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Assessing larval food quality for phytophagous insects: are the facts as simple as they appear?

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Summary

1. We argue here that host plant quality affects many life-history traits of herbivorous insects and these traits often interact. Studies that look only at a limited number of traits often fail to determine the overall effect of plant quality on larval performance and adult fitness. Parameters such as mating success and adult longevity are frequently neglected even though they are affected by larval feeding and are crucial to overall fitness.

2. To illustrate this, we examined a whole suite of life-history traits of the moth *Lobesia botrana* after rearing larvae of this grape pest on three different grape cultivars.

3. Development time, mating success, fecundity, egg size and fertility were differentially affected by the cultivar on which the larvae were reared. Our results highlight that decreased fecundity can be balanced by an increase in egg size and fertility.

4. Throughout this study, we emphasize that drawing conclusions about larval food quality can be spurious when based on too few life-history traits. Therefore, it is essential to study all fitness related life-history traits to fully understand the effects of larval food quality on herbivore fitness.

Key-words: Female reproductive output, grapes, larval performance, *Lobesia botrana*, trade-off

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Introduction

Larval food quality is the determinant for the fitness of phytophagous insects with a non-feeding adult stage. In those species, reproductive potential critically depends upon resource accumulation during the larval stage. The amount and the quality of the food ingested by the larva strongly influence the storage of resources that are allocated to reproduction (Slansky & Rodriguez 1987; Awmack & Leather 2002).

Numerous studies have examined the influence of larval food quality on fitness components such as larval and adult performance (Awmack & Leather 2002 for a review; Kaspi *et al*. 2002 for an example). However, most of these studies consider only a few fitness parameters (see Tammaru, Esperk & Castellanos 2002 for an exception). Larval performance has often been estimated in terms of larval growth and survival, ignoring the influence of larval food on the timing of adult emergence, which can be an important determinant of reproductive success (Fagerström & Wiklund 1982; Tikkanen, Niemela & Keranen 2000). For example, when the time-lag between emergence of males and females becomes critical with regard to their life span, mating success can be impaired, leading to a reduced overall individual fitness. This may turn critical, especially in adults with a short life span. Potential fecundity measured as the number of eggs in the ovarioles is often used as a component of adult performance (e.g. Wint 1983; Savopoulou-Soultani, Nikolaou & Milonas 1999; Tikkanen *et al*. 2000). However, it often fails to predict achieved fecundity (number of eggs laid by a female during her lifetime) (Leather 1988; Awmack & Leather 2002). Another important trait that is seldom examined is the time between the date of emergence and a successful mating, which directly influences the number of fertile eggs that a female will lay during her lifetime. For a given life span, the earlier a female will mate, the longer she may be able to lay eggs. Moreover, among existing studies, female mating success (measured as a probability for a female to mate) has often been ignored (i.e. Leather *et al*. 1998;

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Yanagi & Miyatake 2002), even though this parameter seems crucial in determining the fitness of phytophagous insects (Awmack & Leather 2002 and references therein). Assessing the proportion of mated females in a population is obviously crucial to determine the proportion of females that contribute to offspring production. Such an omission is thus surprising since it is well known that larval food quality can influence mating probability by affecting the production and quality of pheromone emitted by females or males (Landolt & Phillips 1997 for a review). Egg size is also considered as a crucial reproductive parameter and, similarly, only a few studies have examined this trait. Generally, females laying larger eggs have higher hatching success and may produce progeny with higher fitness than females laying smaller eggs (Nakasuji 1987; Karlsson 1989; Fox & Czesak 2000; Roff 2002; Torres-Vila & Rodriguez-Molina 2002).

For insects with a non-feeding adult stage, a limited amount of resources will result in a trade-off in the allocation of these resources to different life-history traits. Thus, a decrease in one trait could be balanced by the increase of another. A classical example is the negative relationship that exists between offspring number and offspring size (e.g. Montague, Mangan & Starmer 1981; Roff 2002; Fox & Czesak 2000 for a review and references therein). In this context of trade-off, there is now good evidence that reproduction reduces female longevity (Chapman *et al*. 1998 and references therein). Because of such trade-offs, herbivore performance could be affected in different ways, and assessing only one component as an estimate of fitness could be misleading. Therefore, for herbivorous insects, larval food quality should be examined taking into account as many life-history traits as possible.

In this study, we examined the influence of larval food quality on a whole suite of life-history traits and we tested how these traits are linked. This was achieved using a polyphagous insect which is the most harmful pest of grapes in Europe, *Lobesia botrana* (Denis and Schiffermueller) (Lepidoptera: Tortricidae) (see Bovey 1966; Roehrich & Boller 1991; Thiéry 2005 for reviews on its biology). Variation in food quality was obtained by offering to the larvae three different cultivars of grapes incorporated into a standardized artificial diet. The effect of larval food quality was measured from egg hatching to individual death. Specifically, we recorded developmental life-history traits such as larval development time, larval survival and pupal mass. Effects of larval food quality on components of adult reproductive success were also measured in terms of mating success, fecundity, egg size, egg hatchability and longevity. The first part of the results describes how cultivars affect different life-history traits and in the second part we examine how the different variables are intercorrelated. In the last part, a discriminant analysis has been performed in order to determine which variables are the most relevant in assessing larval food quality.

Materials and methods

STUDY SYSTEM: MOTHS, THEIR ORIGIN AND MAINTENANCE

The strain of *L. botrana* (INRA Bordeaux) used for this study originated from individuals collected in a French Sauternes vineyard (cultivar White Sauvignon). This culture is based on important number of adults caged (several thousands per week), in order to avoid genetic bottlenecks. The stock culture is maintained on a semi-artificial diet at 24 ± 1 °C, 60 ± 10% r.h. with a photoperiod of 15:8 h light : dark + 1 h of dusk. The first 15-photophase hours were at 1000 lux luminosity and the last one (dusk) at 25 lux. All following tests were performed under these same conditions.

LARVAL DIET TREATMENTS AND GENERAL PROCEDURE

The grape cultivars were compared using a standardized procedure adapted from Mondy & Corio-Costet (2000) and similar in principle to that described elsewhere (Thiéry & Moreau 2005; Moreau, Benrey & Thiéry 2006a; Moreau *et al*. 2006b). This procedure uses an artificial medium in which freeze-dried fine powder of plant material is incorporated. It has at least three main advantages: (a) feeding isolated larvae prevents competition and subsequent food deprivation, (b) it prevents differences in grape bunch compactness, which has an effect on larval feeding behaviour (J. Moreau, B. Benrey and D. Thiery, unpublished observation) and on the climatic environment of the larvae (Pieri & Fermaud 2005) and (c) it also prevents the incidence of infections by fungi on grapes which affect larval fitness (Savopoulou-Soultani & Tzanakakis 1988; Mondy & Corio-Costet 2000).

Larvae were raised individually to pupation in Eppendorf tubes filled with 1·5 ml of a medium composed (for 100 Eppendorfs): 150 ml water, 5 g agar, 6 g cellulose powder, 4 g vitamin-free casein, 3·5 g glucose, 2 g mineral salt, 0·12 g cholesterol, 0·12 g maize oil, 0·25 g benzoic acid, 0·1 g nipagine and 12 g freeze-dried plant powder. The plant powder was obtained from bunches of *V. vinifera* cv. 'Pinot', 'Merlot' and 'Riesling'; all harvested from our 'gene collection of grape plants' 'Domaine de la Grande Ferrade', INRA-Bordeaux. The bunches were collected at the beginning of the growing season (beginning of May 2003) at phenological stages 23–27 (Eichhorn & Lorenz 1977), which correspond to the grape phenology on which the first annual generation of *L. botrana* occurs.

The Eppendorf lids were pierced to allow air circulation. Using a fine brush, newly hatched larvae (age < 24 h) were transferred individually to the diets in each Eppendorf, 100 larvae per diet. Neonate larvae issued from eggs oviposited from thousands of caged females were randomly chosen and assigned to the different diets. Eppendorf tubes were randomized in

the Eppendorf racks and racks were moved every 3 days in the climatic chamber in order to minimize the effect of possible climatic gradients.

LARVAL PERFORMANCE

The 100 larvae obtained from each diet were monitored daily until pupation; pupae were then carefully removed from the diet and weighted to the nearest 0·1 mg. Because adult moths are difficult to be weighed with enough accuracy, we used the mass of living pupae as an index of adult body size. Pupae were placed individually in glass tubes (70 mm × 9 mm diameter) covered with a cotton plug and stored in the test room until emergence. Adults were sexed upon emergence. We recorded the following variables: (1) total survival (larval + pupal) (= percentage of adults emerged), (2) pupal mass, (3) total development time (larval + pupal) and (4) adult sex-ratio.

ADULT PERFORMANCE: REPRODUCTIVE LIFE-HISTORY TRAITS

All newly emerged adults resulting from the three larval diets were used to evaluate the reproductive output of females. Newly (less than 1-day-old) emerged females were individually confined in 0·5-l transparent cellophane bags as mating and oviposition chambers and provided with water *ad libitum* through a soaked cotton dental wick. One or 2-day-old virgin males originating from the same diet were added to each caged virgin female 1 h before dusk, which is just before their sexual activity (Bovey 1966). Males were randomly assigned to females, paired and caged in these bags until the death of both sexes.

Females could behave and oviposit freely inside the cellophane bag until death. At the end of the experiment, eggs on the walls of the cellophane bags were measured with an ocular micrometer. Their surface (estimated as an elliptic surface, $S = \pi \times a \times b$ (mm^2)), where a and b are the ellipse semi-axes) was used as an index of egg size. Mean egg size was estimated for each female from a randomly chosen sample representing 15% of the total number of eggs laid. All eggs were measured for females that laid less than 15 eggs during their lifetime.

To estimate fertility (= hatching success), each measured egg was incubated at 22 °C for 10 days until hatching. Mated but unfertilized females were excluded from later analyses.

Seven variables were considered: (1) % of mated females (female mating success) assessed by the production of fertile eggs (non-mated female are able to lay some infertile eggs at the end of their life), (2) delay before the first egg is laid (in days), (3) total achieved fecundity (number of eggs laid), (4) duration of egg-laying (in days), (5) overall mean egg size, (6) female fertility (% of hatched eggs) and (7) longevity of mated females (in days). From egg size and the number of eggs laid, we calculated an index termed reproductive output (= egg size × fecundity), considered as an important parameter in the estimation of the investment of female resources (Yanagi & Miyatake 2002).

CALCULATION OF 'FITNESS INDEX'

The life-history traits assessed during this study allowed us to estimate the fitness of *L. botrana* females. First, we calculated the mean number of larvae produced per mated female using fecundity and fertility. From this, we calculated the number of females that emerged by using larval + pupal mortality and the sex ratio. Finally, from the mating success, we calculated the number of mated females that contributed to the production of the next generation. We then used this estimation as an overall measure of fitness (= fitness index).

STATISTICAL ANALYSIS

All statistical tests were performed using JMP software (Version 3·2.2, SAS institute Inc.), except the discriminant analyses which were performed using SPSS software (Version 7·5). A stepwise analysis (backward procedure) was used to remove non-significant ($P > 0·05$) effects and interactions (SAS Institute 1995). Only the resulting models are presented here. Kruskal–Wallis or Wilcoxon rank sum non-parametric tests were used when data did not meet assumptions of normality (Shapiro–Wilks' test) or homogeneity of variance (Levene's test). Two-tailed tests of significance were used throughout the analysis. To evaluate the effects of larval diet on development time until emergence of the moths, we used parametric survival analysis since the assumption for the Weibull distribution was not violated.

A large number of variables were assessed during this study. We first considered whether the different variables are intercorrelated. To this purpose, we performed a multiple correlation of Spearman simultaneously among life-history traits assessed in *L. botrana* females. The correlations between the different variables were tested using the whole set of data generated with the three cultivars. This was allowed by a preliminary analysis among cultivars which indicated the same relationships in the three treatments (MANOVA, $F_{2,112} = 15·62$, $P = 0·38$). In order to reduce the incidence of false positives, we applied the Bonferroni correction (procedure described in detail in Sokal & Rohlf 2001).

Secondly, a discriminant analysis was classically used to screen out the core variables that described from the whole set which ones are interrelated. A stepwise discriminant analysis was thus carried out using a 'forward' procedure, which begins with no variables in the model and adds the variables with the greatest discriminating power (procedure described in detail in Legendre & Legendre 1998). This analysis screens out

Table 1. Larval and pupal life-history traits of *Lobesia botrana* according to the larval food. Values are presented with mean ± SEM. We started with 100 larvae per cultivar

Life-history traits		Larval food			Statistical test	
		Pinot	Merlot	Riesling	χ^2_2	*P*
Development time (days)	Female	29·0 ± 0·3	29·9 ± 0·2	31·2 ± 0·2	18·7	< 0·001[a]
	Male	27·0 ± 0·4	27·9 ± 0·2	28·7 ± 0·3	6·5	0·04[a]
Pupal mass (mg)	Female	14·3 ± 0·3	14·2 ± 0·2	13·6 ± 0·3	4·9	0·08[b]
	Male	9·66 ± 0·3	9·7 ± 0·2	9·8 ± 0·3	0·4	0·83[b]
% of emergence		89	98	96	4·6	0·09[c]

[a]Parametric survival analysis, [b]Kruskall-Wallis test, [c]Pearson χ^2.

the variables that allow differentiation among cultivars. The input parameters for the discriminant analysis were group variables (Pinot, Merlot and Riesling) and independent variables (all life-history traits measured during experiments). Qualitative variables, such as mating success, were transformed in numerical variables (see Legendre & Legendre 1998 for statistical details).

Individuals are named further according to their diet of origin (e.g. Riesling larvae, Riesling females …).

Results

LARVAL AND PUPAL TRAITS

For the three cultivars, males reached adulthood significantly faster than females, and development time of both sexes varied slightly, but significantly, according to the cultivar (Table 1) (parametric survival analysis: general model: $\chi^2_5 = 80{\cdot}74$, $P < 0{\cdot}0001$; effect of cultivar: likelihood ratio (L-R) $\chi^2_2 = 37{\cdot}46$, $P < 0{\cdot}0001$; effect of sex: L-R $\chi^2_1 = 44{\cdot}84$, $P < 0{\cdot}0001$; effect of their interaction: L-R $\chi^2_2 = 0{\cdot}24$, $P = 0{\cdot}89$). No significant difference was found in the pupal mass of males and females according to the cultivars. However, in all cultivars, female pupae were always heavier than male pupae (two-way ANOVA: general model: $F_{5,272} = 68{\cdot}37$, $P < 0{\cdot}0001$; effect of sex: $F_{1,272} = 333{\cdot}89$, $P < 0{\cdot}0001$; effect of cultivar: $F_{2,272} = 0{\cdot}49$, $P = 0{\cdot}61$; effect of their interaction: $F_{2,272} = 1{\cdot}13$, $P = 0{\cdot}32$). The proportion of moths that emerged from the three cultivars was not significantly different (almost 90% of larvae reached the adult stage).

ADULT REPRODUCTION

There was no effect of diet on the sex ratio of the emerging adults (Table 2). Female mating success, measured as the number of mated females, was significantly different among cultivars. Particularly, 'Riesling' females were less successful at mating than females of other origins. Among mated females, the first egg was laid 4·5 days after presenting males to females, and this was not affected by the grape cultivar. The duration of the egg-laying period was however, slightly affected by the cultivar. Fecundity, egg size and fertility of mated females were strongly affected by the cultivars but not in the same way. The 'Riesling' females laid fewer but larger eggs than females from the other cultivars. Thus, 'Riesling' females exhibited a higher hatching success. If we consider a trade-off between the number of eggs laid and their size, the overall reproductive output of females from the three cultivars did not differ. Finally, the longevity of mated females was not different among cultivars.

FITNESS INDEX

The values obtained with the fitness index (Table 3) showed that 'Merlot' females produced more females participating to the next reproduction than females from the other two cultivars (Kruskal–Wallis test, $\chi^2_2 = 23{\cdot}26$, $P < 0{\cdot}0001$).

CORRELATIONS AMONG VARIABLES

A prolonged development time resulted in heavier females that lived longer than smaller ones (Table 4). Fecundity was positively correlated with both female pupal mass and duration of laying, and negatively

Table 2. Adult life-history traits of female *Lobesia botrana* according to the larval food. Within each line, values (mean ± SEM) with the same letter are not significantly different ($P > 0{\cdot}05$) after a non-parametric PLSD test. We started with 100 larvae per cultivar

Life-history traits	Larval food			Statistical test	
	Pinot	Merlot	Riesling	χ^2_2	*P*
Female sex ratio	62·9	48·9	54·2	3·6	0·16[a]
% of mated females (no. females tested)	78·2 (55)	97·7 (43)	65·4 (52)	15·0	<0·001[a]
Delay before laying (days)	4·5 ± 0·4	4·33 ± 0·4	4·8 ± 0·5	1·1	0·58[b]
Duration of laying (days)	6·0 ± 0·3 A	5·7 ± 0·4 AB	4·8 ± 0·3 B	7·1	0·03[b]
Fecundity (= no. eggs laid)	123·4 ± 9·2 AB	131·6 ± 9·8 A	104·3 ± 6·5 B	8·7	0·01[b]
Egg size (mm^2)	1·4 ± 0·02 A	1·4 ± 0·1 A	1·6 ± 0·1 B	34·1	<0·001[b]
Fertility (= % of hatched larvae)	69·6 ± 2·2 A	72·3 ± 1·1 A	89·7 ± 1·6 B	48·0	<0·001[b]
Reproductive output	186·2 ± 13·9	195·4 ± 14·1	168·4 ± 10·8	4·5	0·10[b]
Longevity of mated females (days)	10·4 ± 0·3	10·0 ± 0·3	9·6 ± 0·3	2·0	0·36[b]

[a]Pearson χ^2, [b]Kruskal–Wallis Test.

Table 3. Number of estimated females (mean ± SEM) participating in reproduction in the next generation of *Lobesia botrana* according to the larval food and to the life-history traits assessed during this study. See 'Calculation of fitness index' in Materials and methods for the details of the calculation

Larval food	Mean no. larvae produced per mated female (using Fecundity * Fertility)	No. adults emerged from larvae produced (using % of emergence)	No. females produced (using sex-ratio)	No. females participating to reproduction (using mating success) = Fitness Index	PLSD test after Kruskal–Wallis tests on fitness index
Pinot	90·0 ± 7·7	80·1 ± 6·9	50·5 ± 4·3	39·4 ± 3·4	A
Merlot	121·3 ± 9·1	118·9 ± 8·9	58·2 ± 4·4	57·1 ± 4·3	B
Riesling	93·1 ± 6·5	89·4 ± 6·2	48·3 ± 3·4	31·4 ± 2·2	A

Table 4. Spearman correlation coefficient among life-history traits assessed in *L. botrana* females

	Female development time	Female pupal mass	Delay before laying	Duration of laying	Fecundity	Egg size	Fertility	Longevity
Female development time	1·00							
Female pupal mass	0·32*	1·00						
Delay before laying	–0·03	–0·008	1·00					
Duration of laying	0·01	0·032	–0·59*	1·00				
Fecundity	0·003	0·26*	–0·63*	0·35*	1·00			
Egg size	0·29*	–0·09	0·06	–0·24*	–0·06	1·00		
Fertility	0·34*	0·01	–0·03	–0·09	0·09	0·28*	1·00	
Longevity	0·003	0·25*	0·60*	0·20*	–0·45*	–0·14	–0·07	1·00

*Indicates a correlation coefficient significant after a correction of Bonferroni.

correlated with the delay before the appearance of the first eggs. In addition, the more eggs a female laid, the shorter her life span was. Surprisingly, egg size was not correlated with fecundity, but fertility was: larger eggs had a higher hatching probability. Interestingly, egg size was also positively correlated with development time. Slower development time resulted in females laying larger eggs and, in turn, with a higher fertility, whereas egg size was not related to pupal mass.

DISCRIMINANT ANALYSIS

A significant discriminant model was obtained when using fertility, egg size, mating success and development time as explanatory variables to distinguish the three cultivars (Table 5, Fig. 1). The variable fecundity was rejected from this discriminant analysis. The graphical representations of the cultivars in the plane defined by the first two canonical functions shows a good separation among cultivars. These results indicate that females originating from the different cultivars could be characterized and discriminated by four life-history traits. The classification matrix of the model obtained indicates a correct global classification of 82·4%.

Table 5. Power of discrimination of life-history traits driving the discrimination of the three cultivars at the end of the analysis

Life-history traits	Exact *F* at last step	*P*
Fertility	56·547	<0·001
Egg size	28·408	<0·001
Mating success	22·864	<0·001
Development time	21·244	<0·001
Fecundity	2·45	NS
Pupal mass	2·06	NS
Longevity	1·12	NS
Delay before lay	0·77	NS
Duration of laying	0·65	NS

Discussion

In this study, larval food quality strongly affected larval performance and female reproductive life-history traits in *L. botrana*, confirming previous results (Savopoulou-Soultani *et al*. 1999; Torres-Vila *et al*. 1999; Thiéry & Moreau 2005; Moreau *et al*. 2006a).

Larval development time varied slightly among the three different diets and the males always emerged approximately 2 days before females. This phenomenon, known as 'protandric emergence', is common in Lepidoptera (Thomas 1989; Wiklund *et al*. 1993; Savopoulou-Soultani *et al*. 1994), and it has the advantage of minimizing the prereproductive period of females as they emerge when most males are available (Fagerström & Wiklund 1982). As a result, most of the females should not have any difficulties in meeting potential partners since males are present at the time of their emergence. However, this is not always the case, and several factors, such as host plants, can significantly

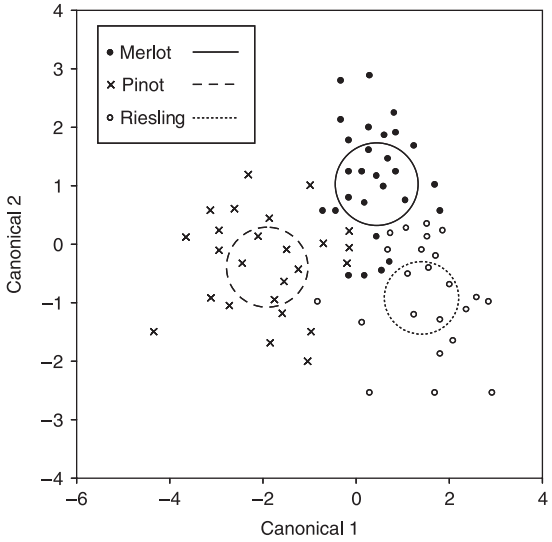

Fig. 1. Representation of the results of the stepwise discriminant analysis: position of three grape cultivars on the plane defined by the canonical variables 1 and 2. The size of the circle corresponds to a 95% confidence limit for the mean. Groups that are significantly different have non-intersecting circles.

disturb the synchrony between the emergence of both sexes (Tikkanen *et al*. 2000) and affect individual mating success (a decrease in the number of mating attempts may increase the risk of dying without mating). Therefore, the assessment of these life-history traits appears important.

In our study, larval survival was high (at least 89% survived and reached adulthood). Such a high larval survival rate is rather exceptional in studies exploring diet influence on larval performance. Typically, previous studies on *L. botrana* have reported larval survival below 50% (Fermaud *et al*. 1996; Savopoulou-Soultani, Nikolaou & Milonas 1999). The unusual high larval survival found in our study could be explained by the procedure we used. In contrast to previous studies, our protocol did not use vine inflorescences or fresh berries to feed the larvae but medium cultivars under strictly controlled conditions in terms of temperature, humidity and stress. Our protocol excluded then potential constraint caused by installation success on bunches and to some extend the incidence of predators.

Our results confirm the importance of larval food quality for adult reproductive success. We found a strong effect of larval food on female mating success strengthens the previous results (Thiéry & Moreau 2005; Moreau *et al*. 2006b) and although this aspect is often ignored in studies of plant–herbivore interactions. In our experiments, low female mating success was observed in 'Riesling' females. This result demonstrates that a proportion of females did not mate during their lifetime, confirming observations made *in natura*, only *c.* 50% of wild-caught females being mated (Charmillot *et al*. 1996). Host plants can affect the reproductive success of phytophagous insects through at least two potential mechanisms (see Landolt & Phillips 1997 for a review). First, plants may provide chemical precursors of the insect pheromones. Second, plant may affect insect physiology, resulting for example in the alteration of the quality of the spermatophores transferred by males to females (Torres-Vila *et al*. 1999), or in a decrease in oocyte production (Kaspi *et al*. 2002). This observation and our experimental results confirm the importance to consider this trait to assess the quality of larval food.

We also found that larval diet affected female achieved fecundity (numbers of eggs laid by a female during her lifetime). Based on the correlation matrix, achieved fecundity depends on three factors. First, it is positively correlated with both pupal mass and duration of egg-laying period. Among insects, such a correlation between female body size and potential fecundity (the number of eggs in the ovarioles) exists in several orders (Honek 1993). However, the relationship between achieved fecundity and female body mass is weaker (Leather 1988; Torres-Vila *et al*. 1999), and sometimes non-existent (Klingenberg & Spence 1997). This difference between the two patterns is easily understandable. Achieved fecundity depends on other factors that usually do not account for the potential fecundity. Here, achieved fecundity is negatively correlated with the delay between mating and laying of the first eggs. In addition, the negative correlation between delay and achieved fecundity is stronger than the positive correlation between achieved fecundity and pupal mass. Based on this negative correlation, we emphasize that achieved fecundity is a more meaningful estimator than potential fecundity since a long delay can significantly reduce the number of eggs laid, especially for a short-lived insect. Then, an interesting related question is to know when the potential fecundity becomes an accurate estimator. It may be a good estimator when oocyte production ceases after the adult moult (e.g. Leather 1983). However, even in that case, many wild insect species do not have sufficient time to lay all eggs contained in their ovaries mainly because of their short life span (see Leather, Watt & Barbour 1985). Longevity would thus appear as an important factor that determines achieved fecundity in Lepidoptera. We believe that the use of potential fecundity rather achieved fecundity is not accurate, unless other factors affecting achieved fecundity are well known. In addition, very few detailed studies have demonstrated that potential fecundity could be used as a reliable estimator of achieved fecundity (e.g. Tammaru, Kaitaniemi & Ruohomäki 1996).

Our results confirm the classical relationship often found in insects between delay before the first egg laid, number of eggs laid and female life span (see references in Torres-Vila, Rodriguez-Molina & Stockel 2002). Indeed, the sooner the female lays eggs, the larger is the number of eggs she produces and the shorter is her life span. Such relationships make sense

for insects that do not feed during the adult stage. Resources are then limited and the energy used for one function is obviously not available for other functions. When mating occurs early, females have enough time to lay all their ovary content. Reduced fecundity, as a consequence of mating delay, has often been explained by the fact that, in non-mated females, oocyte production is stopped or attenuated and by the occurrence of egg resorption (Bell & Bohm 1975). These mechanisms may increase female longevity through reallocation of resource from reproduction to general body maintenance (Rosenheim, Heimpel & Mangel 2000). On the other hand, if females lay their all complement of eggs, no energy can be reassigned to general maintenance and this explains the shorter life expectancy. There is now good evidence that reproduction reduces female longevity because of high energetic demands on available resources (Chapman *et al*. 1998; Foley & Luckinbill 2001; and reference therein, but see Kotiaho & Simmons 2003 for exception).

Achieved fecundity alone is not sufficient to estimate the overall quality of the larval food. As previously shown for other Lepidoptera (Wickman & Karlsson 1989; Karlsson & Wickman 1990), we found that a decrease in fecundity was balanced by an increase in egg size. The assumption of a trade-off between egg size and number of progeny is generally supported by empirical studies in arthropods, especially in species that use larval-acquired resources for egg production (Fox & Czesak 2000). Numerous studies have examined the relationship between egg size and fitness components of the progeny. They often demonstrate that within a species, small eggs are less likely to hatch (Fox & Czesak 2000 and references therein). Our study confirmed this general trend. Egg size is positively correlated with hatching success. Thus, females that lay fewer but larger eggs may have a higher reproductive output. This was true for 'Riesling' females which had the lowest fecundity, but the highest egg hatchability. In contrast, 'Pinot' and 'Merlot' females showed high fecundity, but low egg hatchability. Larger eggs are often associated with more nutritional provisions (Berrigan 1991; Fox & Czesak 2000) and we can hypothesize that 'Riesling' females invest more lipids and proteins to their eggs than 'Pinot' ones, thus compensating for the low number of eggs by a higher hatching efficiency. Further biochemical analyses of egg contents would be necessary. By considering the achieved fecundity alone we would have drawn the conclusion that for *L. botrana*, Pinot was more suitable than Riesling. This would have been wrong because there is a trade-off between the number of eggs and their size. Therefore, reproductive effort (fecundity × egg size) that estimates a female's resource allocation to reproduction is more meaningful than egg size or egg number alone.

We found that achieved fecundity depends on the larval diet and that there is a positive correlation, although weak, between achieved fecundity and pupal mass. However, we did not found any effect of the diets on pupal mass. This may suggest that, despite body size usually enhancing fitness value (Stearns 1992), pupal mass cannot be used as an estimator of fecundity as already pointed out elsewhere (Leather 1988 and reference therein). In addition, there is now growing evidence that body size does not affect or even affect negatively fitness components (Klingenberg & Spence 1997 and references therein). Given the ecological and physiological diversity of insects, one may not be able to draw general rules about the relationship between size and reproductive traits.

We stress the importance of estimating both achieved fecundity and egg hatchability, as well as mating success. In the case of *L. botrana*, mating success appears even more important than fecundity. However, more typically, mating success and egg hatching have high values (e.g. Tammaru *et al*. 2002). We also consider larval developmental time and time until mating as particularly important traits. Percentage of emergence and sex ratio of the emerging adults could be useful since both contribute directly to the proportion of females available in the population for reproduction. Longevity of mated female is also of importance as it could significantly be affected by larval food quality and it may increase the possibility for a female to disperse her eggs across the habitat.

In summary, conclusions concerning larval food quality based on a few life-history traits only can be spurious, because some traits are intercorrelated and compensate for each other. However, some exceptions are possible. For example, in a detailed study like ours, Tammaru *et al*. (2002) showed that pupal weight is a sufficient and reliable index of adult fitness since no component of fitness was negatively correlated to body size (see also Leather 1988 for a review). But to determine which traits are meaningful for fitness requires a good knowledge of the ecology of the studied species. We believe that in order to obtain an accurate view of the effect of larval food on herbivore fitness, future studies need to consider as many life-history traits as possible.

Finally, our study considered the effects of chemical composition extracted from different cultivars on insect fitness and this was the only varying factor. We demonstrated here that this factor, on its own, explains the relatively large variation of insect life history and demonstrate the complexity of interacting traits and their possible trade-offs. The chemical differences between the cultivars we used to prepare the larval foods are not known. Further analyses would be needed to know what are the main biochemical components that affect insect life-history traits. However, as pointed by Janzen (1985) and Bernays & Graham (1988), the host plant is not merely something fed on, it is something lived on. Therefore, plant chemistry is not the sole driving force in determining the performance of phytophagous insects. Here, we excluded potential differences between cultivars in cellular

structure and growth form, parameters which have certainly a great influence on fitness performance of *L. botrana* under field conditions. For an even more complete assessment further studies should take into account these parameters but also examine the effects of larval food on the dispersal behaviour of the moth and on the susceptibility to natural enemies, such as predators, parasitoids and pathogens.

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