Chemoecology 2 (1991) 15–19

Chemical defence in ladybird beetles (Coccinellidae). II. Amount of reflex fluid, the alkaloid adaline and individual variation in defence in 2-spot ladybirds (*Adalia bipunctata*)

Peter W. de Jong, Graham J. Holloway, Paul M. Brakefield, and Helene de Vos

Section of Evolutionary Biology, Department of Population Biology, University of Leiden, Schelpenkade 14a,
2313 ZT Leiden, The Netherlands

Received April 14, 1991 / Revision accepted July 10, 1991

Summary

2-spot ladybirds secrete alkaloid (adaline)-rich defence fluid (reflex blood) in response to predator attack. Reflex fluid was collected from individual ladybirds and weighed and the alkaloid content measured by GC. The amount of fluid produced built up rapidly following winter hibernation in animals feeding on aphids. The concentration of adaline in the fluid was highest in the first bleeding after winter hibernation. A large sample of beetles was reflex bled several times. Significant among beetle variation was found in the amount of fluid produced and the concentration of the reflex blood. The results are discussed in relation to the possibility that 2-spot ladybirds are Batesian mimics of 7-spot ladybirds and to the possible functions of adaline.

Key words

chemical defence, mimicry, reflex bleeding, variation, alkaloid, adaline, Coleoptera, Coccinellidae, *Adalia bipunctata*

Introduction

Many species of ladybirds are distasteful as a consequence of the mobilization of self-synthesized alkaloids (*e.g.* Pasteels *et al.* 1973). Perhaps as a result of this distastefulness, most ladybird species, such as the 7-spot ladybird, *Coccinella septempunctata* L., are aposematically coloured (see Guilford 1988). Ladybirds defend themselves, at least during the months when breeding is a possibility (Holloway *et al.* 1991), with the aid of a behaviour known as reflex bleeding (Cuenot 1896; Hollande 1911; Frazer & Rothschild 1960; Happ & Eisner 1961). This behaviour involves the exudation of reflex fluid through pores in the knee joints of all six legs which serves to transport defensive compounds to the surface. Holloway *et al.* (1991) showed that the amount of fluid ejected by 7-spot ladybirds can be remarkably high; up to about 20% of the fresh body weight. Holloway *et al.* (1991) argued, therefore, that reflex bleeding is probably an energetically expensive exercise and, consequently, may induce selective pressure to produce automimics (Brower *et al.* 1967, 1970; Pough *et al.* 1973; Gibson 1984). Here an individual becomes a Batesian mimic of its distasteful conspecifics by retaining its warning coloration, but saves energy that would otherwise be used for defensive purposes. This energy could then be redeployed to, for example, produce more eggs or extend lifespan and in doing so increase lifetime fitness (Sibly & Calow 1986).

Holloway *et al.* (1991) found substantial individual phenotypic variation in the 7-spot ladybird, both in the amount of reflex blood produced and the amounts of the alkaloids, coccinelline and precoccinelline (Tursch *et al.* 1971, 1975, 1976), therein. However, the above two characters were normally distributed about their means and, thus, provided no evidence for the operation of automimicry in 7-spot ladybirds, leading to a polymorphism of defended and undefended beetles.

In Western Europe, the 2-spot ladybird, *Adalia bipunctata* L., differs from the 7-spot ladybird in a number of ways. There are several different colour forms of the 2-spot ladybird that fall broadly into two categories: red with black spots (typica) and black with red spots (melanic). It has been proposed that the latter forms are melanic in response to lower sunshine levels and spatial climatic variation maintains clines in the frequency of melanics (Brakefield 1984). It is also possible that the different forms fall into different mimetic assemblages (Brakefield 1985a; Marples 1990). Furthermore, the 2-spot ladybird synthesizes a different alkaloid, adaline (Pasteels *et al.* 1973). Adaline renders the 2-spot ladybird mildly distasteful to bird predators, but certainly does not protect 2-spot ladybirds against bird predation to the same extent as coccinelline does 7-spot ladybirds (Marples *et al.* 1989).

The 'typica' form of the 2-spot ladybird is considered to be a member of the mimicry ring containing 7-spot ladybirds (Brakefield 1985a). In view of the 2-spot ladybirds' degree of unpalatability, it has been suggested that 2-

spot ladybirds are Batesian mimics of 7-spot ladybirds (Brakefield 1985a; Marples 1990). If this is the case then it may be that there is considerable selective pressure to reduce the amount of energy invested in defence, perhaps even to zero if predation comes primarily from vertebrates. This would particularly be true if the mimicry of 7-spot ladybirds was mainly of the aposematic coloration and did not extend to behavioural or chemical mimicry. However, at least some 2-spot ladybirds quite clearly reflex bleed.

The goals of the study were:

1. to establish how much material 2-spot ladybirds invest in defence and whether it is comparable with the amount invested by 7-spot ladybirds, and
2. whether, as a result of the arguments laid out above, some 2-spot ladybirds invest very little or nothing in defence.

Materials and Methods

Insects

Adult 2-spot ladybirds were collected from plane trees (*Platanus* x *hybrida*) in Zevenbergen (The Netherlands) in the late summer and early autumn of 1989, during which time they form aestivating aggregations in curled-up dead leaves. They were kept in a cage (40×40×40 cm) in a garden shed so that the temperature was ambient. The ladybirds overwintered in the cage in dead leaves in plastic pots and on some branches still bearing dead leaves. In February 1990, 210 ladybirds (form 'typica') were taken from this cage and each assigned at random to one of four groups. Three groups (A, B, C) contained 20 ladybirds each and group D contained the remaining 150 insects. The ladybirds were kept individually in plastic petri-dishes (5 cm diameter) containing a filter paper (4.5 cm diameter) in a constant climate cabinet (20 °C, 80% relative humidity and 18:6 light:dark cycle). The experimental insects were sexed under a stereo microscope (×25) using ventral surface characters (Majerus & Kearns 1989). In addition to the characters illustrated by Majerus & Kearns (1989), we discovered another useful character. In male 2-spot ladybirds the trailing edge of the fifth sternite curves towards the head (as shown in Majerus & Kearns 1989), but in females the trailing edge of this sternite frequently and obviously curved away from the head. In some females this curvature was very pronounced so that a little median knob was formed. The ladybirds were fed daily with an ample supply of fresh pea aphids *(Acyrthosiphon pisum)* from a laboratory stock. The ladybirds were frequently transferred to clean petri dishes to reduce the risk of infection. Eggs laid by females during the course of the experiment were counted. Most of the females laid eggs which served as a convenient check of accuracy of the sexing procedure and demonstrated that we were nearly always correct.

Extraction and collection of reflex blood

The technique used to collect the reflex blood is described in detail in Holloway *et al.* (1991), the only difference being that 1.2 mm diameter tubing was used rather than 2.3 mm since 2-spot ladybirds are considerably smaller than 7-spot ladybirds. The amount of reflex blood produced by each ladybird was weighed to the nearest 0.01 mg using a Mettler AE160 top pan balance. All ladybirds were weighed to the nearest 0.1 mg after being reflex bled. Ladybirds from groups A, B and C were examined on days 1, 3 and 5, respectively, to determine the rate at which the ability to reflex bleed could build up following winter hibernation and to estimate an appropriate time interval between successive bleedings of the group D ladybirds. The ladybirds in group D were used to examine among individual variation in the amount of reflex blood produced and the concentration of the adaline therein. All group D individuals were examined on days 8, 11, 14 and 17, yielding four values of fluid weight, fresh body weight and amount of adaline for each beetle. These values were used to establish whether each beetle produced a consistent amount of material through time, in other words, whether the amount invested in defence could be physiologically determined. After the final bleeding and weighing on day 17, all beetles were frozen at −30 °C.

Alkaloid analysis

The amount of adaline in each extract was measured using a Hewlett Packard 433 Gas Chromatograph (GC). The GC settings and the extraction and measurement techniques are described in detail in Holloway *et al.* (1991).

A test was performed to assess the level of efficiency with which alkaloid could be transferred from the capillary tubes to the methanol prior to GC injection. This involved reducing capillary tubes from which the alkaloid had already been extracted to a fine powder using a pestle and mortar. This was washed in chloroform and the chloroform was injected into the GC. The analysis revealed that, on average, more than 99.5% of the alkaloid had already been extracted following the method described by Holloway *et al.* (1991).

Results

The mean amounts of reflex blood produced by the ladybirds in groups A to D are shown in Figure 1A. Under the experimental conditions the ladybirds achieved maximum reflex bleeding potential after about three days. This value was used to set the time period between subsequent bleedings of the ladybirds in group D. However, in practice, three days of feeding only enabled these ladybirds to achieve 80% of their apparent maximum (assuming that their post-winter hibernation maximum could have been re-attained).

The amount of adaline carried in the reflex blood is shown in Figure 1B. Apparently, the rate of adaline production was not as high as the rate of fluid production. Consequently, the concentration of the alkaloid in the subsequent bleedings declined to a steady value during bleedings on days 11, 14 and 17 (Fig. 1C). The results show that large amounts of adaline are present in overwintering 2-spot ladybirds despite the inability to reflex bleed.

Of the 150 ladybirds originally assigned to group D, 130 survived and produced reflex blood on days 8, 11, 14 and 17. Of the remaining 20, 2 were transferred to maintain 20 animals in group C and 18 died of unknown causes. The sex ratio of the surviving ladybirds was significantly biased in favour of females (86 *vs* 44, $\chi^2 = 13.6$, $p < 0.001$). Female 2-spot ladybirds are on average considerably heavier than males (16.2 mg *vs* 12.0 mg on day 8, 16.1 mg *vs* 11.6 mg on day 11, 15.7 mg *vs* 11.5 mg on day 14 and 16.0 mg *vs* 11.6 mg on day 17 ($F_{1,128} > 86$, $p < 0.001$ in all cases)).

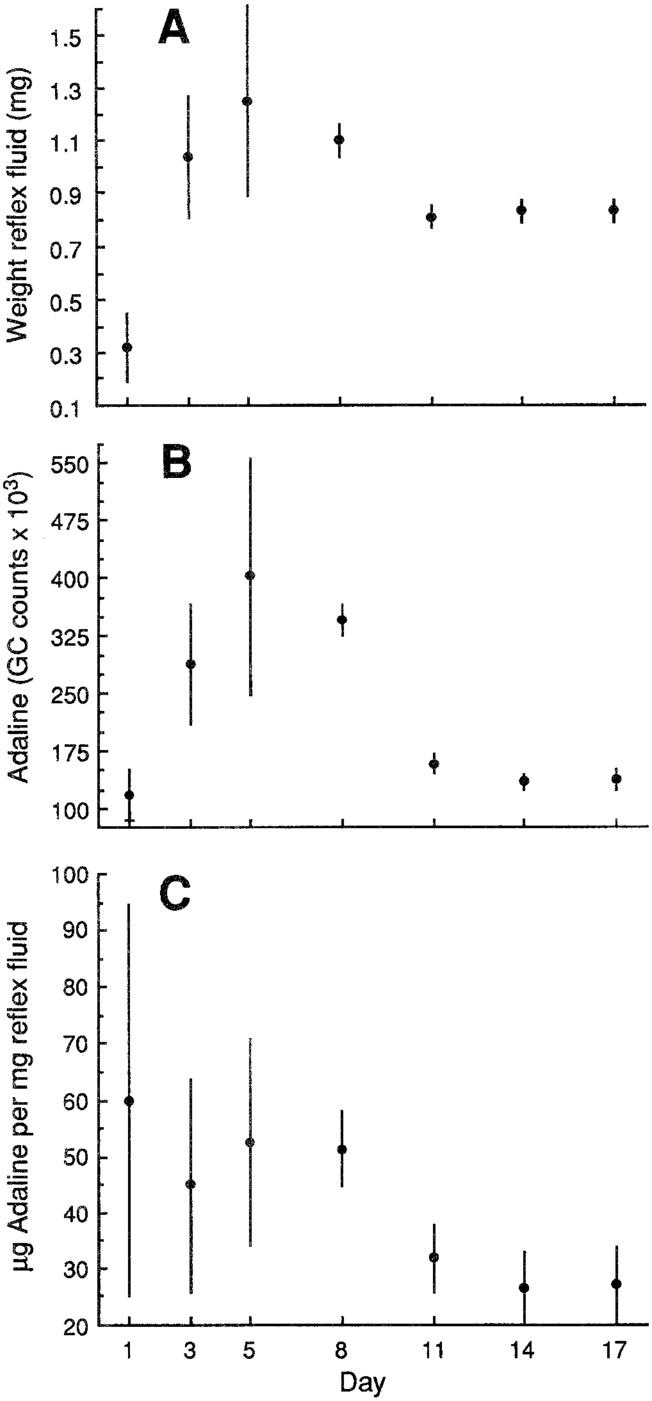

Fig. 1 Change through time in A) amount (mg) of fluid produced, B) amount (GC counts) of adaline produced and C) adaline concentration (µg/mg) of the reflex blood. Mean values with 95% confidence intervals. For days 1, 3 and 5 n = 20, for days 8, 11, 14 and 17 n = 130

As noted by Brakefield (1985b) and suggested by Holloway *et al.* (1991) with respect to 7-spot ladybirds, the sex ratio could be a consequence of differential mortality of (lighter) males during the winter. However, recent studies (G. J. Holloway *et al.*, unpubl.) have shown that this sex ratio bias is still present when animals are reared from egg to adult in the laboratory.

As a result of their larger size, females produced on average more reflex fluid than males (1.2 mg *vs* 0.92 mg on day 8, 0.85 mg *vs* 0.72 mg on day 11, 0.88 mg *vs* 0.73 mg on day 14 and 0.91 mg *vs* 0.68 mg on day 17 ($F_{1,128} > 9.6$, $p < 0.002$ in all cases)). However, the mean (± standard error) amounts of reflex fluid produced on days 8, 11, 14 and 17 by males and females as a proportion of body weight was less variable (0.076 ± 0.0024 *vs* 0.074 ± 0.0019, 0.062 ± 0.0023 *vs* 0.053 ± 0.0014, 0.063 ± 0.0023 *vs* 0.056 ± 0.0013 and 0.058 ± 0.0023 *vs* 0.057 ± 0.0014, respectively). The pairs of values from days 8 and 17 were not significantly different from each other, but there were significant differences between the pairs of means from days 11 and 14 ($F_{1,128} = 11.1$, $p < 0.001$ for day 11 and $F_{1,128} = 6{,}93$, $p < 0.01$ for day 14; all analyses were carried out on angular transformed data). The maximum proportion produced by a male at any one time was 0.109 and by a female 0.12. The means of the four values from days 8, 11, 14 and 17 of fluid weight as a proportion of body weight for each ladybird were taken and displayed as histograms for each sex (Fig. 2) to illustrate the shapes of the distributions about the means. There was no evidence of polymodality.

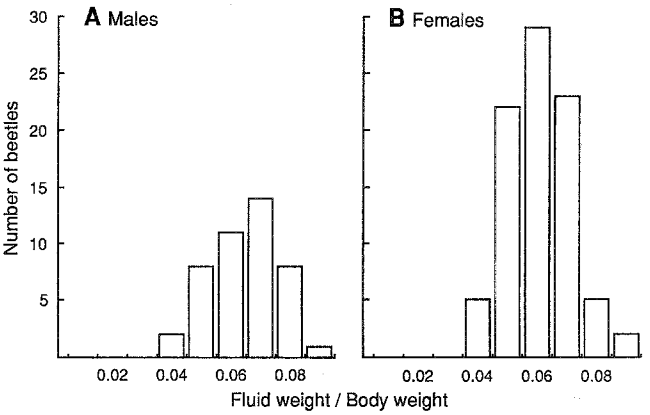

Fig. 2 Distribution of means of the four amounts of fluid produced by each beetle on days 8, 11, 14, and 17 as a proportion of fresh body weight. For males n = 44, for females n = 86

The amounts of adaline per mg reflex fluid for males and females on day 8 were 53.5 ± 2.07 µg and 53.0 ± 2.1 µg (not significantly different, n.s.), respectively. On day 11, males produced fluid containing 28.0 ± 1.71 µg and the females $35.0 \pm 1{,}27$ µg ($F_{1,128} = 10.4$, $p = 0.002$), respectively. On days 14 and 17 the adaline concentration for males dropped further to 19.5 ± 1.09 µg and 18.0 ± 1.21 µg (n.s.), respectively, whilst females produced fluid containing 30.1 ± 0.88 µg and 31.0 ± 0.95 µg (n.s.), respectively. From day 14 to 17 the adaline concentrations reached steady values, but females produced significantly higher concentrations of adaline during that time than males (day 14 $F_{1,128} = 53.5$, $p < 0.001$ and day 17 $F_{1,128} = 67$, $p < 0.001$).

Analyses were carried out to determine whether the amount of fluid produced varied among beetles, so that some beetles always tended to produce more fluid than the overall mean and others less. The amount of fluid produced was always closely correlated with body weight so to remove the influence of body weight, the amount of fluid produced was regressed on fresh body weight and residuals about the regression line were taken. Sets of residuals were obtained for males and females separately on days 8, 11, 14 and 17 and analyses of variance were carried out on the male and female sets of residuals. The residuals varied significantly among both males ($F_{3,43}=4{,}29$, $p<0.001$) and females ($F_{3,85}=3.78$, $p<0.001$).

Similar analyses were carried out on the concentration of the adaline in the reflex fluid, although concentration was corrected for both fluid weight and fresh body weight. Again, significant variation was found among both males ($F_{3,43}=3.3$, $p<0.001$) and females ($F_{3,85}=4{,}76$, $p<0.001$).

We could find no effect of the number of eggs laid on the amount of fluid or adaline produced by females.

Discussion

The study reported here extends the work carried out by Holloway *et al.* (1991) and enables comparisons to be made between the reflex bleeding behaviours of the 7-spot and 2-spot ladybirds. One of the most notable aspects of the studies is the qualitative similarity of the two sets of results. Both of the species were able to exude large quantities of reflex blood as a proportion of their body weights, but only after the beetles had fed on aphids for several days following winter hibernation. Immediately after hibernation, neither species produced very much fluid (Fig. 1A), but the material that was produced was highly concentrated. All individuals reflex bled and the distributions of the values of reflex blood as a proportion of body weight about the means were normal, with no indication of polymodality (Fig. 2). Both 2-spot and 7-spot ladybirds produced substantial amounts of reflex fluid as a proportion of their fresh body weight (0.075 and 0.11 on day 8, respectively). In both species there was statistically significant variation among beetles with respect to the amount of reflex blood produced and the alkaloid concentration of the fluid. This implies that internal factors within an individual determine how much effort is invested in defence. One possible factor contributing to this variation is additive genetic variation (Falconer, 1981; G. J. Holloway *et al.* unpubl.). Furthermore, in both studies, group D animals produced reflex fluid containing less alkaloid in bleedings subsequent to the first post-winter hibernation bleeding (day 8). This can easily be explained if the day 8 bleedings contained newly synthesized alkaloid, together with alkaloid carried over from the previous autumn. However, what is more interesting, and more difficult to explain, is why in the bleedings after day 8, and only on these bleedings, females produced fluid containing a higher concentration of alkaloid than males? The difference between the sexes was always highly significant and strongly implies that it is a real phenomenon. Perhaps the explanation lies in sex limited physiological constraints. Clearly the roles of the sexes differ in the perpetuation of genes to subsequent generations and, as a result, they may partition available energy in different ways among the various important fitness functions (Sibly & Calow 1986; Holloway *et al.* 1990a, b). It is perhaps surprising that we found no effect of egg production on the amount of fluid and alkaloid produced by females, but then the experiment was not designed specifically to search for this. Other experimental designs may be more appropriate.

There were also some differences between the two studies. For example, the rate at which 2-spot ladybirds replenished their reserves of fluid was considerably faster than 7-spot ladybirds. 7-spot ladybirds are around three times as heavy as 2-spot ladybirds, therefore there could conceivably be a size related explanation. Perhaps the most remarkable difference, though, concerned the amount of alkaloid synthesized by the two species. In µg/mg fluid, 2-spot ladybirds produced from 6 to over 8 times as much adaline as 7-spot ladybirds produced coccinelline. In view of the distastefulness and toxicity of 7-spot ladybirds to vertebrate predators (Marples *et al.* 1989), this result serves to highlight the highly noxious nature of coccinelline.

Overall, the two species seem to operate their defence systems in similar ways which, in both species, almost certainly involves a considerable investment of energy. As explained in the introduction, it has been argued that the red forms of 2-spot ladybirds are Batesian mimics of 7-spot ladybirds and this formed part of the incentive for carrying out the study. As Figure 2 shows, there was no category of animals which invested nothing or very little in chemical defence. All 2-spot ladybirds are capable of producing large quantities of reflex fluid and also synthesise substantial amounts of adaline. These observations suggest that the 2-spot ladybird is not relying for protection, at least not entirely, on its aposematic colour resemblance to 7-spot ladybirds. In view of these similarities between the two species discussed above, the discussion laid out in Holloway *et al.* (1991) on the evolution and maintenance of aposematic coloration in the 7-spot ladybird probably also applies to the 2-spot ladybird. Mimicry can, of course, be extended to the chemical level, although the spectrum of volatile pyrazine production in the two species is very different (Moore *et al.* 1990), so that smell mimicry is unlikely to play a role. However, if a predator picks up a 2-spot ladybird, despite its resemblance to 7-spot ladybirds, the adaline in the reflex blood could be used to mimic the taste of coccinelline. The ingestion of coccinelline can have severe consequences for bird predators (Marples *et al.* 1989), so that a taste reminiscent of coccinelline may be sufficient to cause a predator to release a 2-spot.

2-Spot and 7-spot ladybirds can be found in similar shrub and herbage habitats during the spring and early summer and are probably exposed to a similar spectrum of predators. From mid to late June, 2-spot ladybirds move into trees, principally Lime trees (*Tylia europaea* L.) (Brakefield 1984), whilst 7-spot ladybirds remain in the herbage (pers. obs.). During the late summer and early autumn, both species aestivate and can frequently be found together in dead leaves on trees (pers. obs.). However, 2-spot and 7-spot ladybirds overwinter in different habitats; 2-spot ladybirds in natural habitats can be found on or under tree bark, sometimes in conspicuous groups (Brakefield 1985b) and 7-spot ladybirds overwinter in leaf litter (Majerus & Kearns 1989; authors pers. obs.). These different overwintering behaviours may expose the two species to very different predators. During the winter, birds such as thrushes (*Turdus* spp.) and titmice (*Parus* spp.) and possibly also small mammals foraging on the ground could find 7-spot ladybirds, whilst potential 2-spot ladybird predators include treecreepers (*Certhia* spp.), nuthatches (*Sitta europeae*) and woodpeckers (*e.g. Dendrocopus* spp.). The winter 2-spot ladybird predators forage almost exclusively on tree trunks and branches and, therefore, have very little opportunity to sample 7-spot ladybirds. Thus the memory of unpleasant experiences with 7-spot ladybirds is unlikely to play a role in defending 2-spot ladybirds during the winter. Consequently, possible mimetic relationships between the two species that exist in the summer break down during the winter. Even so, overwintering 2-spot ladybirds appear to suffer rather little from bird predation (Brakefield 1985b).

In view of the non-overlap in winter habitat, the amount of material and, presumably, energy invested in defence by the 2-spot ladybird and the very similar qualities of the reflex blood in the two species, it is more likely that the 2-spot ladybird is able to defend itself to a degree against bird predators in the absence of 7-spot ladybirds.

Adaline may be effective against other predators, such as invertebrates, although adaline is also less effective as an ant deterrent than coccinelline (Marples 1990). Although vertebrate predation must be a significant selective force (aposematic coloration could not have evolved otherwise), the largest biotic mortality factor in adult ladybirds may be parasitization. Majerus & Kearns (1989) summarize some of the parasites of ladybirds and point out that the most important hymenopteran parasite is *Perilitus coccinellae.* 16% of the adult 7-spot ladybirds were parasitized by *P. coccinellae* in the study of Holloway *et al.* (1991) and the level of parasitism can reach 50% in some populations (N Marples, pers comm). Majerus & Kearns (1989) list the species known to be parasitized by *P. coccinellae*; they include three *Coccinella* spp: 5-spot *(C. 5-punctata)*, 7-spot and 11-spot *(C. 11-punctata)* ladybirds, all of which produce coccinelline (Pasteels *et al.* 1973). Of the three species named as not being parasitized by *P. coccinellae*, two of them are the British species of *Adalia:* 2-spot and 10-spot *(A. 10-punctata)* ladybirds and, indeed, none of the 2-spot ladybirds from the present study were parasitized. Majerus & Kearns (1989) suggest that the latter species are too small to be favoured by *P. coccinellae,* but 11-spot ladybirds are the same size as 2-spot ladybirds. Therefore, another possible function of adaline is to reduce hymenopteran parasite loads in *Adalia* spp.

Another important question concerning adaline production is why do 2-spot ladybirds produce so much alkaloid in the reflex blood? If adaline deters *P. coccinellae,* then it is not needed in the reflex blood *per se* at such high concentrations as a defence against parasitism. The same is true if adaline is toxic to *P. coccinellae* eggs and/or larvae. Work has been carried out comparing the relative noxiousness of coccinelline and adaline (Marples *et al.* 1989) and the conclusion is that adaline is less toxic and repellent than coccinelline to birds. This does not mean that adaline has no repellent properties at all against all potential bird predators. As already argued, 2-spot ladybird adults are probably able to defend themselves against attack, but only if adaline (or some other principal) has an effect. It is possible, therefore, that the amount of adaline synthesized is high in order to compensate for the lower repellency/toxicity.

In summary, the chemical defence system with respect to alkaloids in 2-spot ladybirds is well developed and this does not appear to be consistent with a Batesian explanation for resemblance to 7-spot ladybirds. However, the chemical defence may be targetted against non-avian predators and it is conceivable that some kind of reciprocal mimicry between these two species operates with respect to different spectrums of natural enemies.

Acknowledgements

We are grateful to Dr. N. M. Marples for her helpful comments for improvements to the manuscript and Marian Joven for technical assistance. Henk Heijn produced the figures. P. W. de Jong was supported by the Foundation for Biological Research (BION, grant #436.031) which is subsidized by the Netherlands Organization for Scientific Research (NWO).

References

Brakefield PM (1984) Ecological studies on the polymorphic ladybird *Adalia bipunctata* in the Netherlands. I. Population biology and geographical variation of melanism. J Anim Ecol 53:761–774

Brakefield PM (1985a) Polymorphic Mullerian mimicry and interactions with thermal melanism in ladybirds and a soldier beetle: a hypothesis. Biol J Linn Soc 26:243–267

Brakefield PM (1985b) Differential winter mortality and seasonal selection in the polymorphic ladybird *Adalia bipunctata* (L.) in the Netherlands. (Biol J Linn Soc 24:189–206

Brower LP, Brower JVZ, Corvina JM (1967) Plant poisons in a terrestrial food chain. Proc Natl Acad Sci USA 57:893–898

Brower LP, Pough FH, Meck HR (1970) Theoretical investigations of automimicry. I. Single trial learning. Proc. Natl Acad Sci USA 66:1059–1066

Cuenot L (1896) Sur la saignée réfluxe et les moyens défense de quelques insectes. Archs Zool exp gén 4:655–680

Falconer DS (1981) Introduction to Quantitative Genetics (2nd ed). London: Longman

Frazer JFD, Rothschild M (1960) Defence mechanisms in warningly coloured moths and other insects. Proc 11th int Congr Ent Symp 4:249–256

Gibson DO (1974) Batesian mimicry without distastefulness. Nature 250:77–79

Guilford T (1988) The evolution of conspicuous coloration. Amer Nat Suppl 131:S7–S21

Happ GM, Eisner T (1961) Hemorrhage in a coccinellid beetle and its repellent effect on ants. Science 134:329–331

Hollande CH (1911) L'autohémorrhée ou le rejet du sang chez les insectes (toxicologie du sang). Archs Anat microsc 13:171–318

Holloway GJ, de Jong PW, Brakefield PM, de Vos H (1991) Chemical defence in ladybird beetles (Coccinellidae). I. Distribution of coccinelline and individual variation in defence in 7-spot ladybirds (*Coccinella septempunctata*). Chemoecology 2:7–14

Holloway GJ, Povey SR, Sibly RM (1990a) The effect of new environment on adapted genetic architecture. Heredity 64:323-330

Holloway GJ, Sibly RM, Povey SR (1990b) Evolution in toxin stressed environments. Funct Ecol 4:289–294

Majerus M, Kearns P (1989) Ladybirds. Naturalist Handbooks 10. Slough, England: Richmond Publishing Co Ltd

Marples NM (1990) The influence of predation on ladybird colour pattern. PhD thesis: University of Wales College of Cardiff

Marples NM, Brakefield PM, Cowie RJ (1989) Differences between the 7-spot and 2-spot ladybird beetles (Coccinellidae) in their toxic effects on a bird predator. Ecol Entomol 14:79–84

Moore BP, Brown WV, Rothschild M (1990) Methylalkylpyrazines in aposematic insects, their hostplants and mimics. Chemoecology 1:43–51

Pasteels JM, Deroe C, Tursch B, Braekman JC, Daloze D, Hootele C (1973) Distribution et activites des alcaloides defensifs des Coccinellidae. J Insect Physiol 19:1771–1784

Pough FH, Brower LP, Meck HR, Kessell SR (1973) Theoretical investigations of automimicry: multiple trial learning and the palatability spectrum. Proc Natl Acad Sci USA 70:2261–2265

Sibly RM, Calow P (1986) Physiological Ecology of Animals: An Evolutionary Approach. Oxford: Blackwell

Tursch B, Daloze D, Dupont M, Hootele C, Kaisen M, Pasteels JM, Zimmermann D (1971) Coccinelline, the defensive alkaloid of the beetle *Coccinella septempuctata.* Chemia 25:307

Tursch B, Daloze D, Braekman JC, Hootele C, Pasteels JM (1975) Chemical ecology of arthropods – X. The structure of myrrhine and the biosynthesis of coccinelline. Tetrahedron 31:1541–1543

Tursch B, Braekman JC, Daloze D (1976) Arthropod alkaloids. Experientia 32:401–407