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Diet-switching by gypsy moth: effects of diet nitrogen history *vs.* switching on growth, consumption, and food utilization

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Abstract

Gypsy moth (*Lymantria dispar* (L.) (Lepidoptera: Lymantriidae)) larvae were reared from hatch on 1.25% N or 3.5% N artificial diet (previous diet) and switched reciprocally to the other diet (current diet) after molting into the second, third, fourth, or fifth instar. The nitrogen concentration of food consumed during previous instars had a strong residual effect on the growth rate in subsequent instars when a diet switch was made during instars two through four, but did not affect growth rate of fifth-instar larvae despite effects on food consumption and utilization. In early instars, larvae reared on 1.25% N artificial diet and then switched to 3.75% N diet had lower mass-adjusted growth rates than larvae continuously reared on 3.75% N diet. Conversely, larvae reared on 3.75% N diet and switched to 1.25% N had higher mass-adjusted growth rates than larvae reared continuously on 1.25% N diet. Relative to larvae previously reared on 1.25% N diet, fifth-instar male larvae previously reared on 3.75% N diet had slightly lower consumption rates, higher net growth efficiency (ECD), and higher gross growth efficiency (ECI). Larvae previously reared on 3.75% N diet tended to have lower food assimilation efficiency (AD) and lower nitrogen assimilation efficiency (AD(N)). Although both previous and current diet nitrogen concentration strongly affected larval growth and food utilization, the interaction term between these was not significant for any response variables except ECD and ECI. Because the interaction term reflects the effect of switching *per se*, the results indicate that there was a metabolic cost associated with switching, but no inherent net cost or benefit of diet-switching to growth.

Introduction

A polyphagous species may be either a monomorphic collection of generalists or a polymorphic collection of specialists (Fox & Morrow, 1981). The success of polyphagous individuals depends on their behavioral and physiological ability to utilize multiple hosts. However, host utilization potentially is affected by previous feeding experience. For example, recent studies of switching (changing from one diet or host to another) generally have concluded that herbivorous insect larvae exhibit reduced growth when switched to a new plant when compared to larvae that are allowed to feed continuously on the same plant (Barbosa & Capinera, 1977; Grabstein & Scriber, 1982; Schoonhoven & Meerman, 1978; Scriber, 1979, 1981, 1982; Hanson, 1976; Barbosa *et al.*, 1986; Hajek, 1989). This implies that there is a behavioral or physiological barrier

or cost which must be overcome in order for switching to be a successful strategy. However, the conclusions regarding feeding strategies and costs that can be drawn from these experiments are limited for two reasons. First, these experiments have used natural foliage. Although the use of natural foliage is appealing because it makes experimental results more applicable to natural situations, foliage varies in a multitude of uncontrolled characteristics. Thus, it is not possible to determine exactly what food characteristics led to the observed responses. Without strict control of nutritional factors it is difficult to deduce mechanisms underlying feeding behavior and physiology that may be applicable to other systems.

Second and more importantly, the design and analysis of these experiments makes it difficult to assess the relative effects of switching *per se*, independent of effects of current diet and previous diet. Previous and current diets may each have their own main effects on growth independent from one another. For example, the effect of a previous diet may be identified as the common response to the diet series A-A and A-B. Similarly, the effect of a current diet can be identified as the common response to the diet series A-A and B-A. But, if the effect of a current diet depends on what diet was consumed previously, then the effect of a change in diet, or switching effect, should be evident as a significant interaction term between previous and current diets. This is identical to saying that response to current diet depends on whether the previous diet was different (insect was switched) or previous diet was the same (insect unswitched) (Fig. 1). If a change in diet is inherently beneficial or detrimental (this need not be the case to have a significant switching effect), then a positive or negative effect should be exhibited regardless of what diet is being switched to or from. For example, if the response to diet B of an insect fed the series A-B is different from the response when fed the series B-B, and the response to diet A when fed B-A is different from the response when fed A-A, then the responses to A and B are non-additive. Statistically, then, an effect of switching *per se* will be manifest by a significant interaction term between

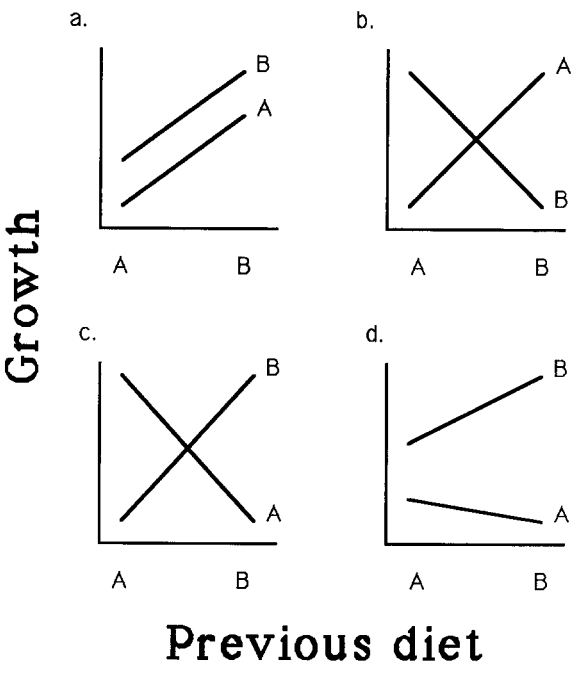

Fig. 1. Manifestation of switching effect as a significant Previous diet × Current diet interaction term. Each panel depicts all four possible Previous diet – Current diet combinations: A-A, A-B, B-B, B-A. Lines connect combinations for which Current diet is the same. Non-parallel lines indicate a Previous diet × Current diet interaction term (effect of switching). a. no effect of switching (lines are parallel). b. switched individuals perform better than unswitched individuals. c. switched individuals perform worse than unswitched individuals. d. switching effect is present, but individuals do not consistently perform better or worse than unswitched individuals.

previous and current diets. Statements regarding switching are prevented in previous studies because switches in different directions (e.g., food A to food B; food B to food A) are analyzed separately, that is, the performance of larvae switched from A to B is compared to larvae switched from A to A (i.e., not switched at all), but not with larvae switched from B to A or B to B. Without direct comparison of different switches an interaction term cannot be calculated, and the effects of switching *per se* cannot be distinguished from effects of previous diet.

Growth of an insect that experiences a shift in food quality will depend on the combined effects of previous diet, current diet, and effects of switching, if any. Previous diet, current diet, and

switching each may exact a cost or benefit to growth independent from the others through changes in larval behavior or physiology. Hence, the roles of previous diet, current diet, and switching in determining growth must be separated in order to properly evaluate insect host range and the potential limitations on feeding specialization/generalization imposed by variation in available food quality.

In this study, I investigated the effects of one-time switches between high and low nitrogen food on larval growth performance, consumption, and food utilization by gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae). The experiments have been designed and analyzed so as to permit the effects of present diet, past diet, and switching *per se* to be evaluated separately. The gypsy moth is a highly polyphagous species, partly as a result of generalist individuals. In nature, it is probably common that individual gypsy moths feed from more than one host plant or species during the juvenile period. For example, after beginning to feed, first-instar larvae may move to a new host species by silking (Mason & McManus, 1981). Later instar larvae are mobile and have also been reported to switch among host plants (Lance & Barbosa, 1982; Liebhold *et al.*, 1986). Under outbreak conditions, defoliation of hosts may force larvae to move to a new host plant or face starvation (Doane & Leonard, 1975). In addition, because rapid changes in foliage quality may be analogous in their effect to switching, insects need not even make active choices to experience changes in diet.

Materials and methods

Insects and artificial diets. Gypsy moth eggs were collected from newly infested forests in Roscommon Co., MI (84° 27′ W, 44° 11′ N) during early April 1990. To kill nucleopolyhedrosis virus adhering to their surface, eggs were surface-sterilized in 4% formalin solution for 1 h, rinsed in running tap water for 1 h, and then air dried. Eggs were hatched and larvae reared individually in 30-ml plastic cups at 25 °C with a L16:D8 light:dark regime. Fresh diet was provided to larvae as needed, usually every two days.

Two artificial diets differing in nitrogen concentration were prepared. The low nitrogen diet (1.25% N, dw) contained 0 g casein/l, and high nitrogen diet (3.75% N, dw) contained 35 g casein/l. Casein was obtained from BioServ, and was vitamin-free with less than 0.25% fat. The diet without casein was not protein-free because of the wheat germ used in the basic diet. It should be noted that, because the ratio of casein and wheat germ protein differed between the two diets, diet effects are not strictly attributable to nitrogen concentration alone and may be related to differences in protein quality. In order to maintain a constant concentration of all other components, non-nutritive alpha-cellulose was adjusted to 92 g and 57 g for low and high nitrogen diets, respectively. The basic diet contained (per liter) the following mixture: stabilized wheat germ (30.6 g), ascorbic acid (5.5 g), *p*-hydrobenzoic acid methyl ester (1.2 g), Beck's salt mix (7.8 g), sorbic acid (2.2 g), dextrose (3.3 g), choline chloride (0.6 g), BioServ Lepidopterous vitamin mix (0.7 g dissolved in 2 ml water), linseed oil (8 g), soluble potato starch (20 g). Dry components were mixed with 25 g of agar and 803 ml of deionized, distilled water.

Growth rates and nutritional indices. Approximately 60 larvae were randomly assigned to each of four switching regimes: high to low N, low to high N, low to low N, and high to high N. This design was replicated using different larvae for switches that occurred during molting into each of the second through the fifth instars. Thus larvae were reared from hatching on either high or low N diet and then either switched to a new diet during one of the four molts, or maintained on the original diet.

To obtain growth rates, fresh weights were taken as larvae entered and exited the instar in which a switch occurred. For example, a larva reared on low N diet during instars one and two might be switched to high N diet just after molting from instar two to three. Growth rate would then be measured during the third instar. Gut contents

did not contribute substantially to weight because larvae terminate feeding and void the gut 24 h prior to molting. Dry mass was measured at the end of the instar in which a switch was made. For the fifth instar, a subsample of 10 larvae was taken from each treatment at the beginning and end of the instar to estimate percentage dry mass of remaining larvae, which were reared to the pupal stage to determine sex.

Nutritional indices were obtained from fifth-instar larvae only. Upon molting into the fifth instar, larvae were weighed and provided with known amounts of fresh diet. The dry mass of diet provided to larvae was determined from 0.5-1.5 g aliquots of diet taken each day while larvae remained in the fifth instar. Following voiding of the gut at the end of the fifth instar, frass and uneaten food were dried to constant mass at 60 °C and then weighed. Nutritional indices were calculated according to Waldbauer (1968). All indices were calculated on a dry mass basis, and relative rates of consumption were based on the arithmetic mean of initial and final larval masses.

Growth rate
= larval mass gained / days

Relative consumption rate (RCR)
= mass of food ingested / (average larval mass × days)

Assimilation efficiency (AD)
= (mass of food ingested – mass of frass) / mass of food ingested

Nitrogen assimilation efficiency (AD(N))
= (nitrogen ingested – nitrogen in frass) / nitrogen ingested

Net growth efficiency (ECD)
= larval mass gained / (mass of food ingested – mass of frass)

Gross growth efficiency (ECI)
= larval mass gained / mass of food ingested.

Statistical analysis. Mean growth rates, consumption, and food utilization indices were analyzed by ANCOVA with previous and current diets as factors, each at two factor levels of 1.25% and 3.75% N. By this procedure, effects of previous diet, current diet, and switching (interaction term) can be determined. Because growth rate depends on initial mass, mass of larvae at the start of growth rate measurement was used as a covariate. When growth rates are corrected for larval mass by the covariate, values from different instars can be pooled and instar effects can be distinguished from mass effects. In particular, different initial masses arose among treatments because of different previous diets. Care must be taken in the application and interpretation of ANCOVAs when a covariate is affected by a treatment (Neter *et al.*, 1985), as it was in this case. However, there was considerable overlap of data points from different treatments across the covariate, so that the influence of previous diet on the covariate did not affect the validity or interpretation of the ANCOVAs.

Use of a covariate to standardize for effects of mass is preferable to analysis of relative growth rate (RGR = larval mass gained/(average larval mass × instar duration) because RGR declines from early to late instars (Slansky & Scriber, 1985) and hence does not satisfy the implicit assumption that growth rate varies isometrically with body size (Packard & Boardman, 1988). In theory, growth rate could be decomposed further into an ANCOVA with both larval mass and instar duration as covariates. This was not possible in this particular case, however, because of unequal slopes (interaction of Current diet with instar duration). Mean growth rates presented in tabular form were calculated for each treatment by solving the least squares regression of growth rate versus initial larval mass. The traditional measure of relative consumption rate (RCR) was analyzed by ANCOVA as dry mass food eaten with initial larval mass and instar duration as covariates. However, RCR is presented in tabular form in conjunction with dry mass eaten for comparison and convenience in interpretation. Because RCR was measured during a single instar, problems posed by non-isometric variation with body size are not as severe as with RGR. Nutritional indices of AD, AD(N), ECD and ECI were analyzed with larval mass as a covariate;

this was done so as to avoid improperly attributing variance associated with mass to a Previous diet effect. The assumptions of homogeneity of variances (via Bartlett's test) and slopes were tested as needed prior to analysis by linear models. Because assumptions become more numerous and difficult to meet in complex data sets such as this, results of tests are provided in several places to render a more complete picture of results.

According to Schmidt & Reese (1986), analysis of nutritional indices ideally is restricted to larvae which consume at least 80% of the food offered to them. In several treatments in this experiment, a substantial number of larvae failed to consume a large percentage of the food provided to them. So as not to bias results by including only larvae with high consumption rates in the analysis, percentage food consumed was not used as a criterion for entry of data into a model. However, complementary analyses (not shown) restricted to larvae which consumed $>70\%$ of the food provided to them yielded nearly identical mean indices, suggesting there was little bias, if any.

Results

Growth rates. When data from all instars were analyzed in a single model with instar included as a factor, the nitrogen concentration of both previous diet (diet consumed during instars prior to growth rate measurement) and current diet (diet consumed during the instar in which growth rate was measured) strongly influenced larval growth rate (Table 1). There was no significant effect of switching on growth rate, as indicated by the insignificant Previous × Current diet interaction term. However, the significant interactions of both previous and current diet with instar make main effects difficult to interpret. The significant interactions with instar were the result of weak responses by larvae in instar five to previous and current diets compared to responses in other instars. Therefore, data were analyzed separately for larvae switched during instars two through four, and during instar five.

When data from instars two through four were analyzed excluding the fifth instar, no interaction was found between instar and either previous or current diet (ANCOVA: $F = 0.01$; $df = 2,287$; $P = 0.986$, NS and $F = 2.08$; $df = 2,287$; $P = 0.126$, NS for Instar × Previous diet and Instar × Current diet interaction terms, respectively), so that responses to previous and current diets were not statistically different among instars two through four. This was confirmed by separate analysis for each instar; results were qualitatively identical among instars two through four. Hence, results are shown for pooled values from instars two through four. For both analyses of instars two through four, and five, cell variances were approximately equal for purposes of ANCOVA, with standard deviations falling within a two-fold range. Homogeneity of slopes was also confirmed prior to analysis by ANCOVA. For instars two through four, interactions with the covariate of initial mass were not statistically significant. Covariate interaction terms with Previous diet, Current diet and Instar, and the three-way Insect mass × Previous × Current diet term yielded, respectively, $F = 0.009$, 1.530, 0.007, 1.766 with corresponding $P = 0.926$, 0.217, 0.992, 0.185 and $df = 1,1,2,1$ where error $df = 290$. Slopes also were approximately equal in instar five. Covariate × Previous diet, Current diet and Sex, and the three-way Covariate × Previous × Current interactions were not statistically significant. Interaction terms yielded, respectively, $F = 2.28$, 0.50, 0.45, 0.47 with corresponding $P = 0.132$, 0.479, 0.505, 0.493 and $df = 1,1,1,1$, error $df = 199$. Linearity of the relationship between the dependent variable and covariate was verified visually by plotting the data. Because model assumptions were satisfied as above, analysis of growth rate by ANCOVA was viewed as an appropriate approach.

Over instars two through four, the nitrogen concentration of the current diet strongly affected growth rate, but the significant main effect of previous diet indicated that growth rate was influenced independently (additively) by the nitrogen concentration of diet consumed in previous instars (Tables 1,2). There was no significant Pre-

Table 1. ANCOVAs for growth rates of larvae switched once during the second through fifth instar between artificial diets (previous diet, current diet) differing in nitrogen concentration*

Instar	Source	ss	df	F	P
All (2–5)	Previous diet	0.841	1	21.56	<0.001
	Current diet	9.538	1	244.45	<0.001
	Instar	0.226	3	1.93	0.124
	Initial mass	1.229	1	31.49	<0.001
	Previous diet × Current diet	0.046	1	1.18	0.278
	Previous diet × Instar	0.714	3	6.10	<0.005
	Current diet × Instar	0.994	3	8.49	<0.001
	Error	19.393	497		
2–4	Previous diet	1.047	1	40.66	<0.001
	Current diet	8.308	1	322.73	<0.001
	Instar	0.226	2	4.40	0.013
	Initial mass	0.697	1	27.09	<0.001
	Previous diet × Current diet	0.003	1	0.12	0.727
	Error	7.594	295		
5	Previous diet	0.018	1	0.76	0.384
	Current diet	1.196	1	50.12	<0.001
	Sex	6.492	1	272.06	<0.001
	Initial mass	0.523	1	21.93	<0.001
	Previous diet × Current diet	0.036	1	1.50	0.221
	Previous diet × Sex	0.069	1	2.89	0.091
	Current diet × Sex	0.092	1	3.84	0.051
	Error	4.739	201		

* Because significant previous diet × instar and current diet × instar interaction terms resulted when data from all instars were pooled, separate analyses are provided for instars 2–4 and 5, as these yielded no significant interactions. Sex was determined for fifth-instar larvae only. Growth rate and initial mass were log transformed to linearize the data.

vious × Current diet interaction. In the fifth instar, growth rate was affected by current diet, but there was no statistically significant main effect of previous diet (Table 1). Current and previous diet affected females more strongly than males (Table 1: marginally significant Previous × Sex, Current × Sex interactions; Table 2: compare mean values). Again, the Previous × Current diet interaction term was not significant, indicating there was no significant effect of switching *per se* on larval growth rate.

Significant treatment differences among raw components of growth rate measures also were found (Table 2). In addition, strong sex differences typical of gypsy moth were evident. Females grew much larger than males, and the fifth instar was the penultimate instar for females, but the final instar for males. Particularly notable is the effect of Previous diet on instar duration. For instars two through four and both males and females in instar 5, instar duration was invariably longer when previous diet was high in nitrogen.

Nutritional indices. Preliminary tests of ANCOVA assumptions indicated that, for males, variances were approximately equal among cells for mass of food eaten, AD and ECD (Bartlett's test: $\chi^2 = 5.1$, df = 3, P = 0.166; $\chi^2 = 3.8$, df = 3, P = 0.285; $\chi^2 = 5.4$, df = 3, P = 0.141, respectively) and that slopes were equal (interactions with covariates all $>$ P = 0.154 excepting mass eaten, which had a Previous diet × initial mass term with P = 0.080). For AD(N), variances were statistically equal among cells (Bartlett's test statistic = 11.78, df = 7, P = 0.134) as were slopes (all interactions with mass covariate had P > 0.281) in a model with both sexes. For ECI of males and all indices for females except for ECI, unequal

Table 2. Mean growth rates of gypsy moth larvae as a function of nitrogen concentration of diet consumed in previous instars (previous diet) and in the instar in which growth rate was measured (current diet)*

Instar	Sex	Previous diet	Current diet	n	Growth** rate	Initial mass	Mass gained	Instar length
2–4	–	1.25	1.25	75	0.69^{a} (0.059)	2.37^{a} (0.233)	3.97^{a} (0.347)	5.77^{a} (0.19)
		1.25	3.75	81	1.43^{b} (0.09)	2.43^{a} (0.21)	5.38^{b} (0.37)	3.87^{b} (0.13)
		3.75	1.25	75	1.67^{b} (0.13)	5.98^{b} (0.59)	10.54^{c} (1.01)	5.90^{a} (0.28)
		3.75	3.75	71	3.73^{c} (0.29)	6.26^{b} (0.62)	15.30^{d} (1.37)	3.97^{b} (0.14)
5	Male	1.25	1.25	19	2.60^{a} (0.21)	26.65^{a} (1.26)	41.56^{a} (2.50)	15.59^{a} (0.51)
		1.25	3.75	16	3.92^{b} (0.33)	28.84^{a} (1.15)	46.90^{ab} (3.16)	11.62^{b} (0.34)
		3.75	1.25	24	3.33^{c} (0.19)	35.27^{b} (0.82)	60.01^{c} (2.51)	17.85^{c} (0.55)
		3.75	3.75	27	3.97^{b} (0.22)	35.68^{b} (0.90)	56.63^{bc} (2.37)	14.03^{a} (0.36)
	Female	1.25	1.25	27	4.97^{a} (0.39)	25.63^{a} (1.25)	39.82^{a} (2.32)	7.76^{a} (0.30)
		1.25	3.75	32	7.84^{b} (0.45)	24.16^{a} (1.14)	46.82^{a} (2.26)	5.82^{b} (0.24)
		3.75	1.25	33	7.94^{b} (0.46)	39.48^{b} (1.19)	76.47^{b} (2.92)	9.62^{c} (0.43)
		3.75	3.75	31	11.76^{c} (1.02)	37.76^{b} (0.87)	83.96^{b} (3.50)	7.32^{a} (0.46)

* Pairwise comparisons (within each instar, sex combination) are by Mann-Whitney U-tests with α adjusted to 0.05/6 to account for multiple tests.

** Growth rate was adjusted for initial mass of each larva by solving the least squares regression within each treatment. Initial mass, mass gained and days are unadjusted components of growth rate.

slopes (at least one interaction with covariate where $P < 0.05$) prevented straightforward analysis by ANCOVA. For this reason, model results for all measures but AD(N) are presented for males only (Table 3). Conservative, nonparametric pairwise comparisons of mean values (Table 4) supplement ANCOVA results and, excepting AD (see below) indicate females did not deviate strongly from males in response to diet treatments.

Current and previous diets affected all nutritional indices of males at a significance level of 0.10 or less (Table 3). The interaction term between Previous and Current diet did not explain significant variance in any of the nutritional indices except ECD and ECI. This suggests a metabolic but not digestive cost of switching. Relative to larvae previously reared on low nitrogen diet, larvae previously reared on high nitrogen diet had a tendency towards lower RCR, higher ECD, and higher ECI. AD was lower or equal after rearing on high nitrogen diet. AD of males previously reared on low nitrogen diet was higher than that of females. But, AD's of males and females were

Table 3. ANCOVAs for food consumption and utilization by fifth-instar male gypsy moth larvae reared during previous instars on artificial diet containing 1.25% or 3.75% nitrogen (previous diet), and fed either 1.25% or 3.75% nitrogen diet during the fifth instar (current diet)*

Nutritional index	Source	ss	df	F	P
Mass eaten	Previous	13.23	1	2.83	0.097
	Current	1259.38	1	269.44	<0.001
	Initial mass	166.08	1	35.53	<0.001
	Days	1.18	1	0.25	<0.616
	Previous × Current	0.95	1	0.20	0.653
	Error	317.84	68		
AD	Previous	0.030	1	13.70	<0.001
	Current	1.976	1	891.33	<0.001
	Initial mass	0.025	1	11.36	0.001
	Previous × Current	0.006	1	2.74	0.103
	Error	0.153	69		
AD(N)	Previous	0.034	1	7.28	0.009
	Current	0.175	1	37.14	<0.001
	Sex	0.121	1	25.604	<0.001
	Initial mass	0.021	1	4.55	0.038
	Previous × Current	0.001	1	0.29	0.591
	Error	0.236	50		
ECD	Previous	0.033	1	7.466	0.007
	Current	0.019	1	4.38	0.040
	Initial mass	0.015	1	3.42	0.068
	Previous × Current	0.027	1	6.06	0.016
	Error	0.303	69		
ECI	Previous	11.11	1	2.97	0.089
	Current	2571.36	1	688.54	<0.001
	Initial mass	0.74	1	0.20	0.657
	Previous × Current	80.77	1	21.63	<0.001
	Error	257.68	69		

* Tests are for males only, except AD(N), for which both sexes were used. AD = assimilation efficiency; AD(N) = assimilation efficiency of nitrogen; ECD = net growth efficiency; ECI = gross growth efficiency. To equalize variances and provide linearity with the covariate, dry mass eaten was square root transformed, ECI was reciprocal transformed, AD and ECD were log transformed and AD(N) was arcsin transformed.

approximately equal when previous diet was high in nitrogen, suggesting that males can better cope with previously low nitrogen diet than can females. Initial larval mass was highly significant for AD (Table 3), and regression indicated a positive relationship between AD and body mass. So as not to introduce bias caused by larval mass, mean AD's in Table 4 are adjusted for initial mass. Larvae currently consuming high nitrogen diet had lower RCR, higher AD, higher ECD, and higher ECI than larvae consuming low nitrogen diet (Table 4).

Percentage nitrogen in frass (includes both digested and undigested nitrogen) was significantly lower when the previous diet was low in nitrogen (Table 5; ANOVA: $F = 11.4$; $df = 1,49$; $P = 0.001$). Although males excreted more nitrogen in their frass than females, a greater proportion of this nitrogen was in the form of uric acid, which is composed of digested nitrogen (Table 5). Despite this, females had higher AD(N), which is corrected for frass uric acid content. Mean values suggest a stronger Previous diet effect on males, although the interaction term with sex was not

Table 4. Effect of diet nitrogen concentration provided during previous and current instars on food consumption and utilization by fifth-instar male and female gypsy moth larvae*

Sex	Percentage nitrogen		n	Nutritional index				
	Previous diet	Current diet		Mass eaten	RCR	AD	ECD	ECI
Male	1.25	1.25	18	923^{a} (24)	2.98^{a} (0.10)	0.145^{ac} (0.003)	0 310^{a} (0.010)	0.044^{a} (0.001)
	1.25	3.75	11	383^{b} (3)	1.47^{b} (0.06)	0 338^{b} (0.009)	0.362^{ab} (0.018)	0.121^{b} (0.005)
	3.75	1.25	23	1158^{c} (25)	2.26^{c} (0.09)	0 142^{a} (0.004)	0.362^{b} (0.009)	0.051^{c} (0 001)
	3.75	3.75	22	524^{d} (17)	1.36^{b} (0.04)	0.302^{b} (0.009)	0.360^{ab} (0 012)	0.108^{b} (0.003)
Female	1 25	1.25	23	664^{e} (25)	4.78^{d} (0.16)	0.110^{c} (0.005)	0.537^{c} (0.031)	0.056^{c} (0.001)
	1.25	3 75	27	255^{f} (8)	2.04^{e} (0.08)	0.288^{b} (0.009)	0.627^{cd} (0.024)	0.177^{d} (0.003)
	3.75	1.25	29	1051^{g} (29)	3.04^{d} (0 09)	0.140^{ac} (0.003)	0.533^{c} (0.013)	0.071^{e} (0.001)
	3.75	3 75	27	406^{b} (6)	1.54^{b} (0.08)	0.316^{b} (0.008)	0.652^{d} (0.022)	0.204^{f} (0.006)

Means are reported with standard errors in parentheses. RCR = relative consumption rate; AD = assimilation efficiency, adjusted for initial larval mass; ECD = net growth efficiency; ECI = gross growth efficiency. The significance of pairwise comparisons within each index was determined by Mann-Whitney U tests; values with different letters indicate means that were significantly different (α set at 0.0018 to adjust for multiple comparisons).

significant (P = 0.099). Overall, AD(N) was significantly higher for larvae previously reared on low nitrogen diet (Tables 3,5).

Discussion

Growth rates. That diet nitrogen content strongly affected growth, consumption, and food utilization of larvae during the instar in which the diet was consumed is not surprising. Higher growth rate, lower RCR, and higher utilization efficiency on high nitrogen food as was found in this study is typical of lepidopteran larvae (Scriber & Slansky, 1981). More interesting is the dependence of larval growth during the second, third, and fourth instars on the diet consumed during previous instars. During instars two through four, larvae reared previously on a low nitrogen diet had lower growth rates than larvae reared previously on a high nitrogen diet (Table 2). Similarly, larvae reared previously on high nitrogen diet had higher growth rates in the subsequent instar, regardless of the current diet. Provided that size effects are largely accounted for by the covariate, this indicates that there was some residual behavioral or physiological effect of previous diet that was carried through to the instar following a switch. Evidence from measures of food consumption and utilization indicates modification of both behavior and digestive physiology (see *Nutritional indices*, below).

In contrast to earlier instar larvae, there was no net effect of previous diet on the growth rate of fifth-instar larvae. Increased length of exposure to a previous diet did not enhance the effect of pre-

Table 5. Mean frass uric acid concentration, percentage nitrogen in frass, and nitrogen digestibility (AD(N), in %) of fifth-instar male and female gypsy moth larvae previously reared on 1.25% N or 3.75% N artificial diet, and switched to or maintained on 1.25% N or 3.75% N diet during the fifth instar*

Percentage nitrogen		Sex	Uric acid in frass (mg/g)		Percentage nitrogen in frass		AD(N)**	
Previous diet	Current diet		Mean	SEM	Mean	SEM	Mean	SEM
1.25	1.25	Male	0.85	0.63	0.64^{a}	0.018	44.9^{ac}	1.4
		Female	0.10	0.10	0.53^{b}	0.026	51.7^{ab}	2.6
1.25	3.75	Male	13.25	0.49	2.60^{c}	0.045	58.2^{bd}	1.0
		Female	1.72	0.54	2.01^{de}	0.098	60.7^{b}	2.4
3.75	1.25	Male	2.58	0.69	0.74^{a}	0.042	40.1^{c}	3.9
		Female	1.71	0.95	0.58^{b}	0.017	52.9^{abd}	1.7
3.75	3.75	Male	13.90	1.20	2.91^{ce}	0.110	50.6^{acd}	2.3
		Female	2.40	0.51	2.07^{d}	0.079	61.1^{b}	1.6

* Percentage data were arcsin and then log transformed to satisfy the assumptions of normality and homogeneity of variances. SEM is the standard error of the mean. Sample sizes are 3,7 and 7 for uric acid, percentage N and AD(N), respectively. Pairwise comparisons are by Tukey's HSD; treatments with different letters are significantly different at $\alpha < 0.05$.
** AD(N) is corrected for uric acid content of frass.

vious diet on growth rate, as might be expected if there is some physiological conditioning on the diet that strengthens over time. One possible explanation is that growth in later instars is via accumulation of lipid rather than protein. Hence, dietary nitrogen may be less important to growth during the fifth instar. Regardless of the cause, the lack of response to previous diet by fifth-instar larvae indicates that the impact of a switch on growth rate can be significantly altered by the timing of a switch between diets. Rapid changes in foliage quality may be analogous in their effect to switching, so that the timing of a switch may be only partially dictated by larvae actively making choices. If so, then disparities in the phenology of foliage and larval development may shift the timing of a switch relative to the developmental age of larvae. In particular, variation among host plants in the timing of a downward shift of foliage quality during larval development could potentially contribute to variation in larval growth rates.

The independent effects of previous diet history, current diet, and larval mass on growth rate have an important implication. Larvae of identical mass and sex that are fed the same diet may not have identical growth rates if previous diet histories are different. Neither current diet nor larval mass alone are necessarily good predictors of growth rate. Investigators should consider this when planning and interpreting experiments in which larval mass is used to correct for differences in growth rates across treatments, especially when larvae are shifted from one diet to another.

Nutritional indices. Many investigators have found preference induction of insects reared on natural foliage (Szentesi & Jermy, 1990). Insects with induced preferences exhibit lower consumption rates when provided with a novel food in a no-choice situation (Stride & Straatman, 1962; Jermy *et al.*, 1968; Ma, 1972, 1976; Hanson, 1976; Barbosa *et al.*, 1979). Larvae in this experiment did not exhibit preference induction; switched larvae did not consume food with novel nitrogen concentration at a lower rate than unswitched larvae. Rather, they exhibited what might be called 'consumption rate induction' in that larvae resisted a change in consumption rate from that

established during the previous instar. Increased consumption rate is a typical response of Lepidopterous larvae to compensate for low food nitrogen (Mattson, 1980; Scriber & Slansky, 1981; Slansky & Scriber, 1985; Simpson & Simpson, 1991), but in the case of switched larvae, the response was suppressed. This is important because it represents an alteration or partial failure of the normal compensatory response to diet nitrogen concentration.

A lack of influence of previous diet on assimilation efficiency (AD) was reported by Scriber (1982) for *Spodoptera eridanea* larvae switched among mountain ash, paper birch, and black cherry. In the present study, however, both AD and AD(N) were affected by previous diet nitrogen concentration (Table 3). This situation indicates that there was a residual effect of previous diet on the digestive process in the gut. Induction of gut enzymes in response to food type and volume has been reported in several species of insects (Engelmann, 1969; Ishaaya *et al.*, 1971; Christopher & Mathavan, 1985; Broadway & Duffey, 1986). The shifts in diet nitrogen concentration and coincident changes in RCR may have been large enough to trigger changes in gut enzyme levels, leading to changes in digestion.

Like digestion, both net and gross growth efficiency were affected by previous diet. Larvae previously reared on high nitrogen diet had higher net growth efficiency (ECD) than larvae previously reared on low nitrogen diet. This indicates that larvae previously on low nitrogen diet allocated relatively more of assimilated food towards energy metabolism than to growth. Gypsy moth larvae reared on low and high nitrogen diets similar to those used in this study did not differ in respiration rates (Stockhoff, 1991), suggesting that the higher ECD of larvae on high nitrogen diet was not caused by lower standard metabolic rate. Because larvae on low nitrogen food had a higher relative consumption rate, the extra metabolic expenditure could be the result of longer periods of activity (feeding) than larvae previously reared on high nitrogen food, which likely spent less time feeding and more time resting.

In instars two through four, previous rearing on high nitrogen diet led to higher growth rates than did rearing on low nitrogen. If previous diet affected food consumption and utilization by fifth-instar larvae, but not growth rate, then why was the growth rate of larvae in earlier instars affected by previous diet? In order to generate the observed growth rates during instars two through four, previous diet effects on food consumption or utilization must be different from that of fifth-instar larvae. Specifically, the product of RCR and ECI must yield higher growth rates on previous diets high in nitrogen. Relative to fifth-instar larvae, one of three conditions is required. First, the effect of previous diet on RCR may be weaker (or opposite), so that effects of ECI predominate. Second, the effect of previous diet on ECI may be stronger, so that the opposite effects of RCR are more than balanced out. Third, both RCR and ECI may be different in the directions described. Put in more general terms, relative to later instar larvae, early instar larvae appear to be less responsive behaviorally or more responsive physiologically to previous diet.

The third hypothesis simultaneously predicts both increased plasticity in behavioral response of early instar larvae relative to fifth-instar larvae (because previous diet had less of an effect on RCR, and response was more directly tied to current diet), and decreased plasticity in physiological response (because the effect of previous diet was greater on ECI). Relatively greater behavioral plasticity of early-instar larvae could be explained by larvae not having as long a previous rearing period to become fixed on some consumption rate. Greater dependence of ECI on the nitrogen concentration of previous diet could be the result of greater lag time in the physiological response to new nitrogen levels. Little is known of the feeding behavior and digestive physiology of early instar Lepidoptera, or ontogenetic changes in these. Study of all juvenile stages would likely be revealing with regard to the above hypotheses and insect-plant interactions in general.

Sex effects. Two important conclusions are supported by the observed responses by sex. First, males and females responded similarly to a shift

in diet nitrogen concentration, so that switching as a strategy (with respect to nitrogen) is not likely to be favored by one sex or the other. However, the marginally significant interactions between sex and previous diet for growth rate and AD(N) suggest that the potential for divergence should not be ruled out. Second, the higher mass-adjusted growth rates, consumption rates, and food utilization efficiency of females are the result of fundamental physiological and behavioral differences between males and females. Assuming statistical removal of size effects was complete, sex differences were not caused by allometric changes associated with the larger body size of females. This is important outside the immediate context of switching because it implies 1) males and females consuming identical diets will utilize the food differently, and by doing so will not achieve identical goals (*e.g.* body size); 2) males and females must choose different diets in order to achieve similar goals. The extent to which the goals of the sexes differ (*e.g.* in gamete production, flight muscle mass) will dictate how the actual nutritional requirements and diet choices of males and females will differ. Although sex differences in nutrition have been recognized (Slansky & Scriber, 1985), the significance of these differences to diet choice is unclear because confounding effects of body mass are rarely sorted out. Because larval mass was used as a covariate (for AD, AD(N), ECD, ECI) or incorporated into the index (for RCR), sex differences are not statistically attributable to differences in body size.

Effect of switching. Net and gross growth efficiency (ECD, ECI) were the only response variables to show an effect of switching. Although statistically significant, the biological significance of the effect of switching on ECI is difficult to identify. First, the effect of switching was not consistently positive or negative. That is, switched larvae did not perform worse or better than unswitched larvae in every case (recall Fig. 1). For example, ECI of females currently eating high nitrogen diet had significantly higher ECI when unswitched (0.204) than when switched (0.177). However, females currently on low nitrogen diet had higher ECI when switched (0.071) than when unswitched (0.056). Second, switching resulted in less than a 3% absolute difference in ECD and ECI (e.g., ECD: $|\{0.627\text{-}0.537\}\text{-}\{0.652\text{-}0.533\}| < 0.03$; ECI: $|\{0.177\text{-}0.056\}\text{-}\{0.204\text{-}0.071\}| < 0.03$). Third, because of the compensatory effect of consumption rate discussed above, there was no net effect of switching on growth rate. The lack of a significant Previous × Current diet interaction term for larval growth rate indicates that there was *no effect of switching per se* on growth rate. Previous and current diet nitrogen effects were additive, so that the effect of current diet nitrogen concentration (*i.e.*, the difference between growth rates of larvae currently feeding on high and low nitrogen) was independent of the previous diet. The biological implication is that, with respect to nitrogen, effects of host-plant switching on growth rate may be attributable solely to the quality of the initial diet rather than to the change in diet quality *per se*.

The lack of a biologically significant effect of switching provides an interesting contrast to Sheppard & Friedman (1990). In their study, third-instar gypsy moths were reared on oak, pine, or a high wheat germ artificial diet, and nutritional indices measured. Main and interactive effects of host plant consumed during the third instar (oak or pine), diet consumed during the second instar (artificial diet or foliage), and phenology of foliage (early or late) were examined. The host plant × diet history interaction term was significant for AD, ECD, ECI, and RCR, but not RGR. This interaction term, however, is not entirely analogous to the Previous × Current diet interaction term discussed in this paper, and cannot be interpreted as a switching effect. Given this limitation, however, that previous and current diets can interact is clear. As the authors point out, precisely what foliage characteristics were involved is not known. The results presented in this paper suggest that nitrogen alone is insufficient to produce interactive effects in gypsy moth.

The costs and benefits of specialization and generalization have received considerable attention by investigators interested in the evolution of insect feeding habits (see Special feature: Insect

host range; Ecology 69:886-915). The costs associated with a polyphagous feeding habit have been investigated primarily in terms of induction and maintenance of detoxication systems. However, plant secondary chemicals are not the only potential source of feeding costs for insects. Because digestive enzyme systems, like detoxication systems, may have induction and operating costs, variability in plant nutrients may be important in the evolution of specialized versus generalized feeding habits. Because no strong negative (or positive) effect of switching on growth rate was found, the results of this study suggest that there is not strong selective pressure against a generalists feeding habit (with regard to food nitrogen) by individual gypsy moth larvae. Similar, comparative studies of specialist species and generalists species composed of specialist individuals will prove interesting.

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