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Reproductive biology of viviparous and oviparous species of the leaf beetle genus *Oreina*

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Abstract

In five species of the genus *Oreina* Chevrolat (Coleoptera, Chrysomelidae) we compared the size of offspring, the fecundity of the females, the timing of offspring production and female investment over the season. Two of the species, *O. elongata* and *O. luctuosa*, laid eggs, while *O. cacaliae*, *O. gloriosa* and *O. variabilis* gave birth to larvae. Offspring size corrected for female size was similar in the two oviparous species and in the viviparous *O. cacaliae*. In the two other viviparous species the larvae were two to three times bigger in relation to the female. The greater size of the offspring was not traded off for lower fecundity in these latter two species, yet the production of bigger larvae was associated with a longer laying period and thereby a spreading of reproductive investment over the season. The prediction of life history theory that higher investment in individual offspring should be traded off for lower fecundity could not be confirmed. The investigation of egg and larval development showed that in one of the oviparous species, *O. luctuosa*, the length of the egg stage was more variable. This corroborates the view that in this species the eggs can be retained for varying times before being laid. Greater size at birth does not necessarily lead to shortened developmental times: the larval periods of *O. cacaliae*, *O. elongata*, *O. gloriosa* and *O. variabilis* were all comparable although the larvae of the first two species were relatively smaller when laid; only the small larvae of *O. luctuosa* needed significantly longer for their development. For all growth parameters examined the differences between species were larger than the differences between populations. A comparison of larval growth of the oligophagous species *O. cacaliae* on three plant genera showed that larval growth rate is influenced by the food plant. However, the plant on which the larvae grew worst is apparently not chosen for oviposition in the field. A comparison with a phylogeny of the species based on allozymes suggests that species with similar reproductive parameters are closely related, yet that viviparity evolved independently in *O. cacaliae* on one hand and *O. variabilis* and *O. gloriosa* on the other.

Introduction

One of the assumptions of evolutionary theory is that selection should result in reproductive strategies that maximize an individual's reproductive success in its particular environment (e.g. MacArthur & Wilson, 1967; Pianka, 1976; Stearns, 1992). The diversity of existing reproductive strategies is striking, and great differences are not only found between distantly related taxa but in some cases between close relatives. At first sight, oviparity and viviparity seem to stand at opposite ends of the spectrum of potential reproductive tactics, with viviparity being rare outside the vertebrates. However, within one genus of leaf beetles, *Oreina* CHEVROLAT (Coleoptera, Chrysomelidae), the whole continuum exists, ranging from oviparous species, via species that incubate the eggs inside the mother, to species that give birth to larvae which have apparently been nourished inside the mother (Champion & Chapman, 1901; Bontems, 1981a, 1984a, 1988).

The consequences of the different modes of reproduction on the reproductive effort and potential reproductive success of the different species has not previously been investigated. Since several species of this genus often occur sympatrically, they also offer the possibility to compare the effectiveness of different reproductive strategies within the same habitat.

Life-history theory offers two explanations for increased investment in individual offspring that could apply to the viviparous *Oreina* species (Stearns, 1992). Firstly, larger size of individual offspring could represent a response to high competition with sibs and nonsibs for essential resources or it could be favored by slower rates of development and shorter season length (Parker & Begon, 1986). Secondly, a developmental stage associated with a higher mortality risk should be shortened by natural selection and more time be spent in a safer developmental stage (Shine, 1978; Sargent et al., 1987). In Shine's original model the egg stage may be shortened in favor of a longer juvenile stage or vice versa, but this idea can easily be extended to a trade-off between time spent inside the mother, duration of the egg stage, and time spent as a larva.

On the other hand, observed life histories might be influenced by phylogenetic constraints and not necessarily by optimal adaptation to the current situation. For example in the case of viviparity the consensus from studies on fishes, reptiles and amphibia is that no reversion to oviparity occurs (e.g. Blackburn, 1992; Wourms & Lombardi, 1992).

In the present study we compared female fecundity and investment and the larval development of three viviparous and two oviparous *Oreina* species. We were especially interested to test whether (a) there is a trade-off between size and number of offspring produced as predicted by life-history theories, and (b) nutrition of the larvae inside the female leads to shortened larval developmental times after birth.

The five *Oreina* species we selected for this study represent the existing variation in mode of reproduction, ecology, and altitudinal range of habitats. Three species, *O. cacaliae*, *O. gloriosa* and *O. variabilis* give birth to larvae, one species, *O. elongata* lays eggs, and the last, *O. luctuosa*, has been classified as facultatively viviparous (Bontems 1981a, 1984a), as females lay eggs containing embryos at different stages of development. All species of the genus feed on herbaceous plants in alpine or subalpine regions of Europe, yet plants from two different families are used as hosts, Apiaceae (*O. gloriosa* and *O. variabilis*) or Asteraceae (*O. cacaliae*, *O. elongata* and *O. luctuosa*). The altitude at which the beetles are found ranges from close to sea level (*O. luctuosa*) to about 3000 m (*O. elongata*). The species also differ in the defensive secretions they produce upon disturbance: in *O. elongata*, *O. luctuosa*, *O. gloriosa* and *O. variabilis* the secretions contain autogenously produced cardenolides, but in *O. cacaliae* and sometimes in *O. elongata* – depending on the host plant – they contain instead alkaloids obtained from the host plant (Rowell-Rahier et al., 1991; Pasteels et al., 1992; Pasteels et al., 1995).

Apart from testing the predictions of life history theory outlined above, we wanted to test whether the reproductive strategies of the insects are influenced by habitat, host plant or mode of chemical defense.

Materials and methods

Beetles and collection sites. Nomenclature of the beetles follows the revision of the genus *Oreina* by Bontems (1978, 1981b, 1984b), the plants are named according to Flora Europaea (Tutin, 1980). The collecting sites used in this study, as well as the field hosts of the beetles and the plants they were fed in the laboratory are presented in Table 1. As far as possible the populations were chosen so as to represent different combinations of sympatrically occurring species to minimize the effects of possibly differing adaptation to different environments.

No common food plant could be chosen for these specialized leaf beetles, so each species was reared on its usual field host. The only exception was *O. luctuosa* from Bayreuth where *Centaurea scabiosa* was replaced by *C. nemoralis*, the field host used by the other populations of this species. In most cases the choice of the field host was unambiguous as the species are either monophagous (*O. gloriosa*, *O. luctuosa*) or oligophagous with one of the potentially accepted plants predominating at the chosen site (*O. elongata*, *O. variabilis*). In *O. cacaliae* the apparently preferred field host is *Adenostyles alliariae*, but at all field sites two other accepted plants (*Senecio fuchsii/nemorensis* and *Petasites albus*) grow in significant numbers and might be used by the beetles.

All beetles were collected as adults just after appearance from hibernation (between beginning of May in *O. cacaliae* and beginning of July in *O. elongata*). At this time females were all mated (with the exception of *O. luctuosa*, where adults emerge from pupae at the beginning of the season and mate about two weeks later). The females were kept individually

Table 1. Collecting sites and host plants in the field of the *Oreina* species used in this study. The populations marked by an asterisk have been used for a study on the influence of the host plant on larval performance. CH - Switzerland, F - France, D - Germany

Species	Locality	Altitude	Field host	Laboratory hosts	
O. cacaliae	Kandersteg, Berner Oberland, CH	1300 m	*Adenostyles alliariae, Senecio nemorensis / fuchsii, Petasites albus,* Asteraceae	*Adenostyles alliariae*	
	Tschiertschen, Graubünden, CH	1800 m	"	"	*
	Lieserwasen, Vosges, F	900 m	"	"	*
	Zastler, Black Forest, D	950 m	"	"	
O. elongata	Mattmark, Berner Oberland, CH	2400 m	*Cirsium spinosissimum,* Asteraceae	*Cirsium spinosissimum*	*
	Col du Lautaret, Haute Savoie, F	2000 m	*Adenostyles alliariae,* Asteraceae	*Adenostyles alliariae*	*
O. gloriosa	Saas Grund, Valais, CH	1800 m	*Peucedanum ostruthium,* Apiaceae	*Peucedanum ostruthium*	
	Col du Lautaret, Haute Savoie, F	2000 m	"	"	
	Tschiertschen, Graubünden, CH	1800 m	"	"	
O. luctuosa	Calmbach, Black Forest, D	500 m	*Centaurea nemoralis,* Asteraceae	*Centaurea nemoralis*	
	Autun, Burgundy, F	400 m	"	"	
	Bayreuth, Bavaria, D	500 m	*Centaurea scabiosa,* Asteraceae	"	
O. variabilis	Zastler, Black Forest, D	950 m	*Chaerophyllum hirsutum,* Apiaceae	*Chaerophyllum hirsutum*	
	Kandersteg, Berner Oberland, CH	1300 m	"	"	
	Tschiertschen, Graubünden, CH	1800 m	"	"	

Table 2. Mean fresh weight (x±SE) of individual females at beginning of reproduction and larvae $\leq$ 24 h old from different geographic populations of different *Oreina* species. The last column gives the weight of the larvae (LW) as percent of the mother's weight (MW)

Species	Population	Female weight[a] [mg]	Larval weight[b] [mg]	LW/MW*100
O. cacaliae	Kandersteg	97.36 ± 5.62	0.62 ± 0.05	0.64 ± 0.06
(viviparous)	Tschiertschen	87.05 ± 3.93	0.81 ± 0.04	0.94 ± 0.05
	Zastler	96.20 ± 2.85	0.73 ± 0.03	0.76 ± 0.03
O. elongata	Lautaret	68.40 ± 2.43	0.71 ± 0.02	1.04 ± 0.03
(oviparous)	Mattmark	59.81 ± 2.09	0.60 ± 0.02	1.02 ± 0.05
O. gloriosa	Lautaret	121.12 ± 3.80	2.63 ± 0.10	2.19 ± 0.11
(viviparous)	Saas Grund	132.99 ± 2.64	2.61 ± 0.09	1.96 ± 0.07
	Tschiertschen	120.10 ± 4.52	2.73 ± 0.09	2.28 ± 0.08
O. luctuosa	Autun	152.13 ± 4.68	1.17 ± 0.04	0.77 ± 0.03
(oviparous)	Bayreuth	161.01 ± 5.06	1.18 ± 0.03	0.74 ± 0.03
	Calmbach	150.50 ± 5.66	1.18 ± 0.05	0.79 ± 0.03
O. variabilis	Kandersteg	166.87 ± 4.32	4.45 ± 0.15	2.69 ± 0.11
(viviparous)	Tschiertschen	154.62 ± 6.92	4.60 ± 0.27	2.97 ± 0.15
	Zastler	169.89 ± 7.02	4.46 ± 0.27	2.67 ± 0.18

[a] n = 10 females except for the Kandersteg population of *O. cacaliae*: n = 5.
[b] n = 20 larvae except for the Kandersteg population of *O. cacaliae*: n = 10.

in plastic containers lined with a thin layer of moist plaster and a filter paper to obtain constant humidity at 10 h light at 18 °C and 14 h dark at 8 °C. This ensured a temperature rhythm concordant with natural conditions at the beginning of the egg laying period for all species, except the lowland species *O. luctuosa*, but did not approximate to a natural photoperiod for the species whose reproduction takes place in the middle of summer. The effect of photoperiod on the fecundity of the females was therefore examined separately (see below). By the beginning of September the breeding season was over for all species and the beetles had stopped feeding. In 1990 and 1991 the plastic containers were then half filled with spruce bark chips and the temperature of the incubator was lowered in steps of 5 °C per week to a constant temperature of 4 °C. During hibernation the substrate was sprayed with distilled water once a week. In May of the following year the temperature was slowly raised to the original day and night rhythm, and the number of emerging beetles was counted.

Offspring size. In 1992, 10 females of each of three populations of *O. cacaliae*, *O. gloriosa*, *O. luctuosa* and *O. variabilis*, and of two populations of *O. elongata* were weighed and two larvae or eggs collected from each female. In the viviparous species the larvae were weighed within 24 h of birth, in the oviparous species the eggs of each female were kept separately in the incubator and checked and moistened daily. The duration of the egg stage was determined and the larvae weighed on the day they emerged.

Female fecundity. The fecundity of 30 females of each of *O. cacaliae* (Zastler population), *O. elongata* (Mattmark population) and *O. variabilis* (Zastler population) was estimated over the summer of 1990. Half of the females of each species were kept singly, while the other half were kept together with a male to observe the influence of the possibility of remating on fecundity. The observations were repeated in 1991 with 20 females of *O. variabilis* that had successfully hibernated and were again distributed in groups with and without males, and with 15 isolated females of *O. cacaliae* (Zastler population), *O. gloriosa* (Saas Grund population), and *O. luctuosa* (Calmbach population). The beetles were fed ad lib, moistened, and checked for offspring every day in 1990 and every second day in 1991. The number of offspring was summed over the season, starting with the first day that a female produced eggs or larvae. Observations were continued for at least three weeks after the last egg or larva was found. Females that produced fewer than 10 larvae or died within two weeks after being brought to the laboratory were assumed to belong to an older generation that had already reproduced the summer before (see below) and were excluded from the analysis.

In 1992, 10 females of two populations of *O. elongata*, and three populations of *O. cacaliae*, *O. gloriosa*, *O. luctuosa*, and *O. variabilis* were collected from the field and offspring production measured, as described above, over a period of 10 days.

Influence of the photoperiod. As the photoperiodic conditions used in the experiment measuring seasonal fecundity did not match natural conditions in the middle of summer, we tested the influence of photoperiod on *O. elongata*, the species for which the deviation from the natural day length during its laying period was largest. Thirty females of the Mattmark population, collected at the beginning of their laying period in July, were ranked by weight and divided into three cohorts, each having a similar weight distribution. Cohorts were kept at 17 °C, under a L10:D14, L12:D12 or L14:D10 photoperiod regime. The number of eggs produced over 10 days was measured for each female, as well as the weight of the females after 10 days. For neither parameter were significant differences between the groups found (egg number $F_{2,25} = 1.17$, $P = 0.326$; weight $F_{2,25} = 0.85$, $P = 0.441$).

Egg development. To compare the variation in egg development time between species and within species, the eggs of 15 females of both *O. elongata* (Mattmark population) and *O. luctuosa* (Calmbach population) were collected in 1991 in the lab at the beginning of the laying period over a two day period. Two weeks later, the eggs of 8 of the females of *O. luctuosa* were again collected, this time over a six day period to obtain similar numbers of eggs. The eggs were kept in small Petri dishes lined with moist plaster and a filter paper under the same conditions as the females and checked daily for hatching.

To compare interpopulation variation within both species, eggs of 10 females from two populations of *O. elongata* and three populations of *O. luctuosa* were collected in 1992 over three days. The time to emergence of the larvae was measured as described above.

Since both the duration of the egg stage and larval growth rates depend strongly on the ambient temperature, data were only compared within each year and among groups which were kept in the same incubator.

Table 3. Fecundity over 10 days (x±SE) in different geographic populations of the investigated *Oreina* species. Within each species populations marked with the same letter do not differ significantly from each other (comparisons by Tukey test, $P > 0.05$)

Species	Population	*n* larvae (+ SE)	*n* females
O. cacaliae	Kandersteg	18.00 ± 2.52a	6
(viviparous)	Tschiertschen	28.10 ± 6.03 a	10
	Zastler	54.27 ± 5.30 b	11
O. elongata	Lautaret	22.46 ± 2.23 a	13
(oviparous)	Mattmark	38.62 ± 2.50 b	13
O. gloriosa	Lautaret	24.13 ± 2.92 a	8
(viviparous)	Saas Glund	30.83 ± 1.52 b	12
	Tschiertschen	32.33 ± 1.17 b	12
O. luctuosa	Autun	38.90 ± 6.39 a	10
(oviparous)	Bayreuth	39.67 ± 6.50 a	15
	Calmbach	34.09 ± 3.39 a	11
O. variabilis	Kandersteg	22.30 ± 0.92 a	10
(viviparous)	Tschiertschen	23.00 ± 1.36 a	10
	Zastler	23.20 ± 1.28 a	10

Table 4. Duration (days) of the egg stage in different populations of *Oreina elongata* and *O. luctuosa*. Populations marked with the same letter are not significantly different from each other (Tukey test $P > 0.05$)

Species	Population	Mean ± SE
O. luctuosa	Autun	16.0 ± 0.2 a
	Bayreuth	16.9 ± 0.2 b
	Calmbach	15.7 ± 0.2 a
O. elongata	Lautaret	18.5 ± 0.1 c
	Mattmark	17.0 ± 0.1 b

Difficulties in reproducing exactly the same environmental conditions in different incubators were thereby avoided.

Table 5. Regression equations of logarithmic larval growth over 25 days of one population of each of different *Oreina* species (c.f. Fig. 3). Species marked by the same letter do not differ significantly in the slopes of the regression line (Tukey test $P > 0.05$)

Species	Slope	Intercept	r^2
O. cacaliae	0.0786	-0.1207	0.979 a
O. elongata	0.0717	-0.1935	0.953 b
O. gloriosa	0.0585	0.3715	0.960 c
O. luctuosa	0.0519	0.1310	0.950 d
O. variabilis	0.0550	0.6107	0.963 c,d

Larval development. To compare the growth rates between the species, 3 larvae of each of 15 females from one population of each species were collected in 1991 and put in individual Petri dishes (5 cm diam.) on the day of birth or hatching. The Petri dishes were lined with a thin layer of moist plaster and a filter paper to obtain constant humidity. The larvae were inspected daily for moltings and every second day were moistened and supplied with fresh food. The food plants of the larvae were the same as for the females. The larvae were weighed on days 1 (day of birth or hatching), 5, 10, 15, 20 and 25. After day 25 the larvae were put in small plastic containers (3 cm diam.) filled 2 cm deep with earth and fed until they burrowed into the soil. In *O. luctuosa* the larvae were weighed again and transferred onto soil on day 30, as few larvae had reached the fourth and last instar by day 25.

To compare the variability between different populations, 2 larvae of each of 10 females of two populations of *O. elongata* and three populations of *O. cacaliae*, *O. gloriosa*, *O. luctuosa* and *O. variabilis* were collected in 1992 and kept individually as described above and weighed on days 1, 3, 7 and 10.

Influence of the food plant. In the oligophagous species *O. cacaliae* we tested the larval growth on 3 potential food plants which all occur in significant numbers at the sites used in this study: *A. alliariae*, the commonest host at all the field sites used for the other experiments with this species, *Petasites albus* and *Senecio fuchsii*. The females were collected in Tschiertschen and Lieserwasen as in this latter population *Senecio fuchsii* is the predominant plant and the beetles could possibly show a different food plant adaptation. Two larvae of each female were raised in an incubator set to 17 °C and L12:D12 on each of the three plants. They were fed and moistened every second day and weighed on day 1, 5, 9, 13 and 17.

Statistical analysis. One-way ANOVAs, Tukey tests and Kruskal-Wallis comparisons of rank sums were carried out using JMP, the SAS version for Macintosh. More complex models were carried out on a VAX 7000-620 with SAS (SAS Institute Inc., 1990). Hierarchical models with random effects were analyzed with the procedures GLM using Type III sums of squares and with the procedure VARCOMP using the restricted maximum likelihood method (REML) for estimating variance components. Larval growth was compared by analysis of covariance with age as the covariate. The weight of the larvae was logarithmically transformed to ensure an even distribution of the residuals and then linear regressions were calculated. A random effect hierarchical model of analysis of covariance was used that tested for different slopes of the regression lines on each level of the model. Multiple comparisons of the slopes were effected by Tukey tests (Zar, 1984).

Results

Offspring size. The weight of larvae relative to females differed considerably between viviparous and oviparous species, as well as within the viviparous species. In the two oviparous species, *O. elongata* and *O. luctuosa*, newly hatched larvae weighed $\leq 1\%$ the mother's weight as did the viviparous species *O. cacaliae*. In contrast the relative weights of the larvae in the other two viviparous species *O. gloriosa* and *O. variabilis* were 2–3% of maternal weight (Table 2).

The between species variability of the relative size of the offspring explained 84% of the variance, while the interindividual and interpopulation variability accounted for 6 and 1% of the total variance (result of a hierarchical ANOVA with the nested effects species, population within species and mother within population: $F_{4,9(species)} = 144.44$, $P<0.001$; $F_{9,121(population)} = 1.54$, $P = 0.142$; $F_{121,135(mother)} = 2.43$, $P<0.001$).

Female fecundity. The cumulative number of offspring starting with the first day a female produced larvae in the lab is given in Figure 1. The mean total number of offspring differed significantly between the species ($F_{4,70} = 9.27$, $P<0.001$). The highest and lowest fecundity values were observed in the two oviparous species, 54 and 90 eggs in *O. elongata* and *O. luctuosa* respectively, while the viviparous species had intermediate values. *O. elongata* females produced significantly fewer eggs than *O. cacaliae*, *O. luctuosa* and *O. variabilis*, and *O. luctuosa* had a significantly higher fecundity than *O. gloriosa* (5% level Tukey – Kramer comparison of means).

The rate at which progeny were produced differed significantly between species ($F_{4,70} = 170.82$, $P<0.001$ for the effect of species in a one-way ANOVA on the time necessary to produce half of the total number of offspring), ranging from 3.1 days in *O. elongata* to 32.4 days in *O. variabilis*. The viviparous *O. cacaliae*, which produces small larvae does not differ significantly from either of the two oviparous species *O. elongata* and *O. luctuosa*. In contrast, *O. gloriosa* and *O. variabilis*, the two viviparous species with bigger larvae took significantly longer than the other species to produce their offspring (Figure 1). The time needed to produce half of the maximal number of offspring correlates well with the relative size of the offspring (c.f. Table 2; a linear regression through the five points gives $R^2 = 0.876$, $F_{1,3} = 21.26$, $P = 0.019$).

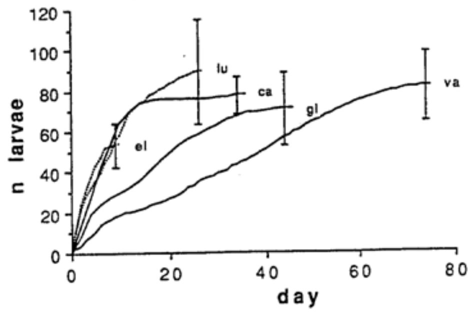

Figure 1. Cumulative fecundity over the reproductive period of females from one population of each of different *Oreina* species. The curves are cut off at the point where they reach the final plateau. At the end points the corresponding standard deviations are given. The solid lines mark the viviparous species, the broken lines the oviparous species. ca – *O. cacaliae*, el – *O. elongata*, gl – *O. gloriosa*, lu – *O. luctuosa*, va – *O. variabilis*

In two species, *O. luctuosa* and *O. variabilis*, no significant differences between the populations appeared (Table 3; $F_{2,33} = 0.26$, $P = 0.775$ and $F_{2,27} = 0.15$, $P = 0.857$). However, considerable differences in the number of larvae produced by females of different populations existed in *O. cacaliae*, ($F_{2,24} = 11.40$, $P<0.001$), *O. elongata* ($F_{1,24} = 23.18$, $P<0.001$), and *O. gloriosa* ($F_{2,29} = 5.22$, $P = 0.012$). This does not necessarily reflect true differences between the populations, but could be due to the difficulty of collecting the females just at the onset of their reproduction in the field. This problem is especially pronounced in the species with short laying periods. For the two species collected at the Col du Lautaret, *O. elongata* and *O. gloriosa*, overheating during transport provoked the females to lay large numbers of eggs or larvae before reaching the laboratory.

The presence of a male did not influence the fecundity of the female during the first season in the three species tested (*O. cacaliae*: $F_{1,23} = 0.02$, $P = 0.879$; *O. elongata*: $F_{1,30} = 1.76$, $P = 0.195$; *O. variabilis*: $F_{1,32} = 0.30$, $P = 0.588$). In contrast in *O. variabilis*

Table 6. Repeated measures analyses on the age at moltings of larvae from one population of each of different *Oreina* species (c.f. Fig. 3). The effect of female is tested against the between subject error (error I), the interaction between time and female is tested against the within subjects error (error II). DF = degrees of freedom, SS = sum of squares, MS = mean square

Source	DF	Type III SS	MS	F-ratio	P-value
O. cacaliae					
female	14	22.10	1.58	3.17	0.004
error I	29	14.44	0.50		
time*female	28	10.58	0.38	4.81	0.001
error II	58	4.56	0.08		
O. elongata					
female	15	135.96	9.06	3.35	0.005
error I	22	59.44	2.70		
time*female	30	14.04	0.47	4.52	0.001
error II	44	4.56	0.10		
O. gloriosa					
female	14	44.84	3.20	3.62	0.002
error I	26	23.00	0.88		
time*female	28	8.01	0.29	1.94	0.019
error II	52	7.67	0.15		
O. luctuosa					
female	14	148.33	10.60	2.20	0.053
error I	20	96.39	4.82		
time*female	28	43.24	1.54	1.27	0.243
error II	40	48.78	1.22		
O. variabilis					
female	14	67.39	4.81	1.06	0.431
error I	29	132.00	4.55		
time*female	28	8.04	0.29	1.35	0.166
error II	58	12.33	0.21		

females that had successfully overwintered the reproductive success in the second year strongly depended on mating. None of eight females that overwintered but had no opportunity to mate since collected in the field at the beginning of the previous season produced any offspring in the second year. Females that had the opportunity to remate in the first or second year or in both, produced larvae in the second year (3 of 4 females in each group). However, the time at which remating occurred was important as females that did so before hibernation produced significantly more larvae (mean 28.88) than those that mated after hibernation (mean 1.25; $P = 0.048$ for significance of the difference in a median test using χ^2 approximation).

Produced biomass. To get a measure for the total female seasonal investment in reproduction, we multiplied cumulative fecundity by the mean weight of the larvae, and divided the product by the mean female weight to correct for the species' differences in size (Figure 2). The two oviparous species *O. elongata* and *O. luctuosa* produce only 50 to 70% of their own weight as offspring, as does the viviparous species *O. cacaliae* (whose larvae are about the same size as eggs) and no significant differences exist between these three species (Tukey – Kramer comparison of means, $\alpha = 5\%$). In contrast the two viviparous species with bigger larvae differ significantly from the former group producing progeny that total 140% (*O. gloriosa*) and 215% (*O. variabilis*) of their own body weight. Furthermore, *O. variabilis* invests significantly more than *O. gloriosa*. However, this greater biomass of offspring is spread out over a longer time, so the daily output of biomass of offspring is not higher in these species (c.f. the slopes of the curves in Figure 2).

Table 7. Regression equations of logarithmic larval growth over 10 days in different populations of the investigated *Oreina* species. In *O. elongata* and *O. gloriosa* an analysis of covariance indicated different slopes between the populations within species, but only in *O. elongata* this difference also appears in the Tukey test ($P < 0.05$)

Species	Population	Slope	Intercept	r^2
O. cacaliae	Kandersteg	0.0973	-0.3097	0.886 a
	Tschiertschen	0.0923	-0.1924	0.902 a
	Zastler	0.0933	-0.2172	0.923 a
O. elongata	Lautaret	0.0716	-0.2105	0.908 a
	Matmark	0.0811	-0.2931	0.886 b
O. gloriosa	Lautaret	0.0628	0.3461	0.879 a
	Saas Grund	0.0723	0.3362	0.926 a
	Tschiertschen	0.0748	0.3662	0.916 a
O. luctuosa	Autun	0.0600	0.0485	0.848 a
	Bayreuth	0.0655	0.0426	0.854 a
	Calmbach	0.0690	0.0424	0.816 a
O. variabilis	Kandersteg	0.0665	0.5752	0.899 a
	Tschiertschen	0.0641	0.5897	0.777 a
	Zastler	0.0641	0.5621	0.834 a

Table 8. Analysis of covariance on the logarithmic growth data of larvae from different populations of different *Oreina* species (c.f. Table 7). The effects female, population and species are nested within each other, the interactions of the effects with age test for different slopes on each level of the model. DF = degrees of freedom, SS = sum of squares, MS = mean square

Source	DF	Type III SS	MS	F-ratio	P-value
species	4	29.51	7.38	1383.71	0.0001
population	9	0.18	0.02	3.84	0.0001
female	122	1.17	0.01	1.80	0.0001
age	1	63.83	63.83	11972.93	0.0001
age*species	4	1.32	0.33	61.79	0.0001
age*population	9	0.14	0.02	2.91	0.0021
age*female	122	0.41	0.00	0.62	0.9993
error	809	4.31	0.01		

Table 9. Regression equations of logarithmic larval growth over 17 days in two populations of *O. cacaliae* raised on the different plants. Within each population larvae on plants marked by the same letter do not differ significantly in the slopes of the regression line (Tukey test, $P < 0.05$)

Population	Food plant	Slope	Intercept	r^2
Lieserwasen	*Adenostyles alliariae*	0.1103	-0.1399	0.957 a
	Senecio fuchsii	0.1094	-0.2838	0.970 a
	Petasites albus	0.0911	-0.2276	0.887 b
Tschiertschen	*Adenostyles alliariae*	0.1148	-0.2428	0.921 a
	Senecio fuchsii	0.1083	-0.3527	0.879 a
	Petasites albus	0.0974	-0.3114	0.924 b

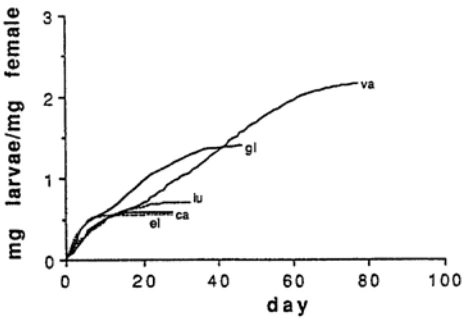

Figure 2. Biomass of larvae produced in relation to female weight for one population of each of different *Oreina* species. The curves are cumulative over the season and are cut off when reaching the plateau. Solid lines for viviparous species, broken lines for oviparous species. ca – *O. cacaliae*, el – *O. elongata*, gl – *O. gloriosa*, lu – *O. luctuosa*, va– *O. variabilis*

Length of egg period. The mean time to hatching of the eggs of *O. elongata* (Mattmark population) and of *O. luctuosa* (Calmbach population) differed significantly: 15.9 ± 0.1 (n = 209) and 14.8 ± 0.1 days (n = 225) in *O. elongata* and *O. luctuosa*, respectively ($F_{1,309} = 52.66$, $P<0.001$ in a Welch analysis of variance allowing for unequal variances). The variance in the length of the egg stage was significantly higher (Bartlett's test $F_1 = 135.96$, $P<0.001$ for different variances) in *O. luctuosa* (range 9–18 days) than in *O. elongata* (range 14–18 days). In both species significant differences existed between individual females (*O. elongata*: $F_{14,194} = 4.89$, $P<0.001$; *O. luctuosa* $F_{14,210} = 7.42$, $P< 0.001$ in a one-way analysis of variance). In *O. luctuosa* eggs produced later in a female's reproductive life developed significantly faster, but the variability in their development time was significantly higher than those produced two weeks earlier (13.9 ± 0.5 days (n = 109); $F_{7,154} = 21.59$ for the effect of female, $F_{1,154} = 19.31$ for the effect of time and $F_{2,154} = 17.19$ for the interaction between both factors, all F-values associated with $P<0.001$ in a two-way analysis of variance).

The variability in the length of the egg stage was higher in all populations of *O. luctuosa* than in *O. elongata* (Table 4). However, when different populations are examined together the duration of the egg stage overlapped in the two species and did not differ significantly between the Mattmark population of *O. elongata* and the Bayreuth population of *O. luctuosa*. Significant female effects were observed within each species (*O. elongata*: $F_{18,215} = 3.71$, $P<0.001$; *O. luctuosa*: $F_{27,469} = 14.78$, $P<0.001$), while the effect of population is significant in *O. elongata* but just fails to be so in *O. luctuosa* (*O. elongata*: $F_{1,18} = 22.37$, $P<0.001$; *O. luctuosa*: $F_{2,27} = 3.24$, $P = 0.054$ in a nested analysis of variance with random effects population and females within population).

Development of larvae. In Figure 3 the increase in larval weight up to the fourth (last) instar is shown for the offspring of one population per species. In all species, except *O. luctuosa*, larval development was completed between 25–30 days, at which time larvae stopped feeding and burrowed in the soil for pupation. In *O. luctuosa* all larvae fed for at least 30 days. The slopes of linear regressions through the logarithmic transformed weight of the larvae (Table 5) of *O. cacaliae* and *O. elongata* were significantly steeper than those of the viviparous *O. gloriosa* and *O. variabilis*, whose larvae start bigger. However, they are also steeper than that of *O. luctuosa*, even though the neonates of all three species have similar size. No significant differences were found between either *O. variabilis* and *O. gloriosa* or between *O. variabilis* and *O. luctuosa*. Although there was a significant female effect on the initial weight of the larvae (see above), there was no significant female effect on the growth rate of the larvae expressed by the slope of the regression lines through the logarithmic weights ($F_{71,1054} = 1.09$, $P = 0.286$ in a hierarchical analysis of covariance testing for different slopes among offspring of the same females within species).

Figure 4 shows the duration of the first three instars and the age at molting for the larvae used in the growth experiments. A repeated measures analysis of variance shows significant interspecific differences in duration of different instars ($F_{4,197} = 218.65$, $P<0.001$), and also a significant interaction between time and species ($F_{8,394} = 103.75$, $P<0.001$). A comparison of the mean age at each molt shows differences between *O. cacaliae*, *O. elongata*, *O. gloriosa*, and *O. variabilis*. In *O. luctuosa*, all three molts occurred significantly later than in the other species. A comparison of the offspring of the same mother shows significant differences between sibling groups in the mean age at molting and the time course of development (described by the interaction between time and female) for all species, except in *O. luctuosa* and *O. variabilis*, where no differences exist between the offspring of different females (Table 6).

Except in *O. luctuosa*, within a given species, larvae that were larger at birth molted earlier than those

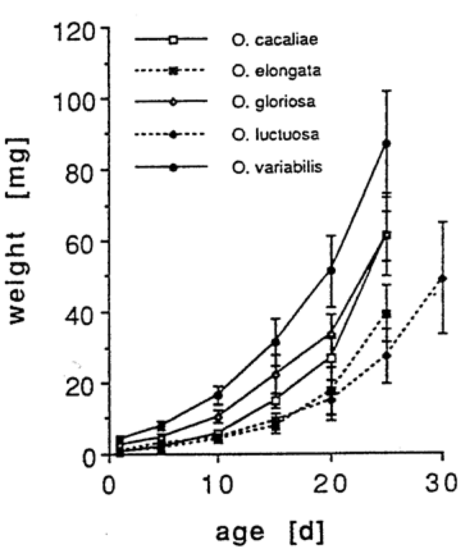

Figure 3. Larval gain in weight for one population of each of five different *Oreina* species. The broken lines mark the oviparous species, the solid lines the viviparous species.

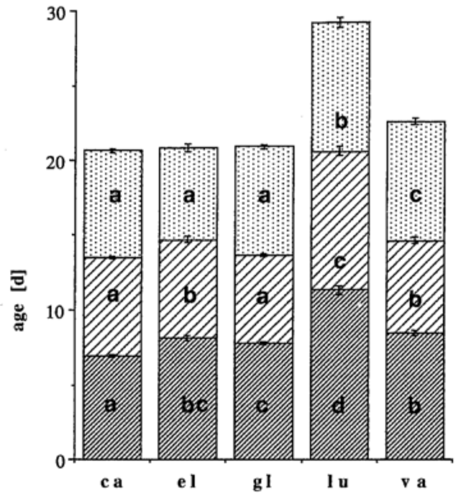

Figure 4. Duration of the first three instars and age at moltings of larvae from one population of each of different *Oreina* species. The different instars are marked by different shadings, the last instar that ends with pupation was not measured. Species marked with the same letter do not differ significantly in the age at a given molt (Tukey tests, P>0.05). The vertical bars give the standard errors. ca = *O. cacaliae*, el = *O. elongata*, gl = *O. gloriosa*, lu = *O. luctuosa*, va = *O. variabilis*

with lower initial weights during the first two instars. In the case of *O. variabilis* this was also true in the third instar (results of analysis of covariance on the age at molting with the effects species, weight at birth and their interaction using Type III sums of squares: 1. molt $F_{1,192} = 15.24$, $P<0.001$; 2. molt $F_{1,192} = 13.31$, $P<0.001$; 3. molt $F_{1,192} = 4.62$, $P = 0.033$ for the effect of weight at birth, the effect of species being always significant and associated with P-values below 0.05 and no significant interaction between the two effects for either of the three molts).

Significant differences in the growth rates also existed between the species when several populations of each species were compared (Table 7 and 8; $F_{4,809} = 61.79$, $P<0.001$). No significant effects existed between the offspring of different mothers ($F_{122,809} = 0.62$, $P = 0.999$) and only in two of the species (*O. elongata* and *O. gloriosa*) did populations differ significantly from one another. In *O. gloriosa* this difference is indicated as significant in the analysis of covariance comparing populations on the species level for different slopes, but the comparison of the slopes by Tukey tests did not reveal any differences, this test being more conservative than the ANCOVA.

Influence of the food plant. In the oligophagous species *O. cacaliae* which at most field sites has a choice between different acceptable plants we tested the influence of the food plant on larval performance (Table 9). At one of these sites (Lieserwasen) *Senecio fuchsii* seems to be the predominantly used plant, while at all the other sites including the one used as comparison here (Tschiertschen) *Adenostyles alliariae* dominates the local plant community. A two factorial analysis of covariance with the effects population and food does not reveal differences in growth rate between populations but between food plants ($F_{1,485} = 1.31$, $P = 0.253$ and $F_{2,485} = 17.08$, $P<0.001$). Larvae from both populations grew best on *A. alliariae* (that has been used for the species comparisons above). Growth on *Senecio fuchsii* was slightly though not significantly worse, while *Petasites albus* supported growth of larvae of the Lieserwasen population significantly less well (Table 9).

Discussion

One of the questions addressed by life-history theory is how much offspring an organism should produce and how much investment should be put into the individual offspring (e.g. Pianka, 1970; Smith & Fretwell, 1974, Brockelman, 1975; Winkler & Wallin, 1987; Sibly & Calow, 1985; Lloyd, 1988).

The leaf-beetle genus *Oreina* includes oviparous and viviparous species, and there are considerable differences in the investment put into the individual offspring after correction for female size. The total production of offspring biomass relative to female weight was significantly higher in the two viviparous species with high investment per offspring than in the three species with low investment in the individual larva (*O. cacaliae*, *O. elongata* and *O. luctuosa*).

The comparison of the fecundity of *Oreina* species with small larvae versus those with big maternally fed larvae does not conform to the often postulated trade-off between offspring size and number (e.g. Pianka, 1970; Smith & Fretwell, 1974; Brockelman, 1975; Sibly & Calow, 1985) which has been found in other taxa (e.g. in fruit flies, Montague et al., 1981; in aphids, Dixon, 1987; in grasshoppers, Kriegbaum, 1988; in ladybird beetles, Stewart et al., 1991).

According to life-history theory, two parameters should strongly influence the optimal effort: per offspring and the optimal total effort adult survival and thus expected future reproductive success, and the increase in juvenile survival rates with increasing effort per offspring (e.g. Winkler & Wallin, 1987). Adult survival over the season has so far only been estimated in *O. gloriosa* (Eggenberger & Rowell-Rahier, 1991) and appeared to be high. This fits with the aposematic coloration, the chemical defense and conspicuous life style of the beetles. Occasionally, marked individuals could be recaptured in the next year, but the percentage survival into the second year has not yet been determined rigorously. Laboratory observations suggest that at least in *O. variabilis* some of the females may have a second reproductive period after hibernation. Juvenile survival rates are much more difficult to measure in the field, yet we attempt to do this in ongoing studies (Rowell-Rahier et al., unpubl.). The egg and larval development times measured in this study may however give a first suggestion of predation risk as a function of times of exposure.

While no risky egg stage exists in the viviparous *Oreina* species, the length of the egg stage has to be added to the total extra utero developmental times in the two egglaying species considered here. In *O. luctuosa* individual females have been described to lay eggs at different stages of embryonic development, the most advanced being ready for hatching (Bontems, 1981a, 1984a). Our data, comparing the length of the egg stage in *O. elongata*, classified as oviparous, and in *O. luctuosa* classified as facultatively viviparous (Bontems, 1988), confirm that the variance in the time to hatching is higher in *O. luctuosa*. However, the mean duration of the egg stage was similar in the two species (for eggs collected early in the season). The shortening of the egg stage and its higher variability in eggs of *O. luctuosa*, collected two weeks later from the same females, agrees with an observation of Chapman (1903). He described for *O. bidentata* (in his work indicated as *O. tristis*; a form that is often considered as the same species as *O. luctuosa*) a shortening of the egg stage with increasing age of the females and differences between individual females. Shortening the egg stage reduces (or in the viviparous species excludes) the risk of egg predation. Avoidance of egg predation by their ant hosts was suggested as the ultimate cause for larviposition in the carabid beetle *Pseudomorpha* (Liebherr & Kavanaugh, 1985). In chrysomelids, cannibalism by young larvae of unhatched eggs as observed in some species (Breden & Wade, 1985; Rank, 1992), might also favor viviparity, yet in the two egg-laying species egg cannibalism was never observed.

Shortening the larval stage might similarly reduce the risk of predation on the young larvae, the more so if the chemically defended mother provides a 'safe harbor', i.e. a developmental stage associated with lower risks (Shine, 1978; Sargent et al., 1987). However, in the *Oreina* species, higher weight at birth in relation to the adult weight does not shorten the time of exposure outside the female. The larvae of *O. cacaliae* and *O. elongata* that are initially smaller relative to their mother (compared to *O. gloriosa* and *O. variabilis* larvae), compensated for this difference by higher growth rates and completed their larval development in a similar time to the two viviparous species born large. *O. luctuosa* started with a small relative weight and, showing a similar growth rate to *O. variabilis*, could not compensate for this difference, but developed and molted significantly slower than all other species.

Of all the parameters examined, growth rates are most likely influenced by changing environmental conditions, especially temperature. As our study was carried out in a common lab environment the conditions do not mirror the situation in the field for all of the species. However, the main comparisons involve two sets of sympatric populations of an oviparous and a viviparous species: *O. elongata* and *O. gloriosa* from adjacent sites in the Valley of the Saas and *O. cacaliae* and *O. variabilis* from the Zastler field site. As frequent visits to this latter field site confirmed, the situation in the laboratory adequately simulated the conditions in the field, i.e. the phenology of beetles and larvae corresponded to the one observed in the laboratory. However, for the lowland species *O. luctuosa* the temperature chosen in the lab was certainly lower than the conditions this species normally experiences in the field and the realized growth rates in the field may lie significantly higher.

The observed relationship between offspring size and developmental rate both in a comparison between sympatric *O. variabilis* and *O. cacaliae* and between *O. gloriosa* and *O. elongata* contradicts theoretical expectations (Sibly & Calow, 1985; Parker & Begon, 1986): neither do larvae of species born large grow faster, nor do all larvae born small grow slow. In contrast to the situation here growth rates in (carnivorous) coccinellid beetles have been shown to be lower in species having smaller eggs (Stewart et al., 1991).

Growth rates in herbivorous insects like the *Oreina* species could strongly depend on plant quality and content of secondary compounds (e.g. Scriber & Slansky, 1981; Tabashnik & Slansky, 1987). In our experiments each species was raised on its usual host plant, *O. gloriosa* and *O. variabilis* on Apiaceae, *O. cacaliae*, *O. elongata* and *O. luctuosa* on Asteraceae. No artificial diet that would allow standardization exists for these specialized beetles. For two of the oligophagous species we tried to assess the influence that rearing the larvae on different acceptable plants has. In *O. cacaliae* two populations with different field hosts reacted similarly to the different plants: for both growth was best on *Adenostyles alliariae* that we have used for all other rearings. Growth on *Senecio fuchsii* was not significantly worse, while the third plant used, *Petasites albus*, supported larval growth significantly less well. The latter plant is probably rarely used for oviposition as choice experiments with females suggest (Doble, unpubl.) and larvae can rarely be found on this plant in the field. In *O. elongata*, the only species in which a significant difference in the larval growth rate appeared between the populations (which were raised on different plants), this can most likely be attributed to host plant dependent performance of an oligophagous species. In a paper addressing the different host plant adaptation of these two populations, we showed that growth is significantly better on *C. spinosissimum* for both populations although one of them lives on *A. alliariae* in the field (Dobler & Rowell-Rahier, 1994).

For our comparison between species these results indicate that the observed reproductive parameters, at least growth rate but maybe also female fecundity, are directly influenced by the host plant. The (untestable) differences of experienced host quality between the groups feeding on Asteraceae versus Apiaceae should be even more pronounced. Differing food quality or secondary plant compounds might also exert different selection pressures in the two groups. The host plants of specialized herbivores, however, constitute part of a historic commitment and cannot be regarded under a purely adaptationist viewpoint. Many of the reproductive parameters correlate well with host plant: *O. cacaliae*, *O. elongata* and *O. luctuosa* which have similar size at birth, rates of offspring production and length of laying period feed on Asteraceae, while *O. gloriosa* and *O. variabilis* which resemble each other in these same parameters and in their growth rates feed on Apiaceae.

At the outset of this study no hypothesis on the phylogeny of these beetles had been formulated. In the meantime we have established a phylogeny estimate based on allozyme electrophoresis to test for the influence of history on the biology of the beetles (Dobler et al., 1996). On this phylogeny the five species investigated here belong to two different clades: *O. luctuosa*, *O. elongata* and *O. cacaliae*, belong to one clade (given in ascending order), and *O. gloriosa* and *O. variabilis* belong to an other derived clade that shares viviparity, maternal nutrition of the larvae and feeding on Apiaceae. Viviparity seems to have evolved independently in the ancestor of *O. cacaliae* and its sister species and at the bottom of the clade including *O. gloriosa* and *O. variabilis*. This seems likely as outside *Oreina* but within the same subfamily (Chrysomelinae) viviparity also evolved several times independently (in *Chrysolina, Doryphora, Gonioctena, Paropsides* and *Pyrgo*) judged by the current classification of the species based on morphology (e.g. Seeno & Wilcox, 1982) or chemical defense characteristics (Pasteels, 1993).

While our data neither confirm several predictions of life history theory nor show a correlation of reproductive characteristics with abiotic habitat, they show instead a strong influence of the phylogeny of the genus on the reproductive biology of the species.

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