PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

A Quantitative Genetic Analysis of an Aposematic Colour Pattern and Its Ecological Implications

Graham J. Holloway, Paul M. Brakefield, Peter W. De Jong, Mart M. Ottenheim, Helene De Vos, Fanja Kesbeke and Lucy Peynenburg

Phil. Trans. R. Soc. Lond. B 1995 **348**, 373-379
doi: 10.1098/rstb.1995.0075

A quantitative genetic analysis of an aposematic colour pattern and its ecological implications

GRAHAM J. HOLLOWAY[1,2], PAUL M. BRAKEFIELD[2], PETER W. DE JONG[2], MART M. OTTENHEIM[2], HELENE DE VOS[2], FANJA KESBEKE[2] AND LUCY PEYNENBURG[2]

[1] *School of Animal and Microbial Sciences, AMS Building, University of Reading, Whiteknights, P.O. Box 228, Reading, Berkshire RG6 2AJ, U.K.*

[2] *Section of Evolutionary Biology, Institute of Evolutionary and Ecological Sciences, University of Leiden, Schelpenkade 14a, 2313 ZT Leiden, The Netherlands.*

SUMMARY

The phenotypic and genetic variability of the aposematic colour pattern of the two-spot ladybird was investigated. There was significant variation in spot size (relative to elytron length) among wild populations of ladybirds. A quantitative genetic analysis revealed high heritability estimates for a number of individual colour pattern elements on the pronotum and elytra. The genetic covariances between the elytral spot and the pronotal spots were generally negative and all of the covariances among the pronotal spots were positive. This pattern of covariances could be explained if natural selection acts so as to maximize the combined anti-predator effect of the pronotal and elytral colour patterns by optimizing the rate of melanin production. An optimization process could also account for the significant levels of additive genetic variation found for each of the pattern elements considered. There was no evidence of any sex-linked gene expression for colour pattern, although female colour pattern may be influenced by maternally inherited factors.

1. INTRODUCTION

The evolution of colour patterns in insects provide some of the clearest examples of the effects of natural selection available. These patterns include cryptic and warning colorations and also eyespots. A number of workers have considered the factors facilitating the development and maintenance of aposematic coloration and mimicry (e.g. Harvey & Paxton 1981; Harvey *et al.* 1982; Wikland & Jarvi 1982; Sillen-Tullberg & Bryant 1983; Guilford 1985, 1988). Research has indicated the necessity for a chemical defence system to render the insect distasteful or toxic to predators as a precursor to the evolution of aposematic coloration (Guilford 1988). Once a warning coloration has become established in the population, natural selection favours uniformity so that all individuals in a population share the same colour pattern (Turner 1977 1984; Sheppard *et al.* 1985). This stabilization process implies a reduction in genetic variation to provide a uniform phenotype.

Ladybirds are both chemically defended and aposematically coloured. Chemical defence systems have already been implicated in the evolution of aposematic coloration (Guilford 1988). Individual ladybirds vary substantially in amount of chemical defence produced and this is true both for both species well defended (seven-spot ladybird) (Holloway *et al.* 1991) and less well defended (two-spot ladybird) (de Jong *et al.* 1991) against vertebrate predators. This variation can be partly attributed to genetic variation maintained through trade-offs with other important fitness characters (Holloway *et al.* 1993*b*). Of course, even though chemical defence and aposematic coloration are linked, variation in defence does not imply that variation in coloration should also exist. However, a similar genetic examination of an aposematic coloration has never been carried out. The genetic studies that have been carried out to date have been largely concerned with the production of unusual variants, presumably through crossing over events within the supergene complex that determines colour pattern (*see* Majerus 1994 and references therein).

The arguments supporting normalizing selection on aposematic colour patterns are convincing, but these colour patterns are almost invariably considered as complete units; the elements that combine to make a warning pattern receive less attention. It is not known whether the components of colour patterns are also subject to these stabilizing effects. In other words, which elements are focused upon by a visual predator. Indeed, a certain amount of phenotypic variation in spot size in ladybirds has been noted (Dobzhansky and Sivertzev-Dobzhansky 1927; Hodek 1973).

The purpose of the current study was: (i) to examine field collected material to determine levels of variation in colour pattern among wild populations of *typica* (red elytra with black spots) two-spot ladybirds; and (ii) to

Phil. Trans. R. Soc. Lond. B (1995) **348**, 373–379
Printed in Great Britain

examine the genetic architecture of the aposematic colour pattern in the same morph. Information obtained from the study was combined to determine whether the component parts of the aposematic colour pattern are also or have been subjected to strong selection pressures as are predicted to influence the pattern as a whole.

2. MATERIALS AND METHODS

Samples of two-spot ladybirds were collected from ten sites in The Netherlands in 1991. The sites lay along an East–West transect across the country from Tilburg in the East to Ouddorp close to the coast in Zeeland in the West. This transect was chosen because it corresponds with the line along which previous studies on two-spot ladybird coloration have been carried out (Brakefield 1984*b*). Each site was visited only once between 23–30 July. Insects were collected from shrubbery and trees around breeding and summer aestivation (principally on plane trees, *Platanus* × *hybrida*) sites. Because of the timing of the collections, most of the insects would have been born in the year of collection.

The insects were examined individually under a Wild binocular microscope. The straight midline length of the left elytron was measured at × 25 while the anterior posterior length of the left black spot on the elytron was measured at × 50.

About 300 two-spot ladybirds were collected in September 1990 in Breda (The Netherlands) from plane (*Platanus* × *hybrida*) and lime (*Tilia* sp.) trees and surrounding shrubbery. All of the animals were phenotypically form *typica* in that they all had red elytra with one black spot on each elytron (although with respect to the colour pattern supergene they may not have all been the same genotype [Majerus 1994]). These ladybirds were split into groups of 15 and placed into 7.5 cm diameter plastic Petri dishes in a constant climate room (20 °C, 16:8 h light:dark). A 7 cm filter paper was placed into the bottom of each Petri dish to facilitate cleaning. The ladybirds were provided with ample pea aphids (*Acyrthosiphon pisum* Harris) daily and, also each day, the dishes were inspected for the presence of eggs. If eggs had been laid the adults were removed and placed in a clean Petri dish to prevent the eggs from being eaten by the beetles. Adults began to lay eggs after about a week and egg laying peaked around two weeks after the beginning of the experiment. They were allowed to lay eggs until all of the adult females died, mainly after four weeks. These adults were discarded.

The larvae produced were reared through to adults under the same constant conditions and were used as the parental generation in the experimental design. Henceforth, these insects will be referred to as the parents. The procedure followed to rear the parents through to adults and the thinning operation used to reduce loss through cannibalism is described in detail in Holloway *et al.* (1993*b*). Newly eclosed ladybirds were kept separately in 5 cm diameter Petri dishes under the same constant conditions to maintain virginity and were supplied daily with fresh aphids. They were fed for three days to allow the cuticle to fully harden before being transferred to a refrigerator at 9 °C. Adult two-spot ladybirds overwinter in temperate climates (Brakefield 1985; Hemptinne 1988) and can often survive at 9 °C for many months since the low temperature induces a state of dormancy. As the eggs were laid over a long period of time, the adults also emerged over an extended period. The use of the refrigerator enabled the pairing of the adults to produce the F_1 to be synchronized.

307 adult insects (parents) were reared successfully. When the last adult insect emerged from the pupa it was fed for three days and then all of the ladybirds were removed from the refrigerator. The ladybirds were returned to the constant climate cabinet and fed daily with fresh pea aphids for seven days and then assigned a unique number between 1–307. The insects were sexed using ventral abdominal characters (Majerus & Kearns 1989; de Jong *et al.* 1991; Randall *et al.* 1992) under a Wild binocular microscope at × 25 and paired up at random to avoid introducing biases into the subsequent quantitative genetics analyses (Gimelfarb 1985). Due to a biased sex ratio (Ottenheim *et al.* 1992) 134 pairs were set up, which were fed daily with fresh pea aphids and allowed to mate and lay eggs. After about three weeks of oviposition the parent insects were transferred to a freezer at −30 °C for storage and analysis at a later date. The F_1 was reared following the same procedure as described above for the parents. F_1 beetles were fed for seven days following emergence to provide sufficient time for the cuticle to harden and the colour pattern to develop completely. They were then transferred to the freezer.

The parental and F_1 adult beetles were examined individually under a Wild binocular microscope. The straight midline length of the left elytron was measured at × 25. As with the field collected insects the length of the black spot (spot 1) on the elytron was also measured at × 50. In addition to this spot three further spots were measured on the pronotum. These are referred to as spots 2, 3 and 4. Figure 1 shows the positions of these spots and the axes along which they were measured.

The quantitative genetic analysis was carried out using offspring–parent regression. Offspring values were regressed on paternal and maternal values separately to provide information on additive genetic and maternally inherited effects. The relationship between both the additive genetic variance (V_a) and covariance (cov_a) and the offspring on father regression coefficient (b) is described by Becker (1984) and Falconer (1989).

The final family size varied (maximum 60) so we used a jackknife resampling procedure (Quenouille 1949, 1956; Tukey 1958; Arvesen & Schmitz 1970; Miller 1974; Sokal & Rohlf 1981; Potvin & Roff 1992) which can be used to calculate V_a, cov_a and their associated errors even when the data set is unbalanced (Holloway *et al.* 1990). Details of all the statistical analyses carried out are described by Holloway *et al.* (1993*b*).

Jackknifing was carried out with data on the five characters described above. Heritability was estimated for all five characters, while cov_a values were estimated

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

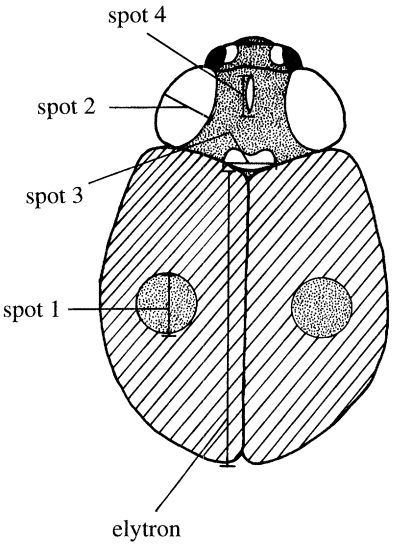

Figure 1. Schematic illustration of a two-spot ladybird showing the positions of spots 1–4 and the axes along which these spots and the elytron were measured. The stippled areas represent black and the hatched areas represent red.

between pairs of spots. Spot size was always phenotypically correlated with elytron length. If elytron length is heritable, then spot size would automatically show variation among families. To correct for this effect the spot size residuals were taken about a regression line of spot size on elytron length. Within-generation values were used to make these (and subsequent) corrections, i.e. offspring spot size on offspring elytron length and father spot size on father elytron length. Spot size residuals were used to calculate the genetic parameters, but are referred to as spot size throughout the text.

The genetic variance/covariance matrices (G-matrices) were compared using maximum likelihood Mahalanobis D^2 tests (Krzanowski 1988). For details see Holloway *et al.* (1993*b*). Jackknifing was carried out using a macro written in Minitab. All other statistical tests (except Mahalanobis D^2) were also carried out using Minitab. Comparisons of mean values were carried out using GLM. Levels of significance are shown.

3. RESULTS

A total of 1150 field collected animals were examined. Table 1 shows a summary of these data. There was significant among site variation in elytron length ($p < 0.001$). Since spot is correlated with elytron length in two-spot ladybirds (see Materials and Methods) the ratio of spot length over elytron length was analysed. This ratio was also found to vary among sites ($p = 0.003$) indicating among site variation in colour pattern. There was no obvious trend in the mean site values along the transect.

A total of 1243 undamaged offspring were reared in the laboratory which were suitable for colour pattern measurement. Of these, 510 were male. Sex ratio biases are commonly found in ladybirds and this phenomenon is discussed elsewhere (Ottenheim *et al.* 1992; Hurst *et al.* 1993). The mean values of the lengths of the elytra and spot sizes are shown in table 2 together with the ratios of the various spot sizes over elytron length. There was a significant difference between the sexes for all of the mean absolute values in table 2 ($p < 0.005$ at least in all cases) with females always having larger values. However, females are larger than males and the sizes of all spots were phenotypically correlated with elytron length ($p < 0.001$ in all cases). Consequently, the ratios of spot 1 and spot 4 over elytron length did not differ between the sexes. The ratios of the other two spots still varied significantly between the sexes ($p < 0.001$ in both cases). The values of the ratios of the elytral spot (spot 1) over elytron length for both sexes were very similar to the values found in the animals collected from the field (see table 1).

The heritabilities of all five characters were estimated for both male and female data sets regressed on father and mother values. Regression on father yields narrow sense estimates whilst regression on mother values may include maternal effects (i.e. broad sense), if there are any. All of the spots were highly heritable (range 0.396–0.807) and all of these estimates were significantly different from zero to $p < 0.001$. Elytron length was less heritable (range 0.045–0.223). All were statistically significant except for the male elytron broad sense ($p = 0.07$) and female elytron narrow sense estimates ($p = 0.69$). There was close agreement

Table 1. *Mean straight midline elytron length, length of black elytral spot and the ratio of spot length over elytron length for two-spot ladybirds collected in The Netherlands in 1991 at the sites mentioned*

(s.e. of length are attached.)

		length/mm		
site	*n*	elytra	spot	ratio
Ouddorp	107	4.277±0.026	0.734±0.011	0.172±0.002
Stellendam	85	4.240±0.032	0.699±0.012	0.165±0.003
Dirksland	137	4.180±0.026	0.683±0.010	0.163±0.002
Middelharnis	171	4.234±0.022	0.691±0.008	0.163±0.002
Oude Tonge	161	4.093±0.023	0.670±0.008	0.164±0.002
Achthuizen	99	4.189±0.033	0.688±0.012	0.164±0.002
Willemstad	121	4.236±0.026	0.718±0.010	0.170±0.002
Zevenbergen	97	4.018±0.032	0.683±0.010	0.170±0.002
Etten Leur	86	4.092±0.035	0.685±0.012	0.168±0.003
Tilburg	86	4.136±0.032	0.660±0.012	0.160±0.003

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Table 2. *Mean straight midline elytron length and the sizes of spots 1 to 4 (see figure 1) for the male and female two-spot ladybirds reared in the laboratory*

(The ratios refer to the size of each spot divided by the length of the elytron. s.e.s are attached.)

	male		female	
	mean	ratio	mean	ratio
elytron length/mm	3.836 ± 0.007	—	4.226 ± 0.008	—
spot 1	0.649 ± 0.004	0.169 ± 0.001	0.707 ± 0.004	0.167 ± 0.001
spot 2	0.667 ± 0.002	0.172 ± 0.001	0.686 ± 0.002	0.163 ± 0.001
spot 3	0.756 ± 0.004	0.199 ± 0.001	0.802 ± 0.004	0.190 ± 0.001
spot 4	0.165 ± 0.007	0.043 ± 0.002	0.191 ± 0.006	0.045 ± 0.001

Table 3. *Narrow and broad sense additive genetic variance/covariance matrices derived from male and female two-spot ladybirds for spots 1–4 (see figure 1). The probability that real value equals zero is given in parentheses. All values* $\times 10^{-3}$

	spot			
spot	1	2	3	4
males: narrow sense				
1	5.62 ($p < 0.001$)	−0.63 ($p = 0.15$)	−0.85 ($p = 0.4$)	−0.44 ($p = 0.85$)
2		0.70 ($p < 0.001$)	0.81 ($p = 0.03$)	1.06 ($p = 0.24$)
3			2.91 ($p = < 0.001$)	1.44 ($p = 0.42$)
4				14.62 ($p = 0.001$)
males: broad sense				
1	5.41 ($p < 0.001$)	−0.68 ($p = 0.21$)	−0.82 ($p = 0.44$)	1.21 ($p = 0.6$)
2		0.97 ($p < 0.001$)	0.68 ($p = 0.14$)	2.43 ($p = 0.03$)
3			4.40 ($p < 0.001$)	4.10 ($p = 0.03$)
4				16.27 ($p < 0.001$)
females: narrow sense				
1	7.57 ($p < 0.001$)	−0.59 ($p = 0.12$)	−0.99 ($p = 0.48$)	−0.64 ($p = 0.77$)
2		0.72 ($p < 0.001$)	0.98 ($p = 0.16$)	0.81 ($p = 0.48$)
3			3.24 ($p < 0.001$	2.86 ($p = 0.13$)
4				15.09 ($p < 0.001$)
females: broad sense				
1	4.51 ($p < 0.001$)	−0.04 ($p = 0.93$)	−1.02 ($p = 0.52$)	1.46 ($p = 0.51$)
2		0.70 ($p < 0.001$)	1.01 ($p = 0.05$)	2.01 ($p = 0.04$)
3			4.77 ($p < 0.001$)	4.80 ($p = 0.01$)
4				23.04 ($p < 0.001$)

between the narrow sense heritability estimates for the male and female spot sizes. The broad sense estimates were not consistently larger than the narrow sense estimates. Table 3 shows the G-matrices for the male and female data sets. In all cases, spot size showed significant V_a (diagonals) ($p < 0.001$). The estimates of V_a were similar in the two data sets and only for spots 3 and 4 was there a suggestion that the broad sense estimates were larger than the narrow sense estimates, indicating the presence of a small amount of maternally derived variation.

None of the 12 covariances in table 3 between spot 1 and the other spots were individually significantly different from zero, but ten of these 12 covariances were negative. The chance of this occurring if there really was no covariance is small ($p = 0.055$). Of the

remaining 12 covariances between spots 2, 3 and 4, six of them were significantly different from zero and all of them were positive. However, given the number of significance tests that have been carried out it is likely that some of these values are significant simply through chance. Adjusting the critical levels for significance using a Bonferroni test (Rice 1989) would undoubtedly reduce the number of significant values. This would not, though, alter the conclusions concerning the patterns of the signs within the matrices. Only for the covariances involving spot 4 were the broad sense estimates consistently larger than the narrow sense estimates.

Overall comparisons of the G-matrices in table 3 were carried out using Mahalanobis D^2 tests (Krzanowski 1988; Holloway *et al.* 1990, 1993*b*). With ten degrees of freedom in each case, the tests produced the following χ^2 values: matrix 4ia versus matrix 4ib = 12.7 (n.s.), matrix 4iia versus matrix 4iib = 23.9 ($p = 0.01$), matrix 4ia versus matrix 4iia = 6.6 (n.s.), and matrix 4ib versus matrix 4iib = 5.6 (n.s).

4. DISCUSSION

The genetic analysis of insect colour patterns can produce information on the types of selective forces that may be acting on the phenotype. Ladybirds are predated by vertebrates and invertebrates (e.g. *Perilitus coccinellae*), but it is likely that vertebrate (visual) predators have influenced the evolution of protective coloration in insects to a greater extent. It is less obvious, however, at what level this selection acts. For example, do predators select on colour only, which European Goshawks, *Accipiter gentilis*, may do (Götmark, 1994), or also the general distribution of those colours, or down to the level of the size or shape of individual elements of a colour pattern. The field data showed that the size of one component of the warning colour varied significantly among sites across The Netherlands. This could suggest that the varying environmental conditions along the transect impose different selection regimes upon the insects which favour different spot sizes. However, the very high levels of genetic variation found may not be wholly consistent with this idea and the lack of, at least strong, selection on this component of the colour pattern might be more likely.

Few studies have been carried out on the genetics of pattern element size in insects. Exceptions include Brakefield (1984*a*), Brakefield & van Noordwijk (1985) and Holloway *et al.* (1993*a*). All of these studies concerned butterfly colour patterns which are selected for defensive purposes in response to vertebrate predation (Brakefield & Larsen 1984). In all cases, spot size was found to be highly heritable. These results, including the present ladybird results, may suggest that while the spots on these particular patterns are necessary elements, their precise size is not greatly important. Consequently, they might not be subjected to high levels of directional or stabilizing selection which would erode genetic variation (Falconer 1989).

The genetic covariance estimates (see table 3) show a non-random distribution of signs, with most of the covariances between the black elytral spot and the white spots on the pronotum being negative, and all of the covariances among the white pronotal spots being positive. Genetic covariances are notoriously difficult to measure with precision, but the probability of obtaining such a distribution of signs through chance is very small. This suggests that the matrices are a true reflection of the genetic architecture of the colour pattern, a conclusion further supported by the genetic covariances that do differ significantly from zero. The production of melanin plays a key role in the development of colour patterns in many ladybird species. The spots on the elytra are black, as is most of the surface area of the pronotum and head in the populations under consideration. Those areas of the pronotum not melanized are the white spots measured in the present study. An individual with a high rate of melanin production may produce large elytral spots and a large black area on the pronotum with, consequently, small white spots. Activity of the biochemical pathway leading to the production of melanin may therefore be sufficient to explain the distribution of signs of the covariances in table 3. It is interesting to note in connection with this that Lus (1932) described a number of pronotal patterns in the two-spot ladybird and came to the conclusion that these patterns were controlled by genes that were tightly linked to the elytral pattern gene locus.

Marples (1990) and Marples *et al.* (1994) have shown that the red/black ladybird elytral colour pattern examined here confers a level of protection against vertebrate predation. Roper & Cook (1989) demonstrated that many bird species show considerable aversion to black. Hence it is possible that these two components of the colour pattern, red elytra with black spots and black pronotum with white spots, combine to enhance protection against vertebrate predation. The rate of melanization may be subject to optimizing selection to produce pronotal and elytral patterns that combine to maximize effectiveness. Selection for optimization can maintain high levels of genetic variation, as found in the present study. There are many examples of optimization processes which promote the maintenance of significant levels of genetic variance in insects (e.g. Rose & Charlesworth 1981; Luckinbill *et al.* 1984; Møller *et al.* 1989; Holloway *et al.* 1990), including two-spot ladybirds (Holloway *et al.* 1993*b*).

Holloway *et al.* (1993*b*) studied the genetics of certain defence and life-history characters in the two-spot ladybird and detected significant levels of sex limited gene expression for all of the characters examined. If different sets of genes operate in the two sexes the structure of the G-matrices derived from the two sexes should vary. The narrow sense G-matrices derived from males and females (see table 3) were not significantly different in the present study, as was the case for the corresponding broad sense matrices. There was, therefore, no evidence for a significant amount of sex limited gene expression affecting the colour pattern of the two-spot ladybirds in the present study. Comparison of the narrow and broad sense matrices within sex provided an indication of the operation of

maternally inherited factors in females, but not in males. The biochemical pathway leading to the production of melanin in insects is relatively simple (Blois 1978) and probably involves only a small number of enzymic steps (although there may be rather more modifier genes involved). If the rate of melanin production does play a key role in determining the G-matrix structure (see table 3), there might be less scope for between sex genetic variation than with life-history characters (Holloway *et al.* 1993*b*).

In conclusion, although the high heritability estimates and the among site variation suggest low levels of selection on spot size in two-spot ladybirds, the G-matrix structure indicate that balancing selection may be involved in maintaining significant levels of genetic variation. Another possibility is that spot size is inherited as a Mendelian character of one or two genes, as are the overall colour patterns (Majerus 1994). This could also account for the high heritability estimates, although spot size shows a continuous distribution and a discrete mode of inheritance is most unlikely (Majerus personal communication). Also, the amount of black in the 14-spot ladybird (*Propylea* 14-*punctata*) has been shown to be polygenic, also with a high heritability (Majerus 1994). More work is needed to determine which of these scenarios is the most likely.

We are very grateful to Mike Majerus, Nicola Marples and an anonymous referee for making valuable suggestions for improving the manuscript.

REFERENCES

Arvesen, J.M. & Schmitz, T.H. 1970 Robust procedures for variance component problems using the jackknife. *Biometrika* **26**, 677–686.

Becker, W.A. 1984 *Manual of procedures in quantitative genetics*, edn 4. Pullman, Washington: Academic Enterprises.

Blois, M.S. 1978 The melanins: their synthesis and structure. In *Photochemical and photobiological reviews*, vol. 3 (ed. K.C. Smith), pp. 115–134. New York: Plenum Press.

Brakefield, P.M. 1984*a* The ecological genetics of quantitative characters in *Maniola jurtina* and other butterflies. In *The biology of butterflies*, pp. 167–190. Symposium of the Royal Entomological Society of London 11. London: Academic Press.

Brakefield, P.M. 1984*b* 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, P.M. 1985 Differential winter mortality and seasonal selection in the polymorphic ladybird *Adalia bipunctata* (L.) in The Netherlands. *Biol. J. Linn. Soc.* **24**, 189–206.

Brakefield, P.M. & Larsen, T.B. 1984 The evolutionary significance of dry and wet season forms in some tropical butterflies. *Biol. J. Linn. Soc.* **22**, 1–12.

Brakefield, P.M. & van Noordwijk, A.J. 1985 The genetics of spot pattern characters in the meadow brown butterfly *Maniola jurtina* (Lepidoptera: Satyridae). *Heredity*, *Lond.* **54**, 275–284.

Conn, D.L.T. 1973 Evidence of restricted mimetic colour polymorphism in the large narcissus bulb fly, *Merodon equestris* Fab (Diptera: Syrphidae), in the Pyrenees. *Heredity*, *Lond.* **36**, 185–189.

de Jong, P.W., Holloway, G.J., Brakefield, P.M. & de Vos, H. 1991 Chemical defence in ladybird beetles (Coccinellidae). II. Amount of reflex fluid, the alkaloid adaline and individual variation in defence in two-spot ladybirds (*Adalia bipunctata*). *Chemoecology* **2**, 15–19.

Dittrich, W., Gilbert, F., Green, P., McGregor, P. & Grewcock, D. 1993 Imperfect mimicry: a pigeon's perspective. *Proc. R. Soc. Lond.* B **251**, 195–200.

Dobzhansky, Th. & Sivertzev-Dobzhansky, N.P. 1927 Die geographische variabilität von *Coccinella septempunctata* L. *Biol. Zbl.* **47**, 556–569.

Falconer, D.S. 1989 *Introduction to quantitative genetics*, edn 3. London: Longman.

Gimelfarb, A. 1985 Is offspring-midparent regression affected by assortative mating of parents? *Genet. Res.* **47**, 71–75.

Götmark, F. 1994 Does a novel bright colour patch increase or decrease predation? Red wings reduce predation risk in European blackbirds. *Proc. R. Soc. Lond.* B **256**, 83–87.

Guilford, T. 1985 Is kin selection involved in the evolution of warning coloration? *Oikos* **45**, 31–36.

Guilford, T. 1988 The evolution of conspicuous coloration. *Am. Nat.* **131**, 7– 21. (Suppl.)

Harvey, P.H. & Paxton, R.J. 1981 The evolution of aposematic coloration. *Oikos* **37**, 391–393.

Harvey, P.H, Bull, J.J., Pemberton, M. & Paxton, M.J. 1982 The evolution of aposematic coloration in distasteful prey: a family model. *Am. Nat.* **119**, 710–719.

Heal, J.R. 1979 Colour patterns of Syrphidae. I. Genetic variation in the dronefly *Eristalis tenax*. *Heredity*, *Lond.* **43**, 229–238.

Heal, J.R. 1982 Colour patterns of Syrphidae: IV Mimicry and variation in natural populations of *Eristalis tenax*. *Heredity*, *Lond.* **49**, 95–109.

Hemptinne, J.L. 1988 Ecological requirements for hibernating *Propylea quatuordecimpunctata* (L.) and *Coccinella septempunctata* (Col: Coccinellidae). *Entomophaga* **33**, 505–515.

Hodek, I. 1973 *Biology of the Coccinellidae*. The Hague, Holland: W. Junk.

Holloway, G.J., Povey, S.R. & Sibly, R.M. 1990 The effect of new environment on adapted genetic architecture. *Heredity*, *Lond.* **64**, 323–330.

Holloway, G.J., de Jong, P.W., Brakefield, P.M. & 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, G.J., Brakefield, P.M. & Kofman, S. 1993*a* The genetics of wing pattern elements in the polyphenic butterfly, *Bicyclus anynana*. *Heredity*, *Lond.* **70**, 179–186.

Holloway, G.J., de Jong, P.W. & Ottenheim, M. 1993*b* The genetics and cost of chemical defence in the 2-spot ladybird (*Adalia bipunctata* L.). *Evolution* **47**, 1229–1239.

Hurst, G.D.D., Majerus, M.E.N. & Walker, L.E. 1993 The importance of cytoplasmic male killing elements in natural populations of the two spot ladybird, *Adalia bipunctata* (Linnaeus) (Coleoptera: Coccinellidae). *Biol. J. Linn. Soc.* **49**, 195–202.

Krzanowski, W.I. 1988 *Principles of multivariate analysis. A users perspective*. Oxford: Clarendon Press.

Lam, P.K.S. & Calow, P. 1989 Intraspecific life-history variation in *Lymnaea peregra* (Gastropoda: Pulmonata). II. Environmental or genetic variance? *J. Anim. Ecol.* **58**, 589–602.

Luckinbill, L.S., Arking, R., Clare, M.J., Cirocco, W.C. & Buck, S.A. 1984 Selection for delayed senescence in *Drosophila melanogaster*. *Evolution* **38**, 996–1003.

Lus, J.J. 1932 An analysis of the dominance phenotype in the inheritance of the elytra and pronotum colour in *Adalia bipunctata* L. *Trudy Lab. Genetika*, *Leningrad* **9**, 135–162.

Majerus, M. 1994 *Ladybirds*. London: Harper Collins.
Majerus, M. & Kearns, P. 1989 *Ladybirds*. Slough, U.K.: Richmond Publishing Co Ltd.
Marples, N.M. 1990 The influence of predation on ladybird colour patterns. PhD thesis, University of Cardiff.
Marples, N.M, Brakefield, P.M. & Cowie, R.J. 1989 Differences between the 7-spot and 2-spot ladybird beetles (Coccinellidae) in their toxic effects on a bird predator. *Ecol. Ent.* **14**, 79–84.
Marples, N.M., van Veelen, W. & Brakefield, P.M. 1994 The relative importance of colour, taste and smell in the protection of an aposematic insect *Coccinella septempuntata*. *Anim. Behav.* **48**, 967–974.
Miller, R.G. 1974 The jackknife – a review. *Biometrika* **61**, 1–15.
Møller, H., Smith, R.H. & Sibly, R.M. 1989 Evolutionary demography of a bruchid beetle. I. Quantitative genetical analysis of the female life history. *Funct. Ecol.* **3**, 673–682.
Ottenheim, M., Holloway, G.J. & de Jong, P.W. 1992 Sex ratio in ladybirds. *Ecol. Ent.* **17**, 366–368.
Potvin, C. & Roff, D.A. 1992 Distribution-free and robust statistical methods: viable alternatives to parametric statistics? *Ecology* **74**, 1617–1628.
Quenouille, M.H. 1949 Approximate tests of correlation in time-series. *Jl. R. statist. Soc.* **B11**, 68–84.
Randall, K., Majerus, M. & Forge, H. 1992 Characteristics for sex determination in British Ladybirds (Coleoptera: Coccinellidae). *The Entomologist* **111**, 109–122.
Rice, W.R. 1989 Analyzing tables of statistical tests. *Evolution* **43**, 223–225.
Roper, T.J. & Cook, S.E. 1989 Responses of chicks to aposematic prey: effects of prey colour and early experience. *Behaviour* **100**, 276–293.
Rose, M.R. & Charlesworth, B. 1981 Genetics of life history in *Drosophila melanogaster*. I. Sib analysis of adult females. *Genetics* **97**, 173–186.
Sheppard, P.M., Turner, J.R.G., Brown, K.S., Benson, W.W. & Singer, M.C. 1985 Genetics and the evolution of Mullerian mimicry in *Heliconius* butterflies. *Phil. Trans. R. Soc. Lond.* B **308**, 433–613.
Sillen-Tullberg, B. & Bryant, E.H. 1983 The evolution of aposematic coloration in distasteful prey: an individual selection model. *Evolution* **37**, 993–1000.
Sokal, R.R & Rohlf, F.J. 1981 *Biometry*, edn 2 Freeman, San Francisco.
Tukey, J.W. 1958 Bias and confidence in not-quite large samples. *Ann. math. Statist.* **29**, 614.
Turner, J.R.G. 1977 Butterfly mimicry: the genetical evolution of an adaptation. *Evol. Biol.* **10**, 163–206.
Turner, J.R.G. 1984 Mimicry: the unpalatability spectrum and its consequences. *Symp. R. ent. Soc. Lond.* **11**, 141–165.
Wiklund, C & Jarvi, T. 1982 Survival of distasteful insects after being attacked by naive birds: a reappraisal of the theory of aposematic coloration evolving through individual selection. *Evolution* **36**, 998–1002.

Received 5 October 1994; accepted 16 December 1994

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES