had better survivorship than upper site larvae at both sites, and (2) mortality during the storm (day 51–64) was significantly greater at the upper site than the lower. There also were marginally significant trends ($0.05 < P < 0.1$) for larvae from the lower site to suffer greater mortality during the storm than larvae from the upper site, and for survivorship prior to the storm to be greater at the upper site

Variation in:	Source of variation	Degrees of freedom	Mean square	F-ratio	Principal trends
Egg survival	Within treatment	12	55.77		
	Site	1	105.06	1.8	
	Source of larvae	1	52.6	0.9	
	Site X Source interaction	1	14.1	0.3	
Survival from day 0 to 51 (before storm)	Within treatment	9	18.7		
	Site	1	70.2	3.8 ^a	Increased mortality at lower site. Larvae from upper site died more frequently.
	Source	1	101.4	5.4*	
	Site X Source interaction	1	15.4	0.8	
Survival from day 51 to 64 (storm)	Within treatment	7	119.9		
	Site	1	3,859.0	32.2****	Increased mortality at upper site. Larvae from lower site died more frequently.
	Source	1	600.4	5.0 ^a	
	Site X Source interaction	1	158.3	1.3	
Survival from day 0 to 64	Within treatment	7	16.2		
	Site	1	31.5	1.9	Larvae survive more frequently at own site.
	Source	1	1.3	0.1	
	Site X Source interaction	1	41.4	2.5 ^a	

beetles had pupated by day 51. Owing to the broad overlap in stages of development at this site, we could not determine the relative development rates of upper and lower site larvae.

Some newly hatched transferred larvae stuck to exposed tape and were killed. Nevertheless, overall effects of the transfer procedures were not statistically significant, as determined by analysis of variance between within-site transfers (LTL and UTU) and controls (LC and UC; $F = 1.4$, $P > 0.25$).

Overall survivorship (day 0–64) varied from 2 to 13% on the different plants (Table 2). Variance in survivorship was high among plants of the same treatment, with a coefficient of variation (Sokal and Rohlf 1981) among arcsine

transformed percentages of 26%. With such large variability, survivorship must have differed among treatments by a large percentage to be statistically significant, and our experimental design could not distinguish slight differences on the order of 2–3%. Due to the strong possibility of type II error we report statistical probabilities at the 10% confidence level as well as those at lesser probabilities (Table 3).

Egg survival rates did not differ significantly among sites or among larvae from different sites (Table 3). Larvae of syrphid flies were observed to be the major egg predator at both sites. Cannibalism was not observed and its incidence is probably low in these beetles (K. Miller, pers. comm.).

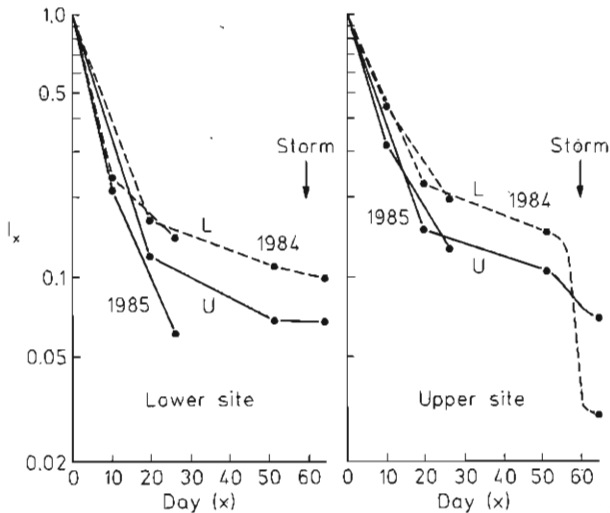


Fig. 1. Survivorship (lx) of transferred *C. aenicollis* at two sites along Big Pine Creek. The source of the transferred larvae is indicated by L (lower site) and U (upper site). During mild summer weather in 1985 and prior to the storm in 1984, lower site beetles on average survived better at both sites. During the storm, these beetles disappeared in disproportionate numbers at the upper site. During mild summer weather, average survival was greater at the upper site than the lower site

During mild summer weather (day 0 to 51), survivorship was marginally greater at the upper site than the lower site, although this was not quite statistically significant (Table 3, site effect, $P=0.08$). During this time, lower site larvae survived more frequently than upper site larvae (Table 3, source effect, $P<0.05$), and no interaction between site and source of larvae was observed ($F=0.8$). Figure 1 illustrates mean survivorship for the different treatments.

During the late-season cold storm, (day 51 to 64), survivorship was greatly reduced at the upper site ($F=32$, $P<0.001$). In addition, LTU beetles suffered marginally greater mortality than UTU individuals during this time interval ($F=5.0$, $P=0.06$). Since we observed that the majority of individuals were killed by being knocked off the plants onto the ground, we suggest that the increased mortality among LTU beetles may have been caused by less favorable attachment to the plants, as a function of either attachment site or strength of attachment.

Summer 1985. The results from 1985 were fully consistent with the trends observed in 1984, except that no cold storm occurred before the final observations on day 26. Larvae from the lower site survived more frequently than larvae from the upper site on *S. lasiolepis* at the lower site, *S. orestera* at the lower site, and *S. orestera* at the upper site (Table 4). Larvae on *S. lasiolepis* died more frequently than larvae on *S. orestera* at the lower site ($P<0.05$; Wilcoxon signed ranks test).

No whole larvae were detected in sticky traps placed under branches which experienced severe larval mortality, and we conclude that mortality was caused by predators which remove or kill the larvae. Since larvae on "bridge" control branches survived approximately as often as larvae on isolated branches (Table 4), we also conclude that the majority of predators were capable of flight or some other method of crossing to the isolated branches. Many bodies of larvae were counted on days 10 and 26, apparently killed

Table 4. Survival of *C. aenicollis* larvae on experimentally isolated *Salix* branches. Treatment codes as in Table 2. Percent survival from day 0 is given in parentheses. Control branches LC and UC have a pine twig "bridge" connecting the branch to the surrounding foliage allowing access and egress for walking insects such as ants and larvae. * $P<0.05$, Wilcoxon signed ranks test. Lower site larvae survived more frequently than upper site larvae at both sites, and there was no consistent effect of branch isolation compared with controls (see text)

Treatment	# branches	# alive on day:		
		0	10	26
<i>S. lasiolepis</i> :				
LTL	21	267	34 (13)	22 (7)
UTL	17	223	15 (7)	5 (2)
LC	9	124	9 (7)	6 (5)
<i>S. orestera</i> :				
LTL	8	147	65 (44)	35 (24)
UTL	9	145	66 (46)	16 (11)
Combined lower site:				
LTL	29	414	99 (24)	57 (14)
UTL	26	368	81 (21)	21 (6*)
Upper site:				
LTU	18	365	166 (45)	73 (20)
UTU	17	345	110 (32*)	46 (13)
UC	9	139	55 (40)	23 (17)

by larvae of syrphid flies (Diptera: Syrphidae), lacewing larvae (Neuroptera: Chrysopidae), and a plant bug (Hemiptera: Miridae), predators which were seen actively feeding. The syrphid and lacewing larvae were either present on the plants prior to their being isolated or they were oviposited by the female fly subsequently. Lacewing larvae were not observed at the upper site.

Predatory wasps (*Symmorphus* sp., Hymenoptera: Eumenidae) were also observed to take whole larvae to a large dead "nest tree" (*Pinus murrayana*). There, 25–35 wasps were observed to place larvae of *C. aenicollis* in bore holes and seal up the holes with mud. No other types of prey were taken. A survey of flowers of *Sphenosciadium capitellatum* ("swamp whiteheads") and *Heracleum lanatum* ("cow parsnip") revealed that this wasp is the dominant predatory wasp between 2,700 and 3,100 m elevation, but is not present at the upper site. The elevational distribution of this wasp corresponded exactly to that of *C. aenicollis* below 3,100 m.

Potential avian predators included the Pygmy Nuthatch, Mountain Chickadee, and the Oregon Junco, but none have been observed foraging on beetle larvae. We also observed two types of parasitoids at both sites. Combined parasitism rate for tachinid flies (Diptera: Tachinidae) and *Schizonotus lotus* (Hymenoptera: Pteromalidae) was moderate (169/604 on day 64, 1984 and 16/232 on day 26, 1985), although difficult to determine since it depends on unknown factors such as the larval stage attacked and the frequency with which parasitized larvae are consumed by predators.

Larval growth rates. Growth rates were a non-linear function of temperature, as expected (Fig. 2). Peak growth rates

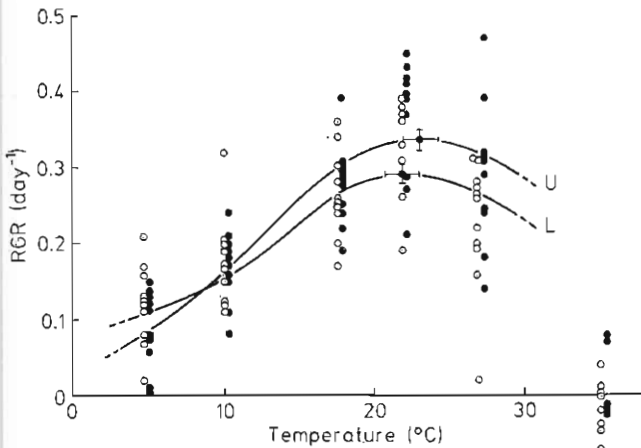


Fig. 2. Relative growth rate (r/day) of third instar *C. aenicollis* larvae, growing on leaves of *Salix orestera* at controlled temperatures. Normal curves were fit to the data (excluding measurements at 35°C) using non-linear regression, which also generated peak height and peak temperature with asymptotic standard deviations (indicated as error bars). L=lower site larvae, U=upper site larvae. Upper site larvae grew significantly faster than lower site larvae, especially at higher temperatures (see text)

Table 5. Non-linear regression of relative growth rate ($\times 100$) of *C. aenicollis* at controlled temperatures, ranging from 5°C to 27°C. Regression minimizes squared deviation from a normal curve with height = $R_m \times 100$ (maximum growth rate), peak at $T = T_m$ (temperature at maximum growth rate), and standard deviation = T_s , as well as minimizing squared deviation from a linear body size effect. The body size effect calculated above for both sites combined was used as a constant in the between-site comparison. Relative growth rate (R_m) was approximately 8% greater in the upper site larvae (see Fig. 2)

Site	Estimated coefficients			
	$R_m \times 100$	T_m	T_s	body size
both combined	39.9 ± 1.9	23.2 ± 1.1	14.5 ± 1.3	7.4 ± 1.4
lower	37.6 ± 0.9	23.9 ± 1.7	15.6 ± 1.8	7.4
upper	40.7 ± 1.3	22.5 ± 1.4	13.2 ± 1.4	7.4

occurred between 20 and 25°C, but growth was almost totally inhibited at 35°C. Mortality of experimental larvae was very low, even at 35°C when 4 of 20 died. This was unexpected, since growth inhibition at high temperatures is usually associated with high mortality rates (Taylor 1981). For this reason, and because growth at 35°C was slower than that expected by extrapolation from lower temperatures, we excluded the 35°C data from statistical analysis of growth rates.

Non-linear regression of growth rates of larvae at 5, 10, 18, 22, and 27°C, revealed a substantial negative effect of body size on growth rate (Table 5). Non-linear regression of growth rate on temperature, incorporating body size effects linearly, revealed that upper site larvae grew approximately 8% faster than lower site larvae (R_m in Table 5), but that differences in T_m or T_s were minimal. The difference in growth rate appeared to be accentuated at warmer temperatures (Fig. 2), and an analysis of variance of growth rate at 17, 22, and 27°C revealed a statistically significant difference between larvae from each site ($F=4.1$, $P<0.05$).

Discussion

During mild summer weather in 1984 and 1985, survivorship of *C. aenicollis* was consistently higher at the upper site as compared with that at the lower site, verifying our prediction that the force of mortality should be reduced at higher elevations when weather conditions remain mild. During the 1984 cold storm, mortality was much more severe at the upper site (55%) than at the lower site (5%; $P<0.001$), dramatically illustrating that episodic climatic factors may be much more severe at higher elevations. As a result of both types of mortality, the combined survivorship probability for 1984 (days 0–64) did not differ significantly at the two sites ($F=1.9$, $P=0.25$). The above findings support the hypothesis that late season cold weather conditions are a more frequent source of mortality at upper elevations, and that warm-weather agents of mortality are more severe at lower elevations.

Our results suggest that warm-weather mortality is caused by predators rather than heat or desiccation. Experiments in the summer of 1983 showed that larvae from both sites can grow successfully in the extremely hot, arid conditions on the Owens Valley Floor at 1,200 m elevation (K. Miller, personal communication), and healthy *C. aenicollis* larvae were not observed to fall off the plant as should occur if they were suffering from desiccation or food of low quality. Cold weather mortality at the upper site during 1984 was usually caused by pupae being knocked off the plant, and our observations suggest it may be partially ameliorated by adaptations for more secure attachment to the plant or more safe pupation sites. Since the probability of cold weather increases as the summer progresses, adaptations for rapid growth should also result in a significant selective advantage at higher elevations.

In spite of the large uncontrolled variation among plants in survivorship of *C. aenicollis*, the transfer experiments and controls of both years showed significant differences between larvae from the two sites. During mild summer weather, survivorship per day was consistently greater for beetles from the lower site as compared with beetles from the upper site. This finding supports the predicted hypothesis that lower site larvae are better adapted to survive under mild weather conditions, probably against predators. During the cold storm at the upper site in 1984, upper site larvae survived more frequently than lower site larvae. Although only marginally significant ($P<0.10$) and caused by an unknown mechanism, this finding supported our prediction that upper site larvae should possess superior adaptations to resist the effects of cold weather. Overall, the data strongly support the hypothesis that populations of these beetles are adapted to the local conditions prevailing at each elevation.

We have not yet determined the mechanisms by which local populations adapt to different elevations, but growth rate seems to be involved. As predicted, upper site larvae grew faster than lower site larvae, both on *S. lasiolepis* at the lower site and on *S. orestera* under controlled conditions. The temperature at which growth was observed to reach a maximum (23°C) was lower than any reported in Taylor's (1981) review of 54 species in six orders, but the temperature tolerance (T_s) of 13°C was not atypical for Chrysomelidae. Apparently, the growth physiology of *C. aenicollis* is unusually adapted to cool conditions prevailing in its natural habitat, and upper elevation

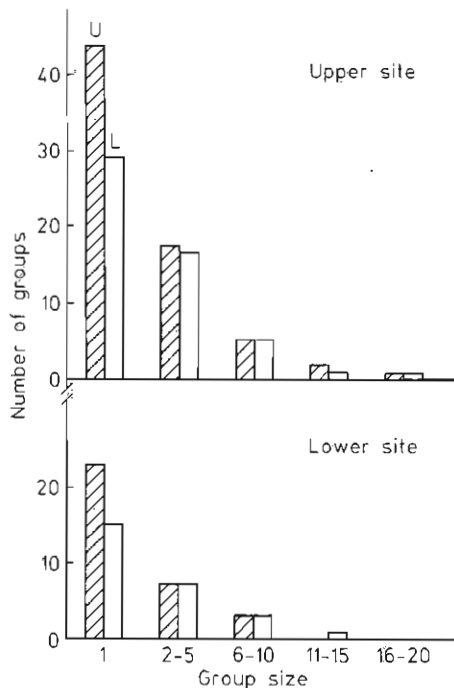


Fig. 3. Number of larvae in aggregations of second instar *C. aenicollis* feeding on *Salix orestera* at two sites. Larvae from the upper site were feeding alone more frequently than larvae from the lower site ($G = 4.81$, $P < 0.03$, loglinear contingency analysis, Dixon 1983)

larvae tend to grow 5–10% faster than larvae from lower elevations.

It is likely that lower site larvae obtain an advantage in predator defense, at the expense of reduced growth rates, by aggregating into larger feeding clusters. Figure 3 shows that lower site larvae were more likely to remain in feeding clusters than upper site larvae, at both sites. Two independent investigations suggest that *Chrysomela* species aggregate to enhance the effects of salicylaldehyde defense (Miller, MS in prep., Wade and Breden, MS in prep.), and *C. aenicollis* larvae along Big Pine Creek aggregate more at lower elevations (K. Miller, personal communication). This suggests that the tendency to aggregate may increase survivorship against predators. Aggregation behavior may affect growth rates as well, since (1) insects which normally aggregate often grow slowly when placed alone (Stamp 1980) as in our controlled growth rate experiments, and (2) insects which feed in groups may forego the most nutritious plant parts or warmest temperatures in order to remain within the group. We therefore suggest that the tendency to aggregate may be under opposing selection pressures at each end of our elevation gradient, and may be a principal mechanism by which local adaptation occurs in this system.

Our study of *C. aenicollis* populations suggests that selective forces change along montane elevation gradients in a predictable way, and that local populations have adapted to local biotic and abiotic conditions. The mechanisms of adaptation were not determined, although there was suggestive evidence that aggregation and feeding behavior is important. We plan to investigate this and other types of adaptation in future work.

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