

The decline and current distribution of *Parnassius apollo* (Linnaeus) in Finland; the role of Cd

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We tested two hypotheses that assume aspects of host-plant quality restrict the current distribution of *Parnassius apollo*. The “foreign host-plant hypothesis” states that host plants from outside the current range of *P. apollo* are, for some reason, unsuitable for the developing larvae. The “heavy-metal hypothesis” specifically attributes this unsuitability to a higher concentration of heavy metals (especially Cd) outside the current species’ range than inside. We fed 60 larvae from two populations with host plants from their place of origin (control) or from an area outside the current distribution of the species (treatment). Treatment plants had lower Cd levels, which allowed us to separate between the two hypotheses. Larvae in the treatment group experienced positive effects on fitness-related traits such as weight at pupation, length of the last instar and food consumption. Hence, Cd appears to have a negative effect on developing *P. apollo* larvae and may therefore restrict the recolonisation of current populations into their former range. However, larvae from one population tolerated a concentration of Cd equally high as what was previously shown to be lethal. Such tolerance undermines the generality of attributing the decline and restrictions of *P. apollo* populations to high Cd concentrations.

Introduction

Butterflies are a group that have suffered severe declines in population abundance and range contractions throughout Europe during the last century (van Swaay & Warren 1999). In Europe, the largest threat to butterflies has been the loss of habitat (New *et al.* 1995, Thomas 1995). In the red data book of European butterflies, the status of the apollo butterfly (*Parnassius apollo*) is listed as decreasing in twelve out of twenty-eight countries and the species is considered extinct in three countries (van Swaay & Warren 1999). Only five

countries report that the apollo populations are stable. In Finland, *P. apollo* began declining after peak abundances in the 1930s (Mikkola 1979) and today *P. apollo* has its stronghold in the southwestern archipelago and on the Åland islands (Huldén *et al.* 2000). Single adult individuals of *P. apollo* are sometimes sighted outside this range (Repo & Kullberg 1996, 1997, 1998). However, there are only sporadic reports of the establishment of new populations through sightings of larvae, and none have thus far been thoroughly studied.

Climate change has also been shown to alter the distribution of butterflies (Parmesan *et al.*

1999). However, due to their limited dispersal abilities and the increasing fragmentation of habitat it is not self-evident that butterflies can track habitat changes due to climate change (Warren *et al.* 2001). In Britain, few butterflies, particularly habitat specialists, have been able to expand through the entire area that has become suitable to them in terms of climate (Hill *et al.* 2001, Warren *et al.* 2001). The limited expansion of the butterflies was due to suitable habitat being too widely dispersed for the butterflies to reach it. Habitat generalists, i.e. many common butterflies, were better able to track the changes due to climate change. What the consequences of climate change for *P. apollo* will be remains to be seen. *P. apollo* is a habitat specialist that depends on small scale heterogeneity in the distribution of adult and larval resources (Brommer & Fred 1999, Fred & Brommer 2003).

The decline of many butterflies including *P. apollo* coincides with the development of industry and changes in agricultural practise. Loss of habitat has most likely been a considerable threat to *P. apollo*. In addition, pollution by heavy metals is viewed as a factor contributing to the historical decline and currently observed lack of re-establishment (Bengtsson *et al.* 1989, Nakonieczny *et al.* 1996, Nieminen *et al.* 2001). The “heavy-metal hypothesis” states that heavy metals (especially Cd) are harmful for the development of *P. apollo* larvae and that current populations are therefore restricted in their distribution because of heavy metals (Nieminen *et al.* 2001). A feeding experiment showed that all larvae

died when they were fed host plants that had higher heavy-metal contents than what the larvae naturally were exposed to in their population of origin (Nieminen *et al.* 2001). Furthermore, the average Cd content of the host plant *S. telephium* in the former range of *P. apollo* is higher than the Cd content in host plants within the species’ current range (Nieminen *et al.* 2001; Table 1). Special emphasis is put on Cd and its antagonist Cu and Zn (Table 1). A similar result as that in the Finnish study was found for *P. apollo* populations in southern Poland, where host plants have much lower concentrations of Cd, Pb and Zn as compared with the plants in the surrounding heavily industrialised areas (Nakonieczny *et al.* 1996). However, the average concentration of Cd in host plants within the current range of the Polish population is much higher than that found in the Finnish study (Table 1). Furthermore, in the Polish study introduced butterflies from the laboratory population that were fed host plant with a very low Cd concentration as larvae, survived in the natural population (Nakonieczny *et al.* 1996). The laboratory-reared animals produced offspring that survived since later studies show an increase in population size due to the introductions (Witkowski & Adamski 1996).

A second hypothesis, the “foreign host-plant” hypothesis, states that re-establishment of *P. apollo* populations is hindered by low suitability of host plants outside the current range for developing larvae (Bengtsson *et al.* 1989). The reason for the unsuitability is not specified. Bengtsson

Table 1. Heavy metal concentrations in the host-plant (*Sedum telephium*) of *P. apollo* found in several studies on the butterfly. Samples are from within the occurrence of the species (inside range), and outside the range of the species (outside range). In the Polish study, heavy metal concentrations are from samples from laboratory-reared host plants. Concentrations are reported in mg kg⁻¹ (ppm) dry weight, and given as means with standard errors.

Authors	Country	Inside/outside current range	Cd ± SE	Pb ± SE	Zn ± SE	Cu ± SE
Nieminen <i>et al.</i> 2001	Finland	Inside range	2.49 ± 1.41		96 ± 30	8.8 ± 2.38
		Outside range	3.51 ± 1.93		144 ± 47	9.4 ± 2.19
Nakonieczny <i>et al.</i> 1996	Poland	Inside range	4.39 ± 0.70	28 ± 3.85	96 ± 20	
		Laboratory	0.30 ± 0.25	6 ± 2.05	73 ± 25	
Bengtsson <i>et al.</i> 1989	Sweden	Inside range	1.64 ± 0.38			
		Outside range	1.44 ± 0.32			
Current study	Finland	Inside range				
		Coastal pop.	4.4	0.7	170	7.3
		Archipelago pop.	1.9	2.0	100	5.0
		Outside range	1.3	1.9	150	7.3

et al. (1989) fed *P. apollo* larvae with host plants from the current *P. apollo* population area and from areas that were not inhabited by the species. They found that larvae fed with the host plants from the current population area grew better than larvae fed with the “foreign” host plants. However, this was only true for the host plants taken from one of the two locations where *P. apollo* did not occur. Furthermore, the heavy-metal contents were not dependent on the location from which the host plants originated (Bengtsson *et al.* 1989; Table 1), and neither was the growth of the larvae influenced by the heavy-metal contents of the host plants in the experiment.

In this paper, we address the two above-outlined hypotheses. Larvae from two populations were fed either host plants from their own population area or host plants from a site within the former range of the apollo butterfly. The Cd content of the host plants was higher in the plants from the current range of *P. apollo* than in the plants from the former range (Table 1). Since the Cd content in the foreign host plants was lower, we were able to separate between the foreign host-plant hypothesis and the heavy-metal hypothesis. The foreign host-plant hypothesis predicts that larval performance in the treatment group would be negatively affected as compared with that in the control since they feed on foreign host plants, whereas the heavy-metal hypothesis predicts that larval performance would be positively affected by treatment as the Cd concentration of foreign host plants is lower.

Material and methods

The collecting and rearing of larvae

The larvae used for the feeding experiment were gathered from two of the strongest *P. apollo* populations in Finland. Both populations have a long history, and are part of the former wider distribution of the species. Twenty larvae were collected from a coastal population in southwestern Finland and forty larvae from an archipelago population from southwestern Finland. Since *P. apollo* is a protected species and has the status of near threatened (NT) in Finland (Rassi *et al.* 2001), we restricted our collection to around

5% of the population size estimated by Mark-Release-Recapture (MRR) data on the adult butterflies. We collected larvae of similar size that we estimated would still moult one more time before pupation. All but eight larvae moulted into their last instar after collection. The larvae collected were about three centimetres long and five millimetres broad. The larvae were placed individually in round plastic containers (\varnothing 20 cm, 10 cm high). The containers had holes in the bottom to allow water to run out, and a large hole on the side to allow for ventilation. The holes were covered with a fine mesh that was glued in place; the container was covered with a lid of fine mesh. The bottom of the container was covered with a sheet of tissue paper. The larvae were all kept under similar conditions, outdoors on a gravel surface in a sunny spot. At night the containers were placed under a well ventilated roof of clear plastic to avoid the containers being flooded in case of rain. The larvae had food available *ad libitum*, and the stems were changed every second day or earlier if they were depleted before that. Until four weeks after pupation the pupae were kept in the containers, after which they were moved to cages. The cages were made of a tube of mesh with a metal rim (\varnothing 40 cm) at the bottom. The cages were high enough (40 cm) to allow the butterflies to climb up and inflate their wings and dry. The cages were hung up above the ground, outside, to prevent any ground dwelling predators such as shrews from eating the pupae. After eclosion the butterflies were marked, sexed, and their condition was noted, after which they were taken back to their patch of origin. Twenty-three butterflies were followed regularly after having been placed out on the patches as part of an ongoing MRR survey. The pupae that did not hatch were dissected in order to sex them and to check for possible parasite infections. Parasites that were found were sent for identification to specialists.

The collecting and administration of the host plant

The host plants were collected with tubers and surrounding soil from the two populations. Because the sampling was destructive, we only collected host plants from patches that had a

high abundance of them, and we scattered the collection over several patches. The host plants collected from the former range of *P. apollo* were collected from a patch with similar conditions as in the actual populations, sunny and exposed. Further, the collection site from the former range was selected to be as similar to the *P. apollo* populations as possible in terms of landscape and human population. The island of Skåldö was selected since it is within the former range of *P. apollo*, geographically not far from the existing populations (40–50 km), and because it is an island in the inner archipelago zone (Häyrén 1900). In terms of landscape it is very similar to the coastal areas inhabited by the *P. apollo* population. After collection the host plants were placed in shallow cardboard boxes with the soil from their site of collection, to avoid their acquiring any heavy metals from the environment where they were kept. The host plants were kept in the same area as the larvae. They were watered with unprocessed drinking water from a well, or naturally by rainfall. The host plants were cut and put in water for 24 hours before being administered to the larvae. To keep the quality of the host plants as even as possible, they were put into vials with water that were closed with a watertight lid with a hole for the stem. More water was added into the vials daily. The host plants were changed after two days or earlier if the leaves had been fully consumed before that.

Measurements and analyses

The daily weighing procedures were carried out indoors on a digital scale with an accuracy of 0.001g. The host plants were weighed before being administered, and after the first and the second day, provided they had not been changed within the last two days. The faeces were collected and weighed daily.

For the analysis of the heavy-metal contents of the host plants from the different locations, in total about ten leaves from different plants were collected per sample. The digestion of leaf material was done in a test tube with < 0.3 g dried leaf material to which 0.5 ml of HNO₃ conc. was added. The tube was heated for 2 h at 50 °C, and for 4 h at 105 °C, and for another 4 h at 180 °C.

The samples were diluted to 25 ml and filtered through filter paper after cooling down. The samples were analysed on a Varian SpectrAA-400 atomic absorption spectrophotometer. The flame technique was used for the analysis of Fe, Zn, Cu, and Mn. The spectrophotometer was equipped with a GTA-96 graphite furnace for the analyses of Cd. Concentrations are reported as dry weight in mg kg⁻¹ (ppm).

The analyses of the larval growth spans over the last instar, from the first day after the moult until the larva had spun a cocoon. For the eight larvae (six males and two females) that did not moult during the experiment, the first day in captivity was taken as the beginning date. In order to test whether the inclusion of these larvae biased the result, we tested whether a variable coding for moulting entered any of the statistical models explained below. Whether an individual moulted or not proved to be unimportant in all models (not reported). The increase in larval weight with age (i.e. days of the last instar) was analysed by fitting a non-linear mixed effects model based on the logistic growth curve

$$\text{Weight} = \frac{A}{1 + e^{-\frac{\text{Age}-I}{S}}}, \quad (1)$$

defined by the three characteristics of the logistic curve, the asymptote *A*, the inflection point *I* and the scale *S*. The asymptote is the asymptotic value of the sigmoid curve, the inflection point gives the age value at which growth is maximal, and the scale gives the overall steepness of the curve. This function fitted the overall growth data well. In a non-linear mixed effect analysis, individual differences in logistic growth are considered random effects in the parameters *A*, *I* and *S*. By incorporating the individual as a random effect, we accounted for the repeated measures on the individuals, and thereby avoided pseudo-replication. In addition, the fixed effects (i.e. larval origin, treatment and sex) on the parameters *A*, *I* and *S* are evaluated by examining to what extent the logistic growth model's fit to the data is improved by incorporating such fixed effects. We followed the approach outlined by Pinheiro and Bates (2000). First we considered whether all random effects were needed and then proceeded to add fixed effects per parameter considered,

keeping only significant effects in the model.

In addition to the full growth data, we considered three larval performance measures; weight at pupation, total consumption of the host plant during the last instar and the length (in days) of the last instar. The analyses on these measures were performed by generalized linear models with normal error distributions and an identity link using a stepwise forward approach (Crawley 2002). The significance of the variables was estimated with the *F* test. All analyses were carried out in S-PLUS (Insightful Corporation).

Results

Heavy metals of host plants

The content of Cd was the highest in the host plants collected from the area where the coastal *P. apollo* population occurs, whereas the plants with the highest concentrations of other heavy metals (Pb and Cu) varied in place of origin (Table 1). The host plants from the site outside the current *P. apollo* distribution had the lowest Cd concentration and a comparatively high concentrations of the Pb and Cd antagonists Zn and Cu, respectively (Table 1). However, acid rains release metals from stable compounds in the soil, for instance Fe and Mn are good indicators for soil acidification (Bengtsson *et al.* 1989, Tyler *et al.* 1989, Nieminen *et al.* 2001). Thus, the elevated Fe and Mn levels in the site from the former range indicate a higher level of acidification there than in the sites where *P. apollo* occurs (Fe former range 180 mg kg⁻¹, coast and archipelago 120 mg kg⁻¹; Mn former range 260 mg kg⁻¹, coast 140 mg kg⁻¹, archipelago 130 mg kg⁻¹) (Nieminen *et al.* 2001). Further, the Pb and Ni levels were relatively high in the site from the former range (Former range Pb 1.9 mg kg⁻¹, Ni 4.0 mg kg⁻¹; Coast Pb 0.7 mg kg⁻¹, Ni 0.7 mg kg⁻¹; Archipelago Pb 2.0 mg kg⁻¹, Ni 0.7 mg kg⁻¹).

Growth data

The growth of the larvae over the last instar describes a typical sigmoid shape curve (Fig. 1). The larvae from the coastal population had

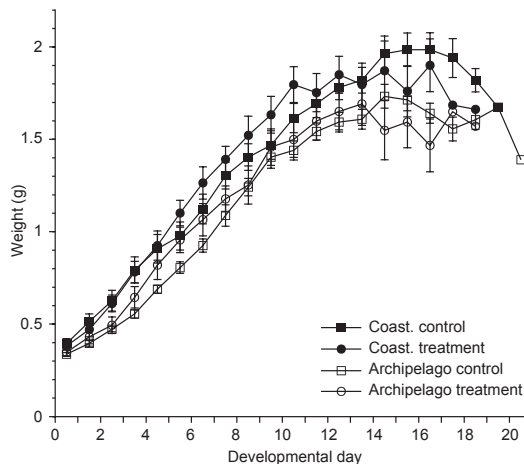


Fig. 1. Growth curve (mean \pm SE) of *P. apollo* larvae in their last instar. Filled symbols represent larvae from the coastal population and open symbols represent larvae from the archipelago population. Squares represent the control groups and circles represent the treatment groups.

a higher asymptotic weight than the archipelago larvae (Fig. 1 and Table 2) and females had a

Table 2. Results of a non-linear mixed effect analysis on the growth data of the larvae in figures 1 and 2. Analysis is based on a logistic growth curve dictated by three parameters, asymptote *A*, inflection point *I* and scale *S* (Eq. 1). Given are the fixed effects that influence these three parameters. Random effects around these parameters are also included in the model.

Variable	Coefficient	<i>t</i>	<i>P</i>
Asymptote (A)			
Intercept	1.81 \pm 0.03	54.20	< 0.001
Origin	-0.09 \pm 0.03	-3.09	0.002
Sex	-0.12 \pm 0.03	-4.34	< 0.001
Inflection point (I)			
Intercept	4.57 \pm 0.17	27.37	< 0.001
Origin	0.20 \pm 0.09	2.20	0.028
Treatment	-0.19 \pm 0.09	-2.08	0.037
Sex	-0.01 \pm 0.09	-0.16	0.874
Origin \times Treatment	0.15 \pm 0.14	1.04	0.297
Origin \times Sex	-0.03 \pm 0.09	-0.30	0.767
Treatment \times Sex	-0.20 \pm 0.09	-2.20	0.028
Origin \times Treatment \times Sex	0.37 \pm 0.09	4.01	< 0.001
Scale (S)			
Intercept	2.92 \pm 0.10	28.20	< 0.001
Origin	-0.00 \pm 0.10	-0.02	0.985
Treatment	-0.21 \pm 0.08	-2.55	0.011
Origin \times Treatment	0.18 \pm 0.08	2.21	0.027

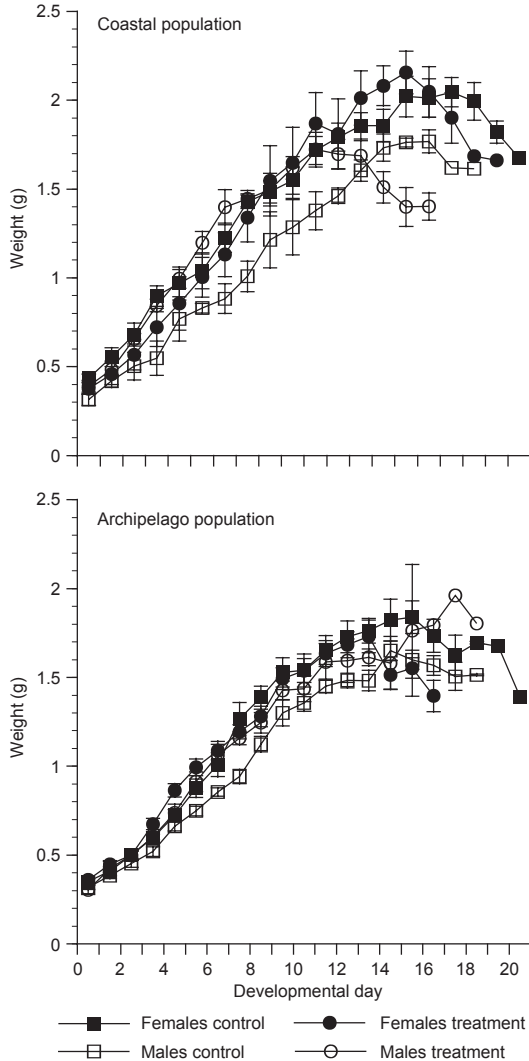


Fig. 2. Growth curves (mean \pm SE) from females and males from the two study populations. The control groups are shown as squares and the treatment groups as circles. Females are shown as filled symbols and males as open symbols.

higher asymptotic weight than males (Fig. 2 and Table 2). The larvae from the coastal population generally grew faster as they had an earlier inflection point in their growth than the larvae from the archipelago population. Similarly, the treatment group had an earlier inflection point in their growth than the control group (Fig. 1 and Table 2). Further, the males in the treatment group in both populations showed a higher rate of weight increase than the control males, whereas

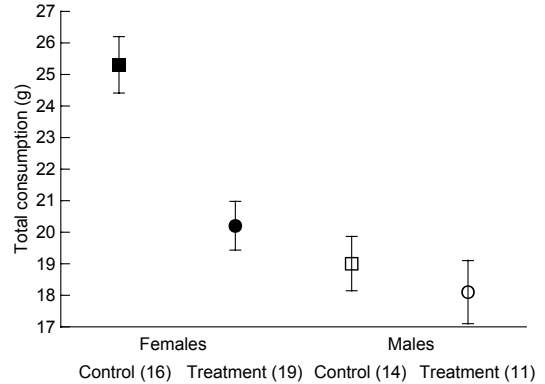


Fig. 3. Least squares means and standard error of total consumption in the different sexes and treatment groups, adjusted for variation in the length of the larval period. The control groups are shown as squares and the treatment groups as circles. Females are indicated with filled symbols and males with open symbols. The number of individuals within each group is given between brackets.

there was no such difference in the females (Fig. 2; treatment \times sex in Table 2). The three-way interaction term between origin, treatment and sex in the inflection point showed that the males in the treatment group in the coastal population grew proportionally faster than the males in the treatment group in the archipelago (Fig. 2 and Table 2). In terms of scale there was a two-way interaction between the origin and treatment (Table 2). The two-way interaction shows that the growth curve of the archipelago population lies below the one of the coastal population, whereas the treatment group lies above the one from the control group (Figs. 1 and 2, Table 2).

Weight at pupation, consumption and developmental time

The larvae from the coastal population were heavier at pupation than the larvae from the archipelago population (Table 3). In addition, females were heavier at pupation than males (Table 3). The total consumption throughout the last instar is naturally higher the longer the last instar was in duration (Table 3). Furthermore, the males consumed less than females and the treatment group consumed less than the control group (Fig. 3 and Table 3). The total consumption in

the control group increased steeply the later the larvae went into pupation, whereas the total consumption stayed almost constant in the treatment group regardless of the date of pupation (Table 3). The total consumption was highest in the control groups for both the males and females, but the difference between the total consumption in the control group and the treatment group was largest for the females (Fig. 3 and Table 3). All the measures of defecation were directly correlated with the corresponding measures of consumption (consumption rate vs. defecation rate, $r = 0.82$, total consumption vs. total defecation $r = 0.88$). Thus, there were no differences between the groups in how the bulk of food was digested. The only factor affecting the length of the last instar was the treatment (Table 3). Developmental time through the last instar of the control group lasted two days longer as compared with that of the treatment group. A summary of the means \pm SE of the total consumption, total weight of faeces,

weight at pupation and length of the last instar is given in Table 4.

The adult butterflies

Of the total of sixty pupae, 18 did not develop properly. However, the developmental problems were not connected with the treatment (GLM with logit link and binomial errors; $\Delta D = 0.7$, $\Delta df = 1$, $F = 0.23$, $P = 0.87$). Seven pupae eclosed, but could not inflate their wings, seven never hatched at all, and four were parasitized. The parasite was the generalist wasp *Pimpla turionellae*. Forty-two pupae hatched into healthy looking adult butterflies. Twenty-three of these butterflies were followed as part of an ongoing MRR study. Although the adults looked healthy their behaviour was different from that of the wild butterflies. Many of the released adults that were monitored tended to be in the same general

Table 3. Stepwise forward generalized linear model with normal error structure and identity link function on factors affecting the weight at pupation, total consumption during the last instar, and length of the last instar. The factors tested were treatment (control = 0, treatment = 1); sex (female = 0, male = 1); place of origin (coastal pop. = 0, archipelago pop. = 1); begin date of last instar, date of pupation, length of the last instar, and adult status (normal = 0, did not inflate wings = 1, did not hatch = 2, parasitized = 3). The deviance D of the null-model (constant only) and the minimal adequate model (variables listed), is given (null vs. m.a.m.: $F_{\Delta df, \Delta df} < 0.001$). The effect of each variable is tested with an F test of the change in deviance (ΔD) and change in degrees of freedom (Δdf) in the model when a particular variable is included. The variable with the highest explanatory power is added, starting with the single terms. Interaction terms are added in order of their explanatory power.

Variable	Coefficient	(Δ) D	F	P
Weight at pupation				
Null model		3.6		
Minimal adequate model		2.6	$F_{2,57} = 10.9$	< 0.001
constant	1.57 \pm 0.03			
place of origin	-0.10 \pm 0.03	0.5	$F_{1,58} = 11.8$	0.001
sex	-0.09 \pm 0.03	0.5	$F_{1,57} = 10.0$	0.003
Total consumption				
Null model		1425		
Minimal adequate model		440	$F_{6,53} = 20$	< 0.001
constant	3.50 \pm 4.40			
length of last instar	0.72 \pm 0.23	506	$F_{1,58} = 70$	< 0.001
sex	-2.12 \pm 0.41	190	$F_{1,57} = 21$	< 0.001
treatment	6.40 \pm 2.66	108	$F_{1,56} = 28$	< 0.001
treatment \times date of pupation	-0.21 \pm 0.07	137	$F_{1,54} = 7.3$	0.001
treatment \times sex	0.93 \pm 0.40	44	$F_{1,53} = 4.3$	0.025
Length of last instar				
Null model		191		
Minimal adequate model		140	$F_{1,58} = 21$	< 0.001
constant	115.6 \pm 0.20			
treatment	-0.92 \pm 0.20	51	$F_{1,58} = 21$	< 0.001

area day after day, and were seldom observed flying. Wild butterflies tend to be active and fly away especially if approached. The released butterflies showed a 4.5% (1/22) between-patch movement rate, whereas the wild butterflies on the same patches showed a 26.6% (11/43) movement rate. Thus, the released butterflies moved less between patches than the wild ones ($\chi^2 = 7.66$, $df = 1$, $P = 0.006$).

Discussion

Host plants, heavy metals and the development of larvae

We found no support for the “foreign host-plant” hypothesis (Bengtsson *et al.* 1989), which predicts that host plants from an area where *P. apollo* no longer occurs are somehow harmful to the animal. Only fitness-enhancing effects on any of the measures concerning growth, food consumption or the length of the last instar could be detected in the larvae that were fed host plants from outside *P. apollo*'s current range. The foreign host plants from outside the current range had, in this case, a lower Cd concentration than the control host plants. Our feeding experiment does not establish a causal link between the plant Cd concentration and larval performance, because the host plants we fed to the larvae were

taken from nature. However, our results are consistent with the notion that current Cd levels may repress certain aspects of larval development in *P. apollo*. Our results, therefore, at least partially support the “heavy-metal hypothesis”.

Females in the treatment group managed to grow similarly and reached the same final size as the females in the control group. However, the treatment group females pupated on some 20% less food, which indicates a higher metabolic efficiency when Cd levels are low. Furthermore, the females that were fed host plant from outside their own population pupated substantially earlier (two days, i.e. 12% of the developmental time in the last instar) than the females in the control group. In general, also males that fed on host plant from outside their current range had a shorter developmental time through the last instar than the control group of males. Males from the coastal population (the highest concentration of Cd in the host plant) increased markedly faster in weight when they fed on foreign host plants (which had a low Cd concentration). This effect was less pronounced in males from the archipelago population, which is consistent with the observation that the difference in Cd concentration is smaller between the archipelago and the foreign site than between the coast and the foreign site. Both sexes were able to complete their development in a shorter time without compromising their weight at pupation.

Table 4. Summary of the mean \pm SE total consumption (g), total weight of faeces (g), weight at pupation (g) and length of last instar (days). Larvae are grouped according to their origin (coastal and archipelago population), control or treatment (fed host plant from their own population or from a foreign location), and sex.

	Consumption	Faeces	Weight at pupation	Developmental time
Coast				
Control				
Females	24.76 \pm 1.53	0.76 \pm 0.02	1.78 \pm 0.06	17.29 \pm 0.61
Males	17.70 \pm 1.42	0.52 \pm 0.04	1.67 \pm 0.03	15.00 \pm 1.16
Treatment				
Females	21.65 \pm 0.67	0.62 \pm 0.03	1.86 \pm 0.14	15.60 \pm 0.68
Males	17.04 \pm 0.92	0.47 \pm 0.04	1.40 \pm 0.06	14.2 \pm 0.37
Archipelago				
Control				
Females	28.05 \pm 1.60	0.86 \pm 0.05	1.61 \pm 0.07	16.78 \pm 0.52
Males	19.79 \pm 1.01	0.63 \pm 0.03	1.43 \pm 0.04	16.18 \pm 0.46
Treatment				
Females	18.58 \pm 0.78	0.55 \pm 0.03	1.49 \pm 0.07	14.43 \pm 0.36
Males	17.24 \pm 1.18	0.50 \pm 0.03	1.41 \pm 0.09	14.83 \pm 0.79

Weight at pupation can be especially important for females, because it is positively related to fecundity (Ochiengodero 1992, Spurgeon *et al.* 1995, Boggs 1997). For both sexes, a shorter developmental time in the conspicuous larval period can be beneficial in terms of avoiding predation and possibly parasites.

We studied the development of larvae through their last instar, when the food intake of the larvae is highest. Earlier instar larvae would potentially be more sensitive to toxins than the later instar larvae that we used. However, since we fed the treatment group with food that had lower Cd concentration than the host plants fed to the control group, the potential higher sensitivity of earlier instars to Cd did not influence the outcome of our experiment. The control group (higher Cd levels) were fed host plants from their own population, and thus the larvae in our experiment had evidently survived the toxin levels in their earlier instars. However, it is possible that mortality due to the high Cd concentration occurs in the earlier instars only leaving the more tolerant individuals to complete development. This must be viewed as a natural process and a basis for the development of tolerance towards heavy metals.

We had no larval mortality in our experiment, and the pupal mortality was only 12% (7/60). If we also take into account the individuals that failed to inflate their wings, or that were parasitized, 30% (18/60) of the pupae did not hatch to normal looking imagines. In large-scale rearing of *P. apollo* in Poland, pupal mortality of 3%–61% was reported for larvae that were collected from the wild and reared in captivity (Witkowski & Adamski 1996). The sedentary behaviour by the adults that we observed has, to our knowledge, not been reported in other studies of this species (cf. Witkowski & Adamski 1996). We can only speculate on what the reasons behind the behaviour are; however, the rearing conditions during the pupal phase were different from the natural conditions because pupae were kept in cages above the ground to avoid predation. In these conditions the pupae received direct sunlight and wind which could have altered several functions during development. The animals were moved out to their place of origin only after eclosion which also prevents any environmental

signalling from the place of origin during the last instar and the pupal development. The animals were also handled, marked with a running number on their wing and taken out of their cage and transported in boxes to the point of release. However, the handling is similar to the handling when marking “wild” butterflies, except for the transportation. Nevertheless, butterflies reared in captivity that have been handled and have experienced a lack of environmental signals from the area of release, have been successfully re-introduced elsewhere (Witkowski & Adamski 1996).

Can cadmium explain changes in the range size of *P. apollo*?

Nieminen *et al.* (2001) suggested that the historical range contraction of *P. apollo* was partly due to an accumulation of heavy metals because of downfall from industrial and mining activities. Furthermore, recolonisation of current *P. apollo* populations back into its former habitat is thought to be hindered by a higher Cd concentration in host plants outside the species' current range than inside (Nuorteva 1999, Nieminen *et al.* 2001).

Due to limited possibilities to experiment with a protected species, we could not extend our experimental protocol to, for example, also include foreign host plants with a higher Cd concentration than in the control host plants. However, Nieminen *et al.* (2001) fed foreign host plants with a high concentration of Cd to larvae from the same archipelago population as we used in this paper. He found that all larvae died when fed host plants with a similar Cd concentration (4.3–4.4 mg kg⁻¹) that we found in the plants from the area inhabited by the coastal *P. apollo* population (4.4 mg kg⁻¹) and that are also found in southern Poland (4.4 mg kg⁻¹; Table 1). Thus, even on a relatively small spatial scale within the current range of *P. apollo* (30 km), the plant Cd levels tolerated by one population (the coastal population) may be potentially lethal to larvae from another population (the archipelago population). Nevertheless, a cross-feeding experiment between the coastal population and the archipelago population is needed to verify this assertion.

The fact that larvae from the coastal population can complete development on host plants from the same area, whereas larvae from the archipelago population suffer severe mortality on host plants with such high concentrations of Cd (Nieminen *et al.* 2001) suggests that *P. apollo* from the coastal population have developed tolerance towards prevailing Cd levels. The historical increase in heavy metal in the environment, which was intensified by acid rains that transform the metals into a soluble form (Nuorteva 1999), is a gradual process. Under such gradually increasing pollution, tolerance towards heavy metals is expected to evolve (Tyler *et al.* 1989, Lindqvist 1994, Ortel 1995, Nascarella *et al.* 2003).

In summary, our results lend partial support for the implications of the heavy-metal hypothesis of Nieminen *et al.* (2001). On one hand, our findings are consistent with a negative effect of Cd on larval development. Although the effects were subtle, they were all effects on fitness-related aspects of development. Even subtle effects on fitness-related traits can potentially have far greater effects on the population in the long term. In general, the performance of tolerant organisms on polluted soils is lower than the performance of non-tolerant individuals on normal soils (Tyler 1984). Any effect that depresses the expected growth rate will especially hinder colonisation, where typically a few individuals, or even just one fertilised female, arrive at a new location. For such a small initial population even small negative effects, such as the one we documented here, could hinder establishment of the colonists. Nevertheless, the large variation in Cd levels within the current distribution of *P. apollo* and the apparently different tolerance of *P. apollo* towards Cd across existing populations make it unlikely that Cd played an important role in the historical contraction and the current lack of recolonisation of *P. apollo* in Finland. Data from 1985 onwards on the Cd concentration in the soil collected by the Finnish Forestry Research Department (<http://www.metla.fi/met-info/metsienterveys/raskasmetalli/index.htm>) show that the Cd concentration in the mainland plants have historically been even higher than it is today. The mainland population is located in the area of Finland with the highest Cd levels

in the soil twenty years ago ($> 0.7 \text{ mg kg}^{-1}$). In general, Cd levels have decreased considerably during the last twenty years (today's level is $0.2\text{--}0.3 \text{ mg kg}^{-1}$ Cd in the soil), but differences between regions have remained proportionally the same. Hence, the mainland population has managed to persist in one of the most Cd-polluted areas of Finland, suggesting that *P. apollo* can overcome high Cd concentrations.

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